Clinical review

Science, medicine, and the future

Prospecting for gold in the human genome

John Savill

Abstract

Doctors struggling with the daily problems of clinical medicine usually have little time for molecular and cell biology. But genetic research is producing an explosion of knowledge which doctors will need to understand in order to join in the ethical and financial debates that will inevitably follow the new treatments discovered. There may, indeed, be therapeutic gold hidden in our genes, but the price for it could be more than we can afford. This is the first of three articles introducing a series which aims to convey the excitement and potential power of biomedical science by speculating how current research will impinge on clinical management of common conditions.

Introduction

Doctors toiling at the coal face of modern managed health care may be entitled to think that optimism and excitement are outmoded states of mind in medicine. But these feelings are major stimuli to ever more rapid and profound developments in the biomedical sciences. As a clinical scientist I can appreciate both points of view. On one hand I am part of an exciting programme of laboratory research into the molecular cell biology of inflammatory disease. On the other, I am a consultant in renal and general medicine. In common with my clinical colleagues I struggle, Canute-like, with a rising tide of acute admissions and all the other joys of the ever changing NHS. But as I plod round the medical admissions ward I feel both excitement and optimism.

Paradoxically, my excitement is based on ignorance. This word is not easily applied to Nottingham's medical senior house officers as they clerk in patient after patient, sifting through the differential diagnosis to arrive at labels such as asthma, stroke, myocardial infarction, or chronic obstructive airways disease. But deep down I know that none of us understands the pathogenesis of these disorders, even when the diagnosis is clear. Whether evidence based or not, too many of the treatments we offer address effect rather than cause, and too few are both safe and reliable.

I feel optimism because there is now a realistic prospect that this frustrating situation will be changed by advances in biomedical sciences. As a "taster" this article is accompanied by Ian Hall's views on the future of asthma.[1] Similar approaches are being applied usefully to a wide range of important clinical problems. In this article I outline a new, genome based philosophy of the biomedical sciences which may greatly alter our approach to disease and treatment.

From gene to function

Biology was once an almost arcadian pursuit. In what might be termed the "pre-industrial" era of biomedical science careful observation and classification chipped away at the edifice of the unknown. However, advances in molecular and cell biology have unleashed a new investigative approach which, like a mechanised army, is systematically crushing ignorance. The epitome of this new biology is the human genome project (box). By as early as 2004 scientists will have located and determined the DNA sequence of nearly all our 90 000 or so genes. This flood of new knowledge may require a profound revision of thinking among biomedical investigators, funding bodies, and the pharmaceutical and biotechnology industries. Many are now openly discussing a systematic "big science" approach toward determining the function of thousands of new genes—perhaps 80% of which are expected to contribute to the unique properties of the human brain—rather than pursuing the "small science" of curiosity driven research.

The human genome project promises unprecedented opportunities for science and medicine. The tools already available to the biomedical community should allow rapid exploitation of new knowledge. Gene function could be explored in several ways. The growing computer based science of bioinformatics allows rapid comparison of novel genes with known genes, prediction of the amino acid sequence of the protein encoded, and thanks to a growing library of three dimensional protein structures solved by nuclear magnetic resonance or *x* ray crystallography, modelling of the three dimensional structure of the gene product (fig 1). This information may allow us to predict protein function. Function can also be inferred from the temporal and spatial distribution of gene expression in tissue samples, which is assessed by detecting messenger RNA or protein product, or both. I will describe these techniques in future articles.

In vitro studies can also give us an insight into gene function. Genes are now routinely expressed in cultured cell lines by using viral based DNA vectors containing complementary DNA (cDNA), a piece of

The human genome project

- This is a global initiative which in Britain has multimillion pound support from the Medical Research Council, The Wellcome Trust, and industry
- Phase one, which is essentially complete, has been to define marker regions of DNA which together constitute a detailed map of the human genome. New genes can now be localised with accuracy to tiny portions of a particular chromosome
- The second phase, already far advanced in particularly interesting parts of the genome, is to define the DNA sequence of all the 90 000 plus human genes
- Success will rely heavily on automation and robotics but most of all on bioinformatics, the computer based science of logging and comparing DNA sequences. Studies of non-human genomes have been particularly profitable
- The rewards of the project are likely to be rich and unpredictable. We can expect a major contribution to understanding of the genetic basis of human disease, but we also need comparable advances in ethics. We may also need to revise current approaches to unravelling disease pathogenesis and developing new therapies

DNA artificially generated by using mature messenger RNA as a template. Therefore, transcription of the cDNA yields the gene's messenger RNA. The protein produced from the mRNA may then confer specific and detectable functions on the "workhorse" cells used to express the gene—for example, a new cell surface adhesion molecule which confers capacity to bind leucocytes. Furthermore, it is possible to manipulate cDNA so that proteins are expressed in a soluble form fused to polypeptide "tags." This allows rapid purification of large amounts of the protein that can be used to raise antibodies, grow crystals for structural studies, or probe protein function in vitro or in vivo in animal models.
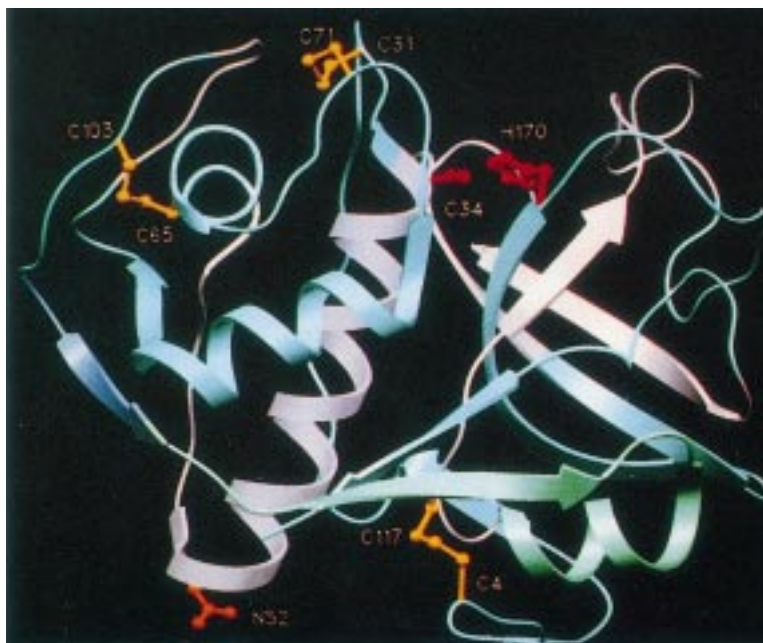

