Science and Medicine

The Science and Medicine Conference was held at the Royal College of Physicians on 6–7 November 1991. This annual Conference, organised by Dr Carol Seymour FRCP