Clinical review

Recent advances

Paediatric anaesthesia

S C S Russell, E Doyle

About one quarter of the population are aged under 15 years and many of them will require anaesthesia and surgery. Several recent developments have contributed to making anaesthesia for children easier, safer, and more pleasant for the child and parents.

Recent advances

- Amethocaine gel, a fast acting and potent topical local anaesthetic, has greatly improved pain relief for venous cannulation and preoperative preparation
- Sevoflurane is replacing halothane as the drug for inhalational anaesthesia. It is absorbed by and cleared from the body quicker than halothane and may eliminate the problem of halothane hepatitis
- Caudal epidural analgesia is often used for postoperative pain relief in children. Adding ketamine or clonidine to the anaesthetic greatly increases the duration of local action

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Topical cutaneous anaesthesia

One of the most unpleasant procedures for children in hospital is insertion of a venous cannula for induction of anaesthesia, taking blood samples, or administering fluid and drugs. To try to reduce the pain associated with the procedure a topically active local anaesthetic preparation is applied such as Emla cream (a mixture of 2.5 mg/ml of lignocaine and 2.5 mg/ml of prilocaine). To work properly Emla cream has to be applied at least an hour before the procedure,[1 2] which means it is not suitable for emergency and some day case patients. Even when applied for 60 minutes it is effective in only 65% of children,[3] although this proportion rises if it is left in place for 90-120 minutes.[4] Emla cream also causes vasoconstriction at the site of application, which can make venepuncture difficult.[5]

The recent development and licensing of a topical cream made with the local anaesthetic amethocaine has greatly improved topical anaesthesia. Amethocaine has a much higher lipid solubility than Emla and penetrates the stratum corneum, the main barrier to absorption of drugs through the skin, with comparative ease. A gel containing 4% amethocaine has been shown to provide analgesia for venous cannulation in over 80% of children compared with 66% of children who had Emla cream applied for the same period.[6] This makes amethocaine particularly useful in outpatient day case surgery. Amethocaine has also been found to cause erythema and vasodilatation at the site of application, which may facilitate venous cannulation.[7]

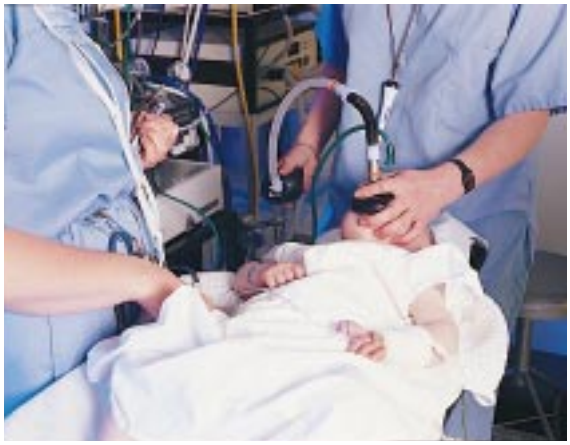
Sevoflurane

One of the ways in which paediatric anaesthesia differs from adult anaesthesia is that inhalational induction is used much more commonly. The characteristics of the volatile anaesthetic agent used are important. It needs to induce anaesthesia quickly, have a low incidence of complications such as coughing, laryngospasm, and hypoxia during induction, and allow rapid recovery. Until recently halothane was the most commonly used agent, and this generally results in smooth, trouble free inductions. However, a new agent, sevoflurane, is now available in Britain which has very low solubility in blood (the blood-gas partition coefficient is 0.47 compared with 2.1 for halothane). Studies have shown that it induces anaesthesia more rapidly than halothane,[8-12] and it is also eliminated more quickly from the blood, producing a faster recovery.[10-14]

Sevoflurane has a pleasant smell and is the least irritant of the volatile agents to the respiratory tract. There is a low incidence of respiratory tract irritation during its use.[15 16] It produces similar degrees of myocardial depression and hypotension to equipotent concentrations of halothane in children[10] and less sensitisation of the myocardium to catecholamines.[17] The degree of respiratory depression is similar to that produced by halothane at equipotent concentrations.[18 19]

Halothane hepatitis

Repeated administration of halothane occasionally results in halothane hepatitis. This can develop into hepatic necrosis, which may be fatal. The condition is probably caused by hapten formation with the trifluoroacetic acid metabolite of halothane and the subsequent production of antibodies and free radicals toxic to the liver. Halothane hepatitis is less common in children than adults[20 21] but is nevertheless important.[22] Since sevoflurane is metabolised to a much lesser extent than halothane (3.3% compared with 20%) and the metabolite trifluoroacetic acid is not produced,[23] the problem of halothane hepatitis should be sig-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Inhalational anaesthesia: sevoflurane is replacing halothane

nificantly reduced or eliminated. The degree of hapten formation with the sevoflurane metabolite hexafluoroisopropanol is much less than with trifluoroacetic acid and no free radical production has been found.[16] Only four cases of hepatotoxicity attributable to sevoflurane have been reported in over two million administrations.[24]

The low toxicity of sevoflurane makes it particularly useful in children since they often require repeated inhalational anaesthesia for multiple procedures carried out close together. The other available volatile anaesthetic agents, enflurane and isoflurane, are not suitable for routine inhalational induction of anaesthesia and are associated with a higher incidence of perioperative complications than halothane.[25 26] A drug which combines rapid trouble free induction of anaesthesia with minimal risk of hepatic damage during repeated administrations is thus a welcome advance in safety.