Fig 1 Computer model of 3D protein structure. The *Der p* I antigen of the house dust mite *Dermatophagoides pteronyssinus* is a major allergen in asthma. This structure was modelled on the basis of amino acid sequence and three known structures in the same cysteine protease family. The coils represent α helices and the arrows depict β strands. (Reproduced with permission of Oxford University Press from C M Topham *et al*, *Protein Engineering* 1996;7:869-94)

The best way to determine function, however, is to manipulate gene expression in vivo. Gene manipulation is most sophisticated in mice because we can obtain pure and self propagating cultures of undifferentiated, pluripotential murine embryonic stem cells. Specific genes in the embryonic stem cells can be inactivated. The manipulated stem cells can be injected into blastocysts to generate chimeric animals from which "knockouts" can be bred. This technique is becoming ever more sophisticated, and it is possible to knock out genes in particular cell lineages or even in adult animals by exposing them to specific triggers. The approach is very powerful and has already been used to generate models of inherited diseases characterised by loss of function such as cystic fibrosis.

From gene to treatment?

The human genome project will provide enormous commercial opportunities for the pharmaceutical and biotechnology industries, which have been quick to realise the potential for new treatments. Traditional problem driven research has produced many possible targets for treatment, and molecular techniques have been rapidly harnessed in the race for new compounds with therapeutic potential. As soon as a cell surface receptor is identified as a possible drug target scientists can clone a family of similar receptors, express these in cell lines, and test a vast panel of reagents for activation or inhibition of a biological response (for example, triggering of calcium influx into the cell). New compounds identified by this high throughput screening approach are already in the therapeutic pipeline.

A particularly enticing prospect is that of rational drug design based on knowledge of the three dimensional structure of the therapeutic targets. Much of this is sophisticated, computer based work but there is already a craving for more structures and more treatment targets. The three dimensional structures of many pathologically important proteins are not yet known, and determining them may suggest new treatments. However, there is growing excitement in pharmaceutical circles that the genome project may identify new genes and proteins that have a role in disease.

The "big science" approach would use all the techniques described above to determine gene function and identify effects that are likely to be important in disease—for example, neurodegeneration induced by knocking out a gene. Genetic research would, however, need to be supplemented by screening for under or over expression of particular genes in diseased tissue. Once new genetic targets are identified the encoded proteins could be expressed, the structure solved, and a drug or gene therapy developed. This is not the pipe dream of an out of touch enthusiast; major pharmaceutical companies have already invested heavily in this thinking.

Problem driven research remains crucial

Fortunately for the constitutively curious such as myself, but less fortunately for our patients, our ignorance of the pathogenesis and treatment of disease will not be eliminated by the biological steamroller I have described above. The least intellectually

satisfying but most powerful reason is that it will probably be too expensive for society or commerce to analyse the effect of knocking out each of the 90 000 genes or solve the structure of 90 000 proteins. Additionally, the steamroller approach may be misleading. For example, make a knockout of the immunosuppressive cytokine transforming growth factor β1 and you observe spontaneous and severe inflammatory responses in the animal's gut. However, the pro-fibrotic properties of this cytokine are not revealed, although these are likely to be crucial to its function. Thus, in a surgical wound threatened by dehiscence increased amounts of transforming growth factor β1 may be a good thing, whereas in an inflamed lung it threatens irreversible scarring and loss of function.

Not only does the big science approach threaten to overlook such subtleties, it may also overlook molecules important in therapy. For example, knockout mice with disrupted expression of the cytokine interleukin 2, a potent activator of T lymphocytes, retain near normal immune responses because other cytokines can substitute for it. But interleukin 2 may still have an important therapeutic role in enhancing cytotoxic T cell responses to tumours.

Consequently, I believe that there will continue to be an important role for investigation driven by clinical problems and aimed at dissecting the complex pathogenesis of disease. However, the human genome project will minimise delay in identifying new proteins and genes or in obtaining specific reagents. Solving a clinical problem such as coronary artery disease will require the coordinated efforts of different "tribes" of investigators with complementary skills. Biochemists, cell biologists, and molecular geneticists cannot work in isolation from cardiologists, pathologists, and radiologists. Clinical scientists wishing to find new treatments will rely more and more on those with skills in design and execution of clinical trials, while epidemiological coups such as the Barker hypothesis of fetal and infant programming for later coronary disease will continue to spark new lines of scientific inquiry.

Conclusions

If we care to raise our eyes above seemingly ever lower clinical horizons it should be apparent that we live in exciting times. The new biology is not a flash in the pan, a passing enthusiasm of academia, or another irritation from central administration. In the next 20 to 30 years the pace of discovery will increase. It seems critical to me that doctors in every discipline should have some knowledge of what is in store—because hard decisions will need to be made about what is affordable or ethical.

1   Hall IP. The future of asthma. *BMJ* 1997;314:45-9.

The future of asthma

Ian P Hall

Abstract

Reports in the media describing the discovery of "the asthma gene" or "a new vaccine for asthma" may be inaccurate and often raise patients' hopes unjustifiably. This article is a personal view of key areas in current asthma research, and it examines how they may impinge on the care of asthmatic patients in the next decade. I have tried to explain the thinking behind some of the approaches under study. I discuss the potential contributions of genetic studies, immunological approaches, and new therapeutic approaches.

Introduction

The molecular sciences have promised so much over recent years, but have they really delivered important advances that have improved the way we practise clinical medicine? Cynics could maintain that the main classes of drugs used for treating asthma have been around for over 20 years, and, despite a vast range of potential targets in the airways having been identified, no new major treatments have arisen as a direct consequence of basic scientific research. However, I think there is genuine reason to be excited about the prospects for the next few years. Research in key areas has reached a stage where we can expect major advances in our understanding of the pathogenesis of asthma. This article is a highly personal view of what I consider to be key developments that will affect asthma treatment over the next decade.

Possible future developments

- Genetic screening for "at risk" individuals
- Targeted gene therapy
- New therapeutic agents
- Immunisation approaches (such as anti-IgE antibodies)

Genetics of asthma

Although the mechanisms underlying the inflammatory response in the airways are still not fully understood, it has become clear that both genetic predisposition and subsequent environmental exposure to allergen are integral to the development of the disease. While studies of twins and families have shown that asthma is a heritable disorder, it is clearly not a disease caused by a single gene defect but rather a polygenic disorder. It is only recently that studies looking at which genes may predispose to the development

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of allergic disease have been performed. Two main approaches have been used: genome screening and identification of potential candidate genes.

Genome screening

In this approach the whole of the human genome is screened by means of markers for regions of DNA (microsatellites) that are highly variable between individuals. By spreading microsatellite markers across the whole of the human genome and looking for an association with markers for allergic disease (such as elevated IgE titres, bronchial hyperreactivity, atopy, or clinical asthma), it is possible to identify genes that may be important in defining each of these subphenotypes of asthma.

In Britain and the United States genome screens have recently been completed in populations of patients with asthma, and genetic loci likely to be important in the development of allergic disease have been identified. At present, these regions of interest in the genome are located only approximately, and the fine mapping of these loci—essential to identify specific genes rather than just regions of human chromosomes—will not be complete for another couple of years. Nonetheless, this approach provides the best hope of identifying novel gene products important in the pathophysiology of asthma.

Candidate gene studies

The second approach is to identify potential candidate genes which might be expected to be relevant to the development of asthma (see box). This has the advantage that if a causal association between a polymorphism or mutation within that gene and the clinical marker for allergic disease is observed then fine mapping will not be necessary.

Examples of potential candidate genes for asthma

- Th_2 cytokines (IL 4,5,9)
- β_2 adrenoceptor polymorphisms
- High affinity IgE receptor (FcϵR1)
- Tumour necrosis factor α
- 5-Lipoxygenase

One problem with this approach has been the relatively small number of subjects included in many studies (increasing the likelihood of false positive results with borderline levels of significance). Another critical point is that mutations or polymorphisms within candidate genes or their promoter regions must be functionally relevant if they are to be causally implicated in the development of disease. Examples of candidate genes that have recently been examined in detail are the β subunit of the high affinity IgE receptor (FcϵR1, situated on chromosome 11q)—in which (in some populations) mutations have been identified—and the β_2 adrenoceptor (chromosome 5q)—for which polymorphisms in the N terminal have been linked with severity of disease in asthmatic populations. Both of these receptors play important roles in controlling airway responses, and hence functionally relevant mutations would be expected to alter airway behaviour.

Technical developments

The ability to perform large genetics studies in asthmatic populations has been radically improved by the development of new techniques in molecular genetics. Large scale assays based on the polymerase chain reaction and automated screening of samples have vastly increased the speed with which these studies may be performed. In addition, the production of much more accurate physical maps of the human genome as part of the human genome project has made interpretation of data far easier.

One particularly useful development is the establishment of an "expressed sequenced tagged" database. Because most of the human genome is composed of non-coding DNA (sometimes called junk DNA) and only 5-10% of the genome codes for genes, fine mapping, which involves screening large regions of DNA for possible genes, has been time consuming. The expressed sequenced tagged database identifies short fragments of genes, which can then be placed on a physical map of the relevant chromosome to increase the speed with which candidate genes can be identified.

Why is asthma genetics important?

If genes important for the development of asthma are identified in the near future, what relevance will this have to the way we care for asthmatic patients? Firstly, knowing the gene products that are important in the development of asthma will provide new potential targets for therapeutic intervention.

Secondly, it seems increasingly likely that early intervention in high risk populations—before development of asthma or perhaps at its earliest stage—may prevent the development of chronic severe asthma and the airway remodelling that accompanies it. If we can define effective interventions (such as early avoidance of allergens) for groups at risk then postnatal screening may become both a practical and cost effective option.