Caudal epidural blockade

Epidural analgesia using a single injection of local anaesthetic to the epidural space via the caudal approach combines the advantages of a simple technique with a high success rate and is one of the commonest local anaesthetic techniques used in children. The technique has a wide range of indications in paediatric practice including orchidopexy, circumcision, and inguinal herniotomy as well as lower limb and pelvic orthopaedic surgery and lower abdominal surgery in neonates and infants. Its main disadvantage is the short duration of action, and even long acting local anaesthetic drugs such as bupivacaine will reliably provide analgesia for only three to four hours.[27] Various additives to local anaesthetic solutions have been used to try to prolong the duration of caudal analgesia provided by a single injection. Opioids mixed with the local anaesthetic are effective at prolonging the duration of caudal epidural analgesia[28] but concerns about late respiratory depression mean that they can be used only for patients who are going to be nursed in a high dependency setting.

Alternative additives which are currently being investigated include clonidine and ketamine. Clonidine is an α_2 adrenergic receptor agonist, a class of drug which is widely used in medicine and anaesthesia as an antihypertensive, sedative, premedicant, and analgesic. Clonidine probably induces analgesia when administered epidurally by stimulating the descending noradrenergic medullospinal pathways. These inhibit the release of nociceptive neurotransmitters in the dorsal horn of the spinal cord. Ketamine hydrochloride is widely used for anaesthesia and analgesia in children. It acts as an antagonist at the subset of glutamate receptors stimulated by the agonist *N*-methyl D-aspartate (NMDA). NMDA receptors are found throughout the central nervous system including the lumbar spinal cord. As well as producing analgesia after systemic administration ketamine exerts profound analgesic actions at the spinal cord level in animal preparations.[29 30] This feature, together with the minimal respiratory depressant effects of ketamine, has stimulated clinical interest in the epidural administration of ketamine to provide postoperative analgesia.

Clonidine and ketamine have both been shown to prolong the duration of the local anaesthetic bupivacaine when used to provide caudal epidural analgesia. The median duration of caudal epidural analgesia with 0.25% bupivacaine is prolonged from 3-4 hours to 9-16 hours when 1-2 μg/kg of clonidine is added to the local anaesthetic solution.[31 32] Similarly, ketamine combined with 0.25% bupivacaine significantly prolongs the median duration of a single shot caudal epidural blockade to 12.5 hours.[33 34] The optimal dose of ketamine for prolonging caudal epidural blockade in children has been shown to be 0.5 mg/kg.[35] No differences have been found between children receiving caudal epidural clonidine or ketamine and control groups in the occurrence of side effects such as significant haemodynamic changes, respiratory depression, motor block, urinary retention, or postoperative sedation.[31-35]

Until new longer acting local anaesthetics that can selectively block sensory rather than motor and autonomic fibres are developed the use of additives is likely to continue. Clonidine and ketamine offer the potential to prolong the duration of single shot caudal injections with minimal risk of side effects. Further studies are needed, however, to compare the optimum regimens of these two additives and to obtain a more complete picture of their benefits and the incidence of side effects in children.

Conclusion

The three developments described here illustrate the ways in which paediatric anaesthesia differs significantly from practice in adults, where none of the above issues are as important. The need for and use of topical cutaneous analgesia is rare in adults and inhalational induction of anaesthesia is also unusual. Caudal epidural analgesia has far fewer indications in adults than children, although the additives described may also prove useful in epidural infusions of local anaesthetic and subarachnoid (spinal) anaesthesia.

1 Hallen B, Olsson GL, Uppfeldt A. Pain-free venepuncture. Effect of timing of application of local anaesthetic cream. *Anaesthesia* 1984;39:969-72.
2 Manner T, Kanto J, Lisalo E, Lindberg R, Vinamaki O, Scheinin M. Reduction of pain at venous cannulation in children with a eutectic mixture of lidocaine and prilocaine (EMLA cream): comparison with placebo cream and no local premedication. *Acta Anaesthesiol Scand* 1987;31:735-9.
3 Ehrenstrom-Reiz G, Reiz S, Stockman O. Topical anaesthesia with EMLA, a new lidocaine-prilocaine cream and the cusum technique for detection of minimal application time. *Acta Anaesthesiol Scand* 1983;27:510-2.