Thirdly, although gene therapy would be inappropriate for most patients with asthma, it is conceivable that this approach could be used in patients with severe disease if potential targets for gene therapy have been identified. The best targets may not be the genes that predispose to the development of asthma but disease modifying genes. Given the important role of airway epithelium in chronic airway inflammation, and the potential for topical administration of genes to the lungs with aerosols, this approach is certainly feasible. Two methods have been used to administer genes to airway cells: first, with aerosols of DNA mixed with charged fatty particles (liposomes), and, second, with aerosols of a modified virus (such as adenovirus) in which the gene of interest has been placed. However, two major problems remain: firstly, obtaining adequately high levels of expression of the gene of interest in the airways and, secondly, preventing inflammatory responses in the airways caused by repeated administration of DNA contained in viral based vectors.

Molecular basis of allergic inflammation

Th_2 cytokines

Although genetic factors are important in determining a person's propensity to develop asthma, it is the inter-

action of these genetic factors with environmental factors that determines the actual prevalence of the disease. Numerous epidemiological studies over the past 20 years have defined environmental factors that are associated with an increased risk of developing asthma—including a Western lifestyle, refined diet, early exposure to allergen, maternal smoking, and patterns of early childhood viral infection. Such studies tend to provide observational rather than mechanistic information.

Knowledge of potentially important mechanisms has arisen as a result of advances in molecular cell biology. It is clear that asthma is an inflammatory disease characterised by up regulation of a specific subclass of T helper cells (Th_2) in the airways. Th_2 driven responses are found in atopic disease and are associated with elevated expression of Th_2 cytokines (such as interleukin 4,5,9) and IgE mediated responses. Initial attempts to characterise the important mediators driving this response were performed in animals. Despite the existence of animal models such as ovalbumin sensitised guinea pigs and inbred strains of mice and dogs with airway hyperresponsiveness, no animal model with all the features of asthma has been available. Partly because of this, over the past five years many groups have performed bronchial biopsy studies in asthmatic patients. Panels of antibodies have been developed against the important cell surface markers, enabling identification of subpopulations of different inflammatory cells, and in situ hybridisation techniques have allowed the mediators being produced by airway cells to be defined. These developments have greatly increased our understanding of the cytokine and mediator networks underlying allergic inflammation in the airways (fig 1).

It is now clear that Th_2 cytokines play a crucial role in both developing and sustaining airway inflammation in response to allergen exposure. Over the next few years antagonists for these cytokines should become available, allowing the individual importance of each cytokine to be defined. The other approach that has been used is to genetically engineer transgenic mice in which the gene of interest is either overexpressed or has been deleted ("knock outs"): these animals are used as models for studying the roles of individual cytokines.

Therapeutic approaches

Although studies of cytokines will be important in defining the mechanisms underlying continued airway inflammation, specific cytokines are not attractive as therapeutic targets. Because there is redundancy in the cytokine network, antagonists of an individual cytokine may well be relatively ineffective on their own. It is clear from biopsy studies that inhaled corticosteroids are able to reduce numbers of inflammatory cells in asthmatic airways and to reduce local production of Th_2 cytokines; thus, it is perhaps naive to imagine that cytokine antagonists will be more effective as first line treatments for asthma than inhaled corticosteroids.

To be truly effective, a therapeutic intervention would have to target the initiating stage in the allergic response. Interventions to reduce exposure to allergens have had limited success. Critical questions that require answering in this area are (a) why some foreign proteins are so allergenic (such as Der p1, the major
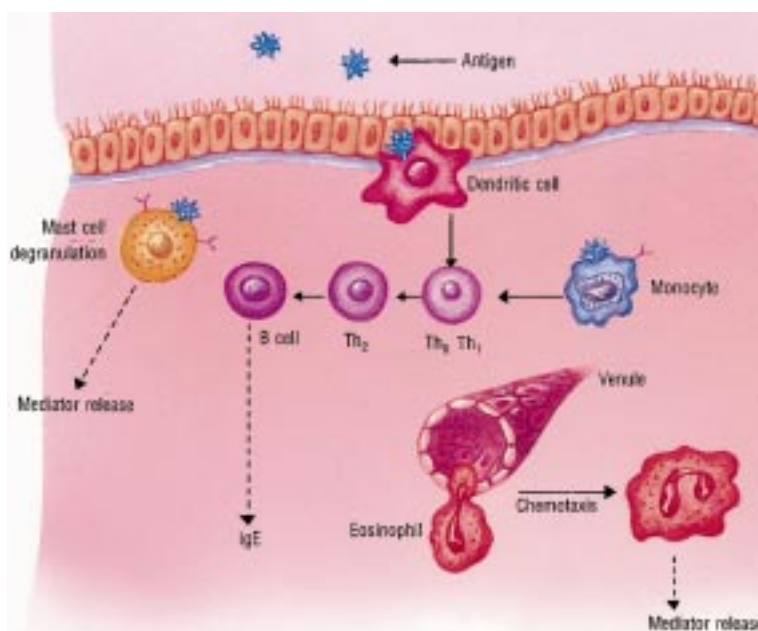

Fig 1 Some key pathways in airway inflammation in asthma

allergen from the house dust mite), (b) what mechanisms control the production of specific IgE in response to allergen exposure, and (c) what the critical antigen presenting mechanisms are in the airways. Some data are becoming available to answer these questions. For example, analysis of the crystal structure of Der p1 suggests that its high allergenicity may be related to its functioning as a protease. The next goal is to identify regions in the molecule that are critical for its biological activity, which might provide targets for vaccine development.

Another therapeutic option currently being studied is to treat patients with anti-IgE antibodies in an
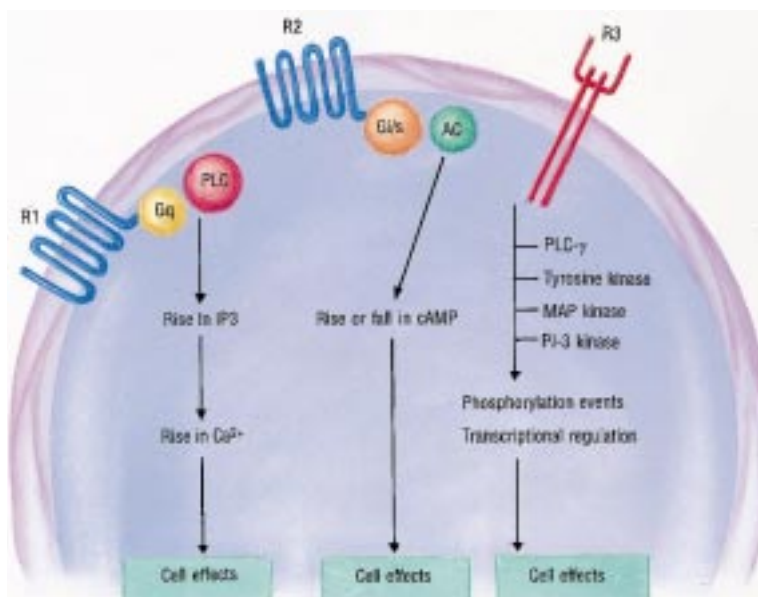

Fig 2 Key signal transduction pathways in airway cells. Agonists act at receptors coupled to specific cell signalling pathways. R1: receptors coupled to phospholipase C (such as histamine H1 receptors) elevate intracellular inositol 1,4,5 trisphosphate (IP3) and hence cause a rise in intracellular calcium. R2: receptors positively (such as β_2 adrenoceptors) or negatively (such as muscarinic M_2 receptors) coupled to adenylate cyclase cause rise or fall in cyclic AMP levels. R3: receptors (such as epidermal growth factor receptors) mediating the action of growth factors or cytokines stimulate a range of kinase pathways to produce their effects on cell function

attempt to damp down IgE mediated inflammatory responses. The difficulty with this approach is that the body may itself develop anti-idiotype antibodies against the immunising antibody. None the less, this kind of approach probably offers the best prospect for a new first line treatment for asthma.

Mechanisms of cell activation

Cell surface receptors and intracellular second messengers

A revolution has occurred in the past 15 years in molecular pharmacology. New drugs used to be discovered by screening compounds for biological activity on animal tissue preparations in organ baths. Now, most primary screening of compounds is done with cell culture systems. Cell surface receptors responsible for transducing the effect of many classic agonists and antagonists to the cell have been identified and cloned. By expressing these receptors in cell lines that do not constitutively express the relevant cell surface receptors, the function of individual receptor subtypes can be clearly defined. These cell lines can then be used to screen lead compounds for activity at individual receptor subtypes. For example, selectivity for β_2 adrenoceptor stimulation could be studied with three cell lines expressing β_1, β_2, or β_3 receptors.

In order to perform these kind of studies a simple readout of receptor activation is required. Many important airway receptors are coupled to one of two major signal transduction pathways, generating intracellular second messengers that mediate the effect of drugs acting on these receptors. These two pathways involve either the activation of phospholipase C or of adenylate cyclase, resulting respectively in a rise in inositol 1,4,5, trisphosphate (and hence intracellular calcium) or cyclic AMP within the cell (fig 2). A rise in intracellular calcium can stimulate mediator release, cell activation, or contraction depending upon the cell type under consideration (fig 3). In contrast, elevation of cyclic AMP levels generally inhibits cell activation—for example, producing relaxation of smooth muscle cells. In addition, some receptors are negatively coupled to adenylate cyclase and can reduce levels of intracellular cyclic AMP. This kind of approach is, of course, not limited to cell surface receptors but can also be used to identify drugs acting at other sites by using appropriate readouts of activation (see box).

Molecular modelling and drug design

Molecular pharmacological techniques have also produced a wealth of information on the mechanisms underlying activation of receptors and their interaction with drugs. The most powerful approach in this area
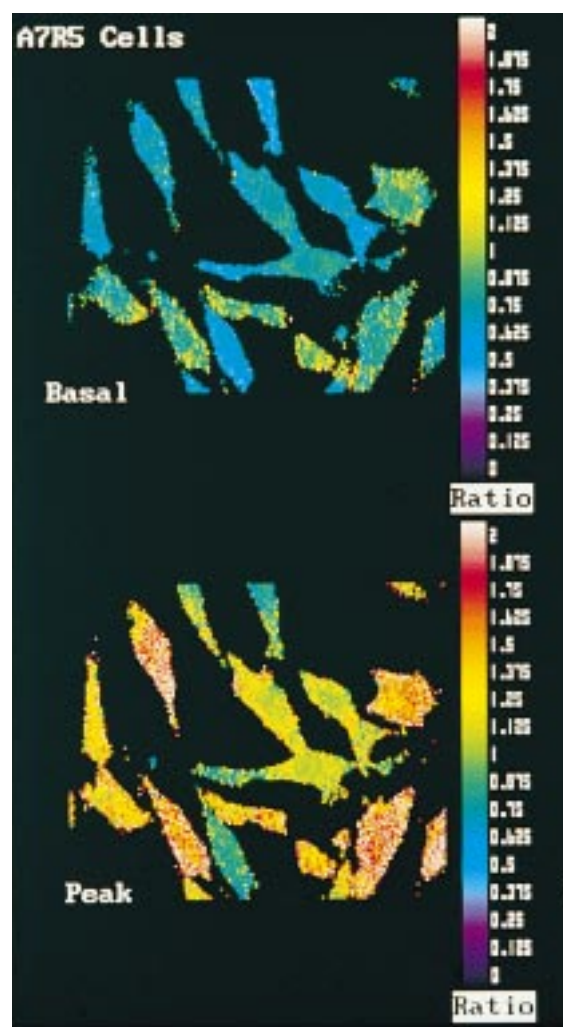