4 Bjerring P, Arendt-Nielson L. Depth and duration of skin analgesia to needle insertion after topical application of EMLA cream. *Br J Anaesth* 1990;64:173-7.
5 Bjerring P, Andersen PH, Arendt-Nielsen L. Vascular response of human skin after analgesia with EMLA cream. *Br J Anaesth* 1989;63:655-60.
6 Lawson RA, Smart NG, Gudgeon AC, Morton NS. Evaluation of an amethocaine gel preparation for percutaneous analgesia before venous cannulation in children. *Br J Anaesth* 1995;75:282-5.
7 Doyle E, Freeman J, Im NT, Morton NS. An evaluation of a new preparation of amethocaine for topical anaesthesia prior to venepuncture in children. *Anaesthesia* 1993;48:1050-2.
8 Black A, Sury MRJ, Hemington L, Howard R, Mackersie A, Hatch DJ. A comparison of the induction characteristics of sevoflurane and halothane in children. *Anaesthesia* 1996;51:539-42.
9 Lerman J, Davis PJ, Welborn LG, Orr RJ, Rabb M, Carpenter R, *et al.* Induction, recovery and safety characteristics of sevoflurane in children undergoing ambulatory surgery. *Anesthesiology* 1996;84:1332-40.
10 Piat V, Dubois MC, Johanet S, Murat I. Induction and recovery characteristics and hemodynamic responses to sevoflurane and halothane in children. *Anesth Analgesia* 1994;79:840-4.
11 Naito Y, Tamai S, Shinghu K, Fujimori R, Mori K. Comparison between sevoflurane and halothane for paediatric ambulatory surgery *Br J Anaesth* 1991;67:387-9.
12 Sarner JB, Levine M, Davis PJ, Lerman J, Cook DR, Motoyama EK. Clinical characteristics of sevoflurane in children. *Anesthesiology* 1995; 82:38-46.
13 Sury MRJ, Black A, Hemington L, Howard R, Hatch DJ, Mackersie A. A comparison of the recovery characteristics of sevoflurane and halothane in children. *Anaesthesia* 1996;51:543-6.
14 Landais A, Saint-Maurice C, Hamza J, Robichon J, McGee K. Sevoflurane elimination kinetics in children. *Paediatr Anaesth* 1995;5:297-301.
15 Eger EI. New inhalational agents desflurane and sevoflurane. *Can J Anaesth* 1993;40:R3-5.
16 Frink EJ, Brown BR. Sevoflurane. *Anesth Pharmacol Rev* 1994;2:61-7.
17 Imamura S, Ikeda K. Comparison of the epinephrine-induced arrhythmogenic effect of sevoflurane with isoflurane and halothane. *J Anaesth* 1987;1:62-8.
18 Doi M, Ikeda K. Respiratory effects of sevoflurane. *Anesth Analgesia* 1987;66:241-4.
19 Mori N, Suzuki M. Sevoflurane in paediatric anaesthesia: effects on respiration and circulation during induction and recovery. *Paediatr Anaesth* 1996;6:95-102.
20 Wark HJ. Post-operative jaundice in children. The influence of halothane. *Anaesthesia* 1983;38:237-42.
21 Warner LO, Beach TP, Garwin JP, Warner EJ. Halothane and children. The first quarter century. *Anesth Analgesia* 1984;63:838-40.
22 Kenna JG, Neuberger J, Mieli-Vergani G, Mowat AP, Williams R. Halothane hepatitis in children. *BMJ* 1987;294:1209-11.
23 Shiraishi Y, Ikeda K. Uptake and biotransformation of sevoflurane in humans: a comparative study of sevoflurane with halothane, enflurane and isoflurane. *J Clin Anaesth* 1990;2:381-6.
24 Ray DC, Bomont R, Mizushima A, Kugimaya T, Forbes Howie A, Beckett GJ. Effect of sevoflurane anaesthesia on plasma concentrations of glutathione-S-transferase. *Br J Anaesth* 1996;77:404-7.
25 Phillips AJ, Brimacombe JR, Simpson DL. Anaesthetic induction with isoflurane or halothane. *Anaesthesia* 1988;43:927-9.
26 Fisher DM, Robinson S, Brett CM, Perin G, Gregory GA. Comparison of enflurane, halothane and isoflurane for diagnostic and therapeutic procedures in children with malignancies. *Anesthesiology* 1985;63:647-50.
27 Kamal RS, Khan FA. Caudal analgesia with buprenorphine for post-operative pain relief in children. *Paediatr Anaesth* 1995;5:101-6.
28 Wolf AR, Hughes D, Wade A, Mather SJ, Prys-Roberts C. Postoperative analgesia after paediatric orchidopexy: evaluation of a bupivacaine-morphine mixture. *Br J Anaesth* 1990;64:430-5.
29 Brockmeyer DM, Kendig JJ. Selective effects of ketamine on amino-acid mediated pathways in neonatal rat spinal cord. *Br J Anaesth* 1995;74: 79-84.
30 Kristensen JG, Hartvig P, Karlsten R, Gordh T, Halldin M. CSF and plasma pharmacokinetics of the NMDA receptor antagonist CPP after intrathecal, extradural and iv administration in anaesthetized pigs. *Br J Anaesth* 1995;74:193-200.
31 Jamali S, Monin S, Begon C, Dubousset A-M, Ecoffey C. Clonidine in pediatric caudal anesthesia. *Anesth Analgesia* 1994;78:663-6.
32 Lee JJ, Rubin AP. Comparison of a bupivacaine-clonidine mixture with plain bupivacaine for caudal analgesia in children. *Br J Anaesth* 1994;72:258-62.
33 Naguib M, Sharif A, Seraj M, El Gammel M, Dawlatly AA. Ketamine for caudal analgesia in children: comparison with caudal bupivacaine. *Br J Anaesth* 1991;67:559-64.
34 Cook B, Grubb DJ, Aldridge LA, Doyle E. Comparison of the effects of adrenaline, clonidine and ketamine on the duration of caudal analgesia produced by bupivacaine in children. *Br J Anaesth* 1995;75:698-701.
35 Semple D, Findlow D, Aldridge LM, Doyle E. The optimal dose of ketamine for caudal epidural blockade in children. *Anaesthesia* (in press).

Science, medicine, and the future

Role of molecular cell biology in understanding disease

John Savill

Abstract

Molecular techniques have revolutionised our knowledge of cell and tissue function in both health and disease. We already have new and powerful treatments based on an understanding of communication between cells by messenger molecules called cytokines. Furthermore, there is great therapeutic promise in defining molecules which regulate cell adhesion, motility, proliferation, survival, and death. Rational manipulation of cell and tissue function for therapeutic ends may be much closer than you think.

Introduction

When I went to medical school in 1975 I did not lie awake at night thinking about cell biology. Indeed, I often cat napped as my teachers struggled to instil an interest in the structure and function of cells through histology. But 22 years later I regularly lose sleep excitedly planning experiments in molecular cell biology. My change in attitude relates to the fashionable and often overused prefix "molecular"; advances in molecular biology and protein chemistry over the past 15 years have fuelled a revolution in the dissection and manipulation of cell and tissue function. This article focuses on a few "hot" topics in molecular cell biology which have far reaching potential in medicine.