Fig 3 Intracellular calcium changes in response to an agonist in smooth muscle cell line loaded with the calcium sensitive dye Fura 2: changes in fluorescence signal in individual cells are shown in pseudocolour (blue=low calcium, red=high calcium)

has been to use site directed mutagenesis or chimeric receptor techniques to alter one or a few amino acids within a given receptor and then to study the characteristics of the altered receptor in cell lines. Such studies require the manipulation of the cDNA for the receptor to specifically alter bases to change the amino acid sequence of the receptor so that the mutated receptor can be expressed in a cell line. The approach is important because it allows the definition of critical parts of receptor molecules for binding of agonists and antagonists. For example, it has been used to determine the mechanism underlying the long duration of action of salmeterol in the airways: this seems to be due to interaction of the long side chain of the molecule with specific amino acid residues in the fourth transmembrane spanning domain of the β_2 receptor.

By determining critical sites for interaction within the receptor drug complex with molecular modelling techniques, the precise molecular characteristics required of a biologically active compound can be identified and lead compounds designed. Conceptually, this "reverse pharmacology" is the opposite of drug screening.

Understanding cell signalling pathways is also important in discovering how cells are activated, both by endogenous mediators such as cytokines and by

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Potential therapeutic targets in the airways

- Phosphodiesterase isoforms
- K^+ channels
- Muscarinic receptors (such as M_3)
- Leukotriene receptors (such as LTD_4)
- 5-Lipoxygenase
- Th_2 cytokine receptors
- Receptors for cell adhesion molecules

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environmental stimuli that can worsen asthma. For example, epidemiological studies have shown that ozone, particulates, and nitrogen oxides may worsen asthma. The mechanism underlying this effect can be studied with cells in culture by looking at the signalling events after exposure to pollutants. Another example is in the study of non-cytokine mediators. Of particular current interest are nitric oxide and oxygen-free radicals. Nitric oxide is released from epithelial cells and inflammatory cells and may have an anti-inflammatory role in the airways, whereas oxygen-free radicals are produced by activated inflammatory cells (such as eosinophils) and may have cytotoxic effects. Unravelling the signalling pathways involved in the release of these mediators, and the pathways involved in their effects on target cells, will help define the role of these agents in more detail and may provide new therapeutic targets in asthma.

Control of cell proliferation

While short term responses in airway cells are dependent on short lived second messengers, longer term effects such as cell proliferation are dependent on activation of other signal transduction pathways such as the MAP (mitogen activated) kinase cascade, which in 2turn increase expression of genes important in progression of the cell cycle. Complex interactions between the different parts of this cascade and other intracellular signalling pathways dictate the ultimate degree of proliferation (and hence remodelling) that occurs in the airways. Many of the longer term effects of corticosteroids are likely to be mediated through inhibitory effects on these pathways. At present, however, no therapeutic compounds targeted specifically at transcriptional activation in airway cells exist, and it is difficult to see how cell specificity could be effected with such a target.

Conclusions

In this review I have highlighted some of the ways in which advances in basic scientific research have led to increased understanding of the mechanisms underlying the physiopathology of asthma. Why is it important for clinicians to know about changing approaches to the study of asthma?

Firstly, it is important that we are able to evaluate the importance of advances in asthma research. Secondly, we need to be able to evaluate the likely importance of new therapeutic drugs. Examples of drugs in the late stage of development include 5-lipoxygenase inhibitors, leukotriene D4 receptor antagonists, and isoform selective phosphodiesterase inhibitors. Each of these classes of drugs will need evaluating against a broad knowledge of the importance of their targets in the pathophysiology of asthma. Thirdly, awareness of current research is crucial in the design of future studies. For example, if the preliminary studies implicating β_2 adrenoceptor polymorphisms in determining airway responses to β_2 agonists prove to be correct, clinical studies involving these drugs will need to take this genetic variable into consideration when randomising patients. Finally, and perhaps most importantly, high quality asthma research in the future requires a combination of a knowledge of clinical asthma together with an understanding of the basic scientific approaches to studying complex disease. Clinical scientists are ideally placed to use techniques of modern molecular medicine to answer focused questions in asthma research.

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WHEN I USE A WORD …
Shalom *Salmonella*

An outbreak of diarrhoea caused, I'm told, by small round structured viruses, reminded me of the time when we admitted several people with acute diarrhoea. They had all eaten salmon from the cold buffet at a wedding. It must be due to salmonella, I told the students. To their credit they didn't believe me; it turned out to be *Escherichia coli*. Of course, you *can* catch salmonella from salmon, but not etymologically.

The Greeks used a sign that we call a breathing over a vowel at the start of a word. A sign that looked like a comma, a smooth breathing, was not pronounced. In contrast, a reversed comma, a rough breathing, was aspirated, as we would pronounce the letter h. In Latin, this rough breathing was replaced by the letter s. For example, salt in Greek was [h]als (whence halide); in Latin sal (saline). In Greek six was [h]ex (hexameter); in Latin sex (sextet, the last six lines of a sonnet). Now, the Greek word meaning to leap was [h]allomai, from which we get halma, a game in which the men jump over one another. The Latin equivalent was salire, whence the name of the leaping fish salmo (English salmon).