Cytokines and cytokine based treatments

In 1975 the messengers in cell communication were mainly elusive factors with confusing names based on activities defined in bioassays. Now these intercellular messengers have found an everyday use in medicine. For example, in haematology and oncology it is now routine to treat life threatening neutropenia with granulocyte colony stimulating factor. This polypeptide messenger or cytokine stimulates the bone marrow to produce and release neutrophil granulocytes needed in defence against infection.

What has changed in the last 20 years? Not the terminology, I fear. The unsystematic and confusing nomenclature of cytokines still depends on how or when the activity was described or the agent was biochemically characterised. The interleukins, now numbered in temporal sequence of identification, are cytokines with many cellular targets but the name reflects their discovery in leucocyte-leucocyte interactions. Interferons regulate many cell functions but were initially identified in studies of viral replication in cells.

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

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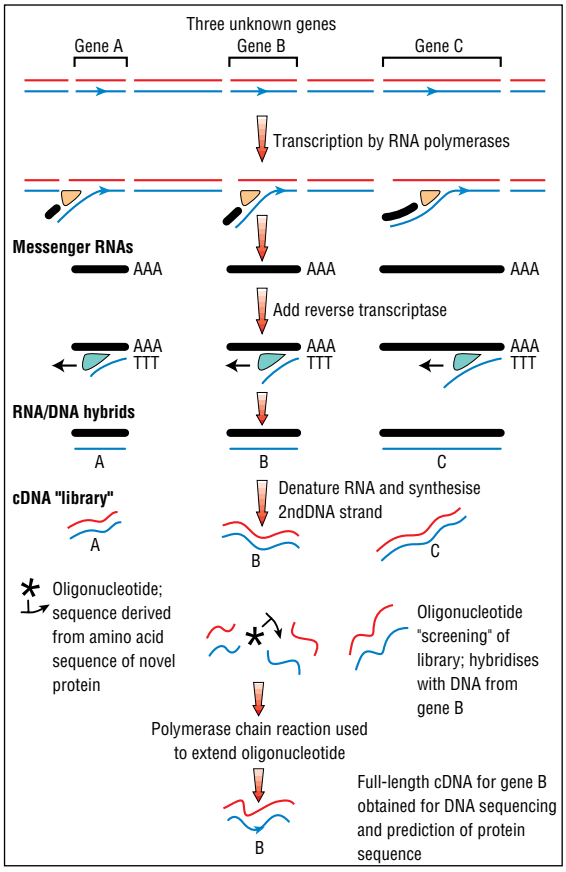

Fig 1 Identifying a novel protein by cloning from a cDNA library. A cDNA library is made by harvesting mRNAs from a tissue of interest. These are reverse transcribed to generate a collection or library of cDNAs which represent genes expressed in the tissue. Purification and amino acid sequencing of a protein fragment allows prediction and synthesis of the oligonucleotide which would encode the fragment. This can be used as a "hook" to "fish" for cDNA strands with complementary sequence and to retrieve, copy (clone), and sequence a full length cDNA allowing prediction of the full protein amino acid sequence

Growth factors, cytokines which stimulate cell division rather than enlargement, include the potent mitogen platelet derived growth factor, which is now known to be produced by many cell types other than platelets. Although transforming growth factor α stimulates epithelial cell division, transforming growth factor β has a different structure and wide ranging properties including inhibition of cell division. Indeed, accidents of discovery have awarded a single cytokine different names. Tumour necrosis factor α, a polypeptide initially defined as killing tumour cells in culture, was also called cachectin because of its catabolic properties in infection and cancer, but its main role seems to be in mobilising the acute inflammatory response.

Table 1 Methods of detecting gene expression

	Messenger RNA	Protein
In homogenised tissue specimens	Northern blotting reverse transcriptase polymerase chain rection (very sensitive)	Western (immuno) blotting
In histological sections	In situ hybridisation In situ polymerase chain reaction (very sensitive)	Immunohistochemistry
In solution	Not applicable	Enzyme linked immunosorbent assay Radioimmunoassay

The advances in cytokine biology have been in protein chemistry and molecular biology. New, sophisticated chromatographic techniques allow the separation of secreted proteins such as cytokines on the basis of size and electrostatic charge. Minute quantities of isolated protein can be "microsequenced" to define short stretches of the amino acid backbone. It is then possible to synthesise short lengths of nucleotide bases that encode the amino acid sequence and use them to clone the cDNA for the cytokine (fig1). Defining the nucleotide sequence of a full length cDNA allows prediction of the amino acid sequence of the whole protein, assignment to a particular cytokine family, and modelling of three dimensional structure. For example, the potent pro-inflammatory cytokine tumour necrosis factor α is a member of the graphically named "jelly roll" family.

Furthermore, the cDNA can be incorporated into viral based vectors which are then introduced into "workhorse" cell cultures that produce the cytokine protein product for harvest. The action of the pure cytokine can then be studied in culture or animal models. Similar information can be obtained by the converse approach of inhibiting cytokine function with neutralising antibodies generated by immunising animals with pure cytokine. Such antibodies are also invaluable in detecting and quantifying cytokine expression in biological solutions such as plasma, in histological sections, and (in experimental settings) in homogenised tissue specimens—complementing techniques based on measuring mRNA expression (table 1).