The word salmonella, on the other hand, was coined by the Frenchman J Lignières in 1900, after the American pathologist Daniel Elmer Salmon (1850-1914). But Salmon's name has nothing to do with the fish. It is one of several corruptions (including Salman, Salmond, and Sammons) of the name Solomon, which in turn comes from the Hebrew word shalom, peace. In II Samuel (12:25) it is recounted how Bathsheba bore a son and how the prophet Nathan called him Jedidiah (beloved of God); but according to the prophet of I Chronicles (22:9), God appeared to David in a dream and told him to call the boy Shlomoh (peaceful one). So, Salman Rushdie is a Solomon, although not perhaps wise in the eyes of his Islamic brethren.

Shalom in Hebrew is equivalent to salaam in Arabic, both used as greetings, from the phrase "peace be with you" (shalom aleikhem or salaam aleikum). A salaam fit, first described by Sir Charles Clarke (1752-1857) and nowadays called infantile spasm, consists of a movement of the arms reminiscent of the movement of making a salaam in greeting. Not a very peaceful type of problem.

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Lesson of the Week
Penetrating intra-oral trauma in children

Robert C Law, Claire A Fouque, Angus Waddell, Eleri Cusick

Be suspicious of apparently non-serious pharyngeal injuries in children

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The incidence of penetrating intra-oral trauma in children is unknown and most cases probably heal spontaneously without being seen by doctors.[1] Occasionally these children develop acute life threatening complications. Retropharyngeal and mediastinal abscesses, mediastinitis, widespread emphysema, internal carotid artery thrombosis, and airway obstruction have all been reported. We recently treated two children with mediastinal sepsis after intra-oral injuries caused by toothbrushes.

Case reports

Case 1

A 13 month old girl presented to the accident and emergency department within two hours of an unwitnessed fall from a standing position with her toothbrush in her mouth. She looked well, with no respiratory distress or stridor but was noted to have a 1 cm abrasion to her right anterior faucial pillar. The tonsil was not pushed towards the midline, as occurs in peritonsillar abscess or bleeding lateral to the tonsillar bed. She was discharged home without treatment but returned to hospital 24 hours later with severe inspiratory and expiratory stridor and severely swollen cervical soft tissues. Radiography showed cervical surgical emphysema, a pneumomediastinum, widening of the prevertebral tissues displacing the trachea anteriorly, and a left pneumothorax.

Her trachea was intubated after gaseous induction, and an examination under anaesthesia showed a retropharyngeal abscess draining into the base of the right pyriform fossa. A right sided neck exploration was performed, the abscess was drained percutaneously, and a left sided intercostal drain inserted.

She was transferred to the intensive care unit, where she was mechanically ventilated for a week and treated with intravenous cefotaxime, penicillin, metronidazole, and fluconazole. The culture swab sent from the operating theatre grew pneumococci, *Haemophilus influenzae*, and yeasts.

She remained feverish, and a right thoracotomy was performed to drain an abscess extending from the posterior oropharynx into the right superior mediastinum. The mediastinal collection did not recur, but she required four further neck explorations to drain retropharyngeal collections. She initially received parenteral nutrition but subsequently was fed through a nasogastric tube and then by percutaneous gastrostomy. She was discharged home nine weeks after the injury and was well at the time of writing.

Case 2

A 2 year old girl was brought to the accident and emergency department after a fall down a flight of stairs with an adult toothbrush in her mouth. This was pulled out by her mother, resulting in some bleeding. The only finding on examination was a small laceration of the soft palate, and she was discharged without treatment. Her parents remained concerned, however, and refused to leave the department.

Over the next two hours she became unwell and was noted to be drooling saliva and to have a swollen neck and poor peripheral perfusion; oxygen saturation varied considerably according to her head position. She was intubated easily after an intravenous induction and was transferred to the intensive care unit. She developed cervical surgical emphysema and right upper lobe collapse but no pneumomediastinum or pneumothorax. Examination under anaesthesia showed a 2 cm full thickness longitudinal tear in the left pyriform fossa, extending down into the superior mediastinum. A nasogastric tube was inserted under direct vision and placed on continuous suction. She was initially fed parenterally and was given intravenous cefuroxime, gentamicin, metronidazole, and fluconazole.

She developed mediastinitis and acute lung injury and required three weeks of mechanically assisted ventilation. Despite a persistent fever no organism was cultured and no focal collections required surgical drainage. She was discharged home four weeks after injury and remained well.

Discussion

Children often put sharp objects in their mouths. The most common items responsible for injuries include toothbrushes, toys, sticks, pens, and pencils, and, in Asia, chopsticks.[2-4] Injuries are most likely to be sustained by toddlers who are still unsteady on their feet and fall on to the object.[2-4] There is a reported strong male predominance.[3 4] Injuries tend to occur in the posterolateral oropharynx, and initial symptoms are usually limited to minor oral bleeding.[2 4]

Several anatomical points are important. The prevertebral fascia divides into two layers in front of the vertebrae: the anterior layer, or alar part, and the posterior layer, or prevertebral part. The alar part fuses with connective tissue on the posterior surface of the oesophagus, thus limiting the retrovisceral space largely to the neck. The more dorsal space between the two layers of prevertebral fascia (danger space) extends from the base of the skull to the diaphragm and may be important in allowing infections to spread into the mediastinum. The carotid artery lies lateral to the tonsil.[5]