Cytokine based treatments will be particularly important in inflammatory, immune, and infective conditions. Human rheumatoid arthritis can be temporarily ameliorated with neutralising antibody to tumour necrosis factor α, and there is every prospect of attaining similar improvements with physiological, non-immunogenic agents such as soluble tumour necrosis factor receptor. Given the importance of tumour necrosis factor α in mediating tissue injury associated with septic shock, such inhibitors may also prove useful in adjunctive treatment of severe infections. Indeed, as we understand more of the endogenous checks and balances of cytokine action we can look forward to anti-inflammatory treatments based on molecules such as the interleukin 1β receptor antagonist, a naturally occurring cytokine which blocks receptors for interleukin 1β during resolution of inflammation.

Adhesion molecules and cell matrix interactions

Cell biologists now have a remarkably detailed understanding of the adhesion molecules that mediate emigration of leucocytes from the blood across the wall of the postcapillary venule and into the tissues. Cell surface adhesion molecules are the Velcro which hold cells and tissues together. Those expressed by leucocytes and endothelial cells are special in that their stickiness is regulated by pro-inflammatory messenger molecules such as cytokines (fig 2).

Anti-inflammatory treatments based on selective inhibition of endothelial-leucocyte adhesion molecules are about to be tested in humans. They have already proved effective in blocking inflammatory responses in

animal models. Furthermore, these approaches may help to prevent bloodborne metastasis of cancer cells, which may occur by similar adhesive mechanisms.

Adhesion molecules are also important conduits of information between the extracellular matrix and cells. The extracellular matrix is composed of proteins and proteoglycans which surround cells in solid organs and form basement membranes in epithelia. It is now clear that the extracellular matrix can greatly affect attached cells—for example, the extracellular matrix protein laminin is critical for differentiation of alveoli in the lactating breast. Extracellular matrix proteins can also signal survival of cells, holding at bay otherwise inevitable cell death by the physiological cell suicide programme of apoptosis. By contrast with accidental cell death or necrosis, apoptosis is a precisely regulated and programmed cell death which amounts to suicide because the cell activates a cascade of death enzymes which kill the cell and mark it for safe clearance by phagocytes. Excitingly, antibody mediated blockade of an extracellular matrix receptor on new blood vessels growing into tumours results in apoptosis of vascular cells and tumour involution.

Cells of many lineages, including tissue cells such as fibroblasts, can also remodel extracellular matrix by secreting matrix metalloproteinases, protein degrading enzymes which digest extracellular matrix providing they are supplied with calcium or other metal ions. This can, for example, enable cells to migrate through tissues. Consequently, it should not be surprising that inhibitors of matrix metalloproteinases are showing promise as antimetastatic agents in cancer. Conversely, naturally occurring inhibitors of extracellular matrix degrading enzymes could be beneficially blocked to prevent postinflammatory scarring.

Cell proliferation, survival, and death

Molecular cell biology could have particularly important implications in cancer. In this article it is sufficient to highlight the important role in carcinogenesis of somatic mutations (that is, arising after fertilisation of an egg). These mutations bring about increased expression of a set of oncogenes, which encode proteins involved in cell proliferation. Examples include erb, which encodes a cell surface growth factor receptor, and c-myc, which gives rise to a nuclear protein essential for cell division.

However, disregulated expression of bcl-2, a ubiquitous gene first characterised in follicular B cell lymphoma, leads to cell accumulation by promoting cell survival rather than cell proliferation. Bcl-2 protein is a potent inhibitor of most stimuli which induce apoptosis, the programmed and physiological form of cell death that normally counterbalances cell birth by mitosis. A disrupted balance of cell survival and cell death by apoptosis may be important in many diseases (box). For example, an important application may be in neurodegenerative diseases such as spinal muscular atrophy. Molecular geneticists discovered that the gene mutated in this condition normally encodes a molecule similar to an inhibitor of an apoptosis protein first found in insects. This protein proved to be the index member of a ubiquitous family of survival proteins and is a candidate treatment for spinal muscular atrophy. Indeed, other nerve cell survival factors are being tested as treatments for commoner conditions associated with unscheduled neuronal death, such as Parkinson's disease.

Role in disease of disordered balance between cell survival and cell death by apoptosis

Probable role:
Cancer
Infections—for example, HIV/AIDS
Neurodegenerative disease
Autoimmunity—for example, systemic lupus erythematosus, rheumatoid arthritis
Inflammatory disorders
Possible role:
Atherosclerosis
Osteoporosis

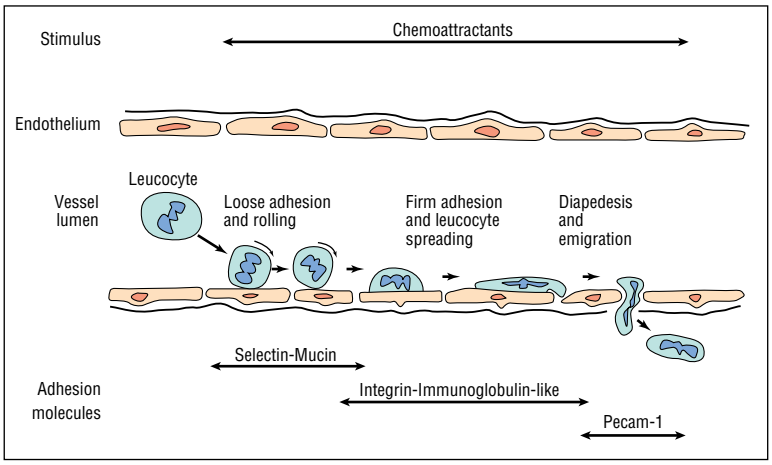

Fig 2 Molecular mechanisms of inflammation. Inflammatory mediators generated by tissue injury act on both leucocytes and endothelial cells. Leucocytes are initially attached to and rolled along the vessel wall, mediated by receptors of the selectin family and their counter receptors of the mucin family. Next, leucocyte receptors of the integrin family mediate firm adhesion and spreading of leucocytes on endothelial cells by binding endothelial cell receptors with structural similarity to the immunoglobulins. A particular receptor in this family (platelet-endothelial cell adhesion molecule 1) mediates movement of leucocytes between endothelial cells and out into the extravascular space

Designer proteins

New skills in molecular and cell biology can be combined to make cultured cells synthesise altered proteins with therapeutically desirable properties. As described in preceding articles, site directed mutagenesis can be used to alter cDNAs so that altered proteins are synthesised when cells are transfected with a viral based expression vector bearing the new cDNA. An example is soluble complement receptor 1, a truncated form of a protein which normally spans cell membranes. Normal or "wild type" complement receptor 1 binds and inactivates injurious pro-inflammatory peptides generated by the complement cascade. Soluble complement receptor 1, which was mutated so that it lacks the membrane-spanning domain, can be harvested from cellular expression systems. After intravenous injection the protein circulates and can be used as a potent anti-inflammatory agent.

Microbiology and immunology

Molecular cell biology has many applications in the prevention and treatment of infectious disease. Defining how micro-organisms adhere to and breach cellular barriers will provide new strategies for treatment. For example, both rhinoviruses and erythrocytes parasitised with malaria can adhere to intercellular adhesion molecule 1, which usually mediates cell-cell interaction in inflammatory and immune responses. The potential usefulness of blocking such adhesion is shown by recent data on the leucocyte β-chemokine receptor type 5; a chemokine is a cytokine which attracts leucocytes. This receptor is used by HIV-1 to infect leucocytes; people with an inherited deficiency in chemokine receptor type 5 resist HIV-1 invasion of leucocytes and may live for many years without developing AIDS.

Vaccine development has also received a boost from the ability to construct and express designer proteins. Many proteins have evolved by evolutionary shuffling of building blocks called domains. It is now possible to shuffle the pack further almost at will by fusing cDNAs which encode protein domains. Fusion proteins in which bacterial protein domains are combined with several copies of a human complement protein domain greatly enhance the immunogenicity of the bacterial material.

Choosing proteins on which to base new vaccines may become much easier because of improved knowledge of the molecular cell biology of the immune response. Antigen presenting cells activate protective immune responses by processing ingested antigenic proteins into short peptides which are then presented at the surface of the cell. The antigenic peptide is displayed to T lymphocytes by being fixed in the "jaws" of major histocompatibility complex molecules on the surface of antigen presenting cells. The presented peptides can be isolated and sequenced or characterised by screening candidate peptides for their ability to promote reassociation in vitro of the isolated subunits of major histocompatibility complex molecules. These last techniques have been used to identify the malarial protein liver stage antigen 1 as a vaccine candidate. Peptides from the protein bind the major histocompatibility complex molecule B53, which seems protective as people with severe malaria possess B53 less often than controls.

Lastly, molecular techniques for manipulating cell function have revolutionised the discipline of immunology, which has pioneered developments in cell adhesion, cytokine biology, and cell death. New approaches to treating autoimmune diseases such as rheumatoid arthritis will be discussed in future articles in this series.

Conclusion

I hope you can understand why molecular cell biology excites me. Understanding the molecular basis of cell and tissue function will provide insights into the pathogenesis of disease. The discipline has, and will, bring basic science to the bedside in the form of new and effective treatments.

Lesson of the week

Prevalence of concomitant disease in patients with iron deficiency anaemia

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Colonic cancer must be excluded in patients with iron deficiency anaemia

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How to investigate patients presenting with iron deficiency anaemia is a matter of debate. Undoubtedly one of the commonest lethal, but potentially curable, causes is colonic carcinoma, the second most common cancer in both men and women in the West. Despite this, the colon may not be investigated if an acceptable cause for the anaemia has been found on upper gastrointestinal investigations.

Clinically important concomitant disease, such as benign upper gastrointestinal disease associated with colonic cancer, has been reported in up to 7% of anaemic patients,[1-3] yet combined upper and lower gastrointestinal investigations are carried out in only a minority of cases with iron deficiency anaemia.[4]

Patients, methods, and results

We examined the records of 89 consecutive patients referred with iron deficiency anaemia and at least two positive faecal occult blood results between 1989 and 1992. Iron deficiency anaemia was defined by a haemoglobin concentration of <110 g/l in association with a mean corpuscular volume of <80 fl and either a serum ferritin concentration of <10 mg/l or an appropriate response to iron supplementation. Patients were either followed up or their case notes were reviewed or their general practitioner was contacted. The case notes of all patients were reviewed in July 1995, a minimum of three years after initial presentation. We identified "acceptable" causes for the anaemia in 67 patients (table 1). Six patients had concomitant disease; in three potentially curative surgery was delayed because of the gastroscopy findings (box). Cases 1-7—and a more recent case (8)—show the importance of not ascribing the cause of iron deficiency anaemia to a lesion in the oesophagus, stomach, or duodenum unless the colon has been investigated.

Discussion

The prevalence of colonic cancer among patients presenting with iron deficiency anaemia varies consider-

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ably, with outpatient studies suggesting rates of 4-11%.[1 3 5 6] We diagnosed malignant disease of the colon in 14 (15%) patients. Of the 13 patients who were initially found to have colonic cancer, six (46%) had "acceptable" upper gastrointestinal causes for their anaemia. The incidence of colonic cancer increases with age, 94% occurring in patients over the age of 50.[7] All our patients were aged over 60, and all cancers were proximal to the splenic flexure. The higher prevalence of colonic cancer in our audit might be attributed to our use of faecal occult blood loss as a selection criterion, in contrast to other studies,[1 3 5 6] but the role of faecal occult blood testing in iron deficiency remains to be evaluated.

The risks of not investigating the colon have been further emphasised by a recent audit of the investigation and outcome of iron deficiency anaemia.[4] Long term follow up identified two patients (at 21 and 18 months) with advanced carcinoma of the colon. Neither patient had had colonic investigations at the time of initial presentation.

Of the six patients with concomitant disease, three had potentially curative surgery delayed because of their gastroscopy findings (cases 1, 2, and 6). The delay in the cases 2 and 6 was particularly lengthy (10 and 12 months, respectively). We suspect that had colonic investigations been carried out at the time of initial presentation, the diagnosis would almost certainly have been made.

The potential for delay in diagnosing proximal colonic cancer was first raised in 1969.[8] Fagan reported that 10 out of 96 cancers of the right side of the colon were initially misdiagnosed as an upper gastrointestinal lesion on the basis of a positive finding on barium meal examination. A more recent study concluded that a delay in referral for investigation was the main avoidable reason for delay in diagnosis rather than the finding of an upper gastrointestinal lesion.[9]

Symptoms did not correlate well with the final diagnosis. This has been shown before.[1 2 3 10] Of the six patients with concomitant disease, three had upper gastrointestinal symptoms while none had proximal colonic symptoms. Only Rockey *et al* reported that colonic symptoms predicted colonic disease.[5]

Table 1 Results of investigations (89 patients)

Diagnosis	No of patients
Oesophagitis	14
Colonic cancer	13
Gastric erosion	13
Erosive gastritis	7
Benign colonic adenomatous polyp	6
Duodenal ulcer	5
Gastric cancer	3
Coeliac disease	3
Colonic angiodysplasia	2
Gastric adenomatous polyp	2
Ulcerative colitis	2
Oesophageal cancer	1
Gastric ulcer	1
Barrett's ulcer	1
Gastric angiodysplasia	1
Crohn's disease	1
No diagnosis	14

Concomitant disease in eight cases of iron deficiency anaemia

Case 1—65 year old man; haemoglobin 83 g/l; no gastrointestinal symptoms. Deep Barrett's ulcer shown by gastroscopy. Iron and ranitidine resolved ulcer and anaemia. Latter recurred after stopping treatment. Barium enema six months later showed caecal mass. Right hemicolectomy for Dukes' C caecal carcinoma.

Case 2—67 year old man; increasing dysphagia; haemoglobin 68 g/l. Previous benign oesophageal stricture. Treated with iron and omeprazole for severe oesophagitis. Anaemia recurred 10 months later; barium enema indicated caecal mass. Palliative hemicolectomy for annular Dukes' C caecal carcinoma.

Case 3—72 year old man; haemoglobin 77 g/l; minimal fresh rectal bleeding. Erosive gastritis at gastroscopy and caecal mass on barium enema. Right hemicolectomy for Dukes' B caecal carcinoma six weeks later.

Case 4—79 year old woman; haemoglobin 107 g/l; no gastrointestinal symptoms. Erosive gastritis at gastroscopy and "apple core" lesion in ascending colon on barium enema. Right hemicolectomy for Dukes' B carcinoma one month later.

Case 5—69 year old man; haemoglobin 104 g/l; epigastric discomfort. Grade 1 oesophagitis and ascending colon filling defect. Right hemicolectomy for Dukes' B carcinoma.

Case 6—75 year old man; haemoglobin 105 g/l; emergency admission; history of haematemesis, melaena, iron deficiency anaemia. Oesophageal ulcer treated with omeprazole. Barium enema for recurrent anaemia showed ascending colonic mass; Dukes' C carcinoma confirmed at surgery.

Case 7—70 year old woman; abdominal mass confirmed as Dukes' B caecal carcinoma. Investigated seven years earlier for iron deficiency anaemia: normal results; iron treatment resolved anaemia. This recurred three years later; gastroscopy and colonoscopy to hepatic flexure only were normal. Repeat barium enema advised but declined by patient.

Case 8—68 year old man; reflux helped by ranitidine; haemoglobin 67 g/l. Chronic duodenal ulcer on gastroscopy and mass at hepatic flexure on barium enema examination. Right hemicolectomy for Dukes' B carcinoma two weeks later. (Note: this case is not included in the original series of 89 patients.)

Follow up of patients in 1995, at least three years after their initial investigations, has not shown serious gastrointestinal disease in patients discharged after negative results on gastroscopy, duodenal biopsy, and barium enema, confirming that this is a safe, limited approach to the investigation of patients presenting with iron deficiency anaemia.[11] Duodenal biopsy is a simple process during gastroscopy, and many series

have shown a prevalence of coeliac disease in iron deficiency anaemia of 3-5%.[1] One patient (case 7) had developed a Dukes' B ascending colon carcinoma at follow up. A barium enema had originally been advised but declined by the patient.

Although colonoscopy is accepted as the best way to visualise the colon,[12] less experienced workers may fail to reach the caecum in up to half the cases.[13-15] Double contrast barium enema may occasionally miss colonic cancer, but these are usually in the sigmoid.[16] We found that colonic cancers presenting with iron deficiency anaemia were proximal to the splenic flexure, suggesting that double contrast barium enema is the investigation of choice, with colonoscopy reserved for persistent or recurrent anaemia or when doubts have been raised by the results of barium enema examination.

We believe that the risk of missing colonic cancer in patients with iron deficiency anaemia is sufficient to justify colonic examination, if findings at gastroscopy are benign. A cut off age of 40 would seem appropriate in view of the evidence that almost all colonic cancers occur over the age of 50.[7] Younger patients without a family history or predisposing condition should probably have colonic investigations for persistent anaemia.

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1 McIntyre AS, Long RG. Prospective survey of investigations in out-patients referred with iron deficiency anaemia. *Gut* 1993;34:1102-7.
2 Cook IJ, Pavli P, Riley JW, Goulston KJ, Dent OF. Gastrointestinal investigation of iron deficiency anaemia. *BMJ* 1986;292:1380-2.
3 Kepczyk T, Kadakia SC. Prospective evaluation of gastrointestinal tract in patients with iron-deficiency anaemia. *Dig Dis Sci* 1995;40:1283-9.
4 Lucus CA, Logan ECM, Logan RFA. Audit of the investigation and outcome of iron deficiency anaemia in one health district. *J R Coll Physicians Lond* 1996;30:33-6.
5 Rockey DC, Cello JP. Evaluation of the gastrointestinal tract in patients with iron deficiency anaemia. *N Engl J Med* 1993;329:1691-5.
6 Gordon SR, Smith RE, Power GC. The role of endoscopy in the evaluation of iron deficiency anaemia in patients over the age of 50. *Am J Gastroenterol* 1994;89:1963-7.
7 Office of Population Censuses and Surveys. *Cancer statistics registrations*. London: HMSO, 1988. (Series MBI, No 16.)
8 Fagan PA. The "red herring" barium meal x-ray examination in carcinoma of the right side of the colon. *Med J Aust* 1969;i:276-8.
9 Stebbing GF, Nash AG. Avoidable delay in the management of carcinoma of the right colon. *Ann R Coll Surg Eng* 1995;77:21-3.
10 O'Reilly D, Long RG. Carcinoma of the colon presenting with dyspepsia. *Postgrad Med J* 1987;63:215-6.
11 Sahay R, Scott BB. Iron deficiency anaemia—how far to investigate? *Gut* 1993;34:1427-8.
12 Sayer JM, Long RG. A perspective on iron deficiency anaemia. *Gut* 1993;34:1297-9.
13 Aldridge MC, Sim AJW. Colonoscopy findings in symptomatic patients without X-ray evidence of colonic neoplasms. *Lancet* 1986;ii:833-4.
14 Irvine EJ, O'Conner J, Frost RA, Shorvon P, Somers S, Stevenson GW, *et al*. Prospective comparison of double contrast barium enema plus flexible sigmoidoscopy v colonoscopy in rectal bleeding: barium enema v colonoscopy in rectal bleeding. *Gut* 1988;29:1188-93.
15 Lindsay DC, Freeman JG, Cobden I, Record CO. Should colonoscopy be the first investigation for colonic disease ? *BMJ* 1988;296:167-9.
16 Dodd GD. Imaging techniques in the diagnosis of carcinoma of the colon. *Cancer* 1992;70 (suppl):1272-5.

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A MEMORABLE PATIENT

Deceived by appearances

He was only a few years older than me, with a friendly smile. His muscular physique and bronzed complexion were, he told me proudly, the result of a working life spent mainly in the fresh air. It was the second week of my first house job, and he was the first patient on the list for minor day case surgery. I was pleased to see a fit young man—clerking him would be an easy job.

His medical history was brief; he had rarely consulted a doctor. I chided him on his smoking habit and moved on quickly to the physical examination. His chest was clear, his cardiovascular system normal. His abdomen was firm and difficult to palpate, but I was confident that it was normal. A brief nervous system examination was similarly unremarkable. As I felt for his apex beat, I had noticed the Elastoplast stuck to his chest, just above his left nipple.

We chatted as he signed the consent form, and he seemed eager to keep me talking. I was equally keen to move on to the next patient, and tried not to feel irritated. After all, I thought, he must be worried about his operation. Eventually I made my escape, but he called me back as I reached the end of his bed.

"Doc, would you like to take a look at this?" With a nervous flourish, he pulled off the Elastoplast.

"I bet you've never seen a thing like this before, Doc."

In fact I had, but only in textbooks. I felt as if I had stepped off a cliff into nothingness; I could almost hear the wind rushing past my ears. I struggled desperately to control my features, and went to take a closer look.

The melanoma filled the area under the Elastoplast and was obviously both malignant and advanced. A large, jet black central nodule had a ragged surface, crusted with dried blood. The margin was mottled with paler hues, and was as irregular as an amoeba. A couple of small moles nearby, which earlier I had barely noticed, now took on the sinister appearance of satellite metastases.

My patient did not seem to find it strange that I wanted to examine him again. With icy fingers and rising nausea, I identified first the craggy mass of nodes in his left axilla and then the suspicious lump above his left clavicle. I still thought that his abdomen was probably normal, but could I just feel a firm liver edge?

He could not remember when the mole had appeared, but it had started to bleed at least two months previously. He knew that he would be coming into hospital soon, so why bother his general practitioner?

He never had his operation, of course. He was transferred to the oncology unit at another hospital, and I never saw him again. Years later, I am still grateful to him for teaching me three invaluable lessons so early in my medical career. Firstly, that the healthiest looking patient may hide the most serious pathology. Secondly, that however busy you are, take the time to look under any dressings or clothing and always examine the whole patient. Above all, remember that the patient who keeps on talking may well have something important to tell you.

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We welcome filler articles of up to 600 words on topics such as *A memorable patient, A paper that changed my practice, My most unfortunate mistake,* or any other piece conveying instruction, pathos, or humour. If possible the article should be supplied on a disk.