One major complication of penetrating pharyngeal injury is infection with abscess formation or mediastinitis, or both.[1 2] Both of our patients developed mediastinal infection secondary to soiling of the retropharyngeal space. In case 1 there were localised abscesses in both the retropharyngeal space and mediastinum and extensive mediastinal emphysema; mediastinitis and a secondary acute lung injury developed in case 2.
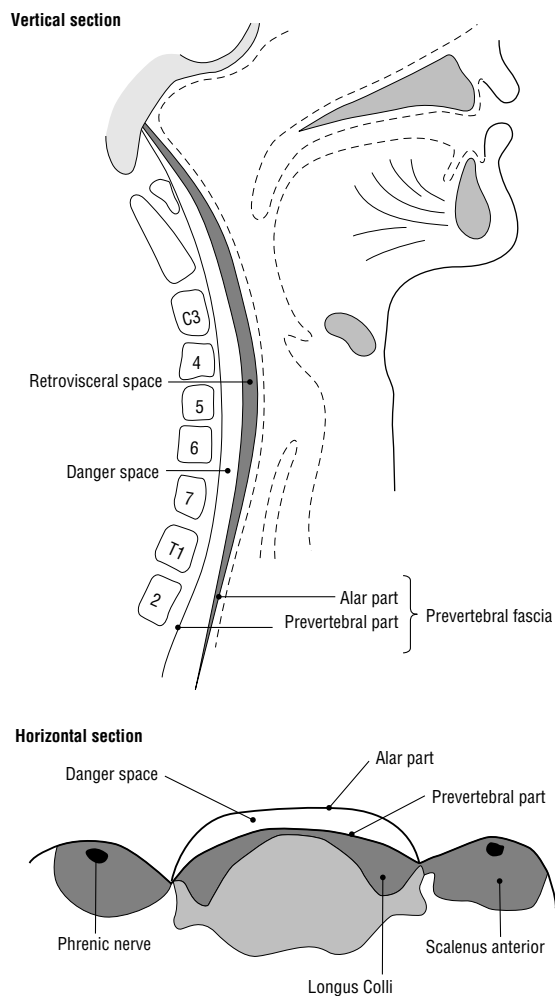
Vertical section

C3
4
5
6
7
T1
2

Retrovisceral space

Danger space

Alar part
Prevertebral part
Prevertebral fascia

Horizontal section

Danger space
Alar part
Prevertebral part

Phrenic nerve
Longus Colli
Scalenus anterior

Fig 1 Sagittal and horizontal sections of the neck showing division of prevertebral fascia. (Sagittal section is based on figs 5-9 and 5-11 from Hollinshead[5] and is adapted by permission of Wiley-Liss. Horizontal section is redrawn from fig 5-5 of Hollinshead[5] and is reproduced by permission of Lippincott-Raven)

Another serious complication is airway obstruction as a result of emphysema or abscesses.[1 2] In both cases the airway was compromised and required intubation. This was largely due to surgical emphysema, which in case 1, was further complicated by anterior displacement of the posterior pharyngeal wall.

Carotid thrombosis is a rare complication occasionally seen in injuries of this kind.[4 6] This is believed to follow an intimal tear caused by compression of the artery against the cervical vertebrae at the time of the injury.[7] The clot may propagate distally and result in widespread cerebral infarction. Classic symptoms are an initial lucid interval followed by deterioration in consciousness level, hemiplegia, and aphasia.[2]

The potential for rare life threatening delayed complications has led to uncertainty about optimal early management. Patients with symptoms must be admitted to hospital, but the management of the larger, symptom free population remains controversial. Some suggest admission for only those with lateral injury or retropharyngeal trauma because they are most at risk of adverse sequelae.[1] Others conclude that

all symptom free children should be managed at home and their parents instructed which symptoms should prompt a return to hospital.[3 4] These conclusions were based on the observation that serious complications were rare and may develop even after discharge following a 48 hour admission.

Three retrospective reviews of penetrating oropharyngeal trauma in children have been published.[2-4] Hellman *et al* described 131 hospital admissions. Their only complication was one case of facial cellulitis. Radkowski *et al* reviewed 23 cases that required hospital admission. Complications included one case of buccal cellulitis, one pneumomediastinum which resolved spontaneously, and one case of pneumonia.[3] Kosaki *et al* reported on 12 patients, three of whom developed complications and were admitted[2]; the nine others were treated as outpatients. One patient developed surgical emphysema, one developed subcutaneous, mediastinal, and retropharyngeal emphysema, and one developed a retropharyngeal abscess requiring surgical drainage.

Care must be taken to ensure that patients who are discharged are able to eat and drink. In inpatients enteral feeding is widely accepted as being preferable to parenteral nutrition, but the risk of gastro-oesophageal reflux, leading to further contamination of the wound, needs to be considered. There is no consensus on the need for antibiotics or surgical exploration in uncomplicated cases, although the urge to suture minor wounds should be resisted.[1 8]

Each of the children we have described here was initially discharged. Hospital staff need to know about the potential complications of such penetrating injuries and be aware that symptoms may be rapid in onset and life threatening. If symptom free children are discharged home parents need to be instructed to observe their child closely for 72 hours and should be given a list of specific symptoms to look out for.[4]

We feel that, as the complications of these injuries may be so devastating, parents need to be made aware of the dangers of allowing toddlers to walk around with sharp objects in their mouths. Toothbrushing must be supervised, and although dental hygiene should be encouraged, toothbrushes must not be presented as toys. We have asked toothbrush manufacturers to place a warning on toothbrush packaging, and several leading companies will be amending their packaging in the near future.

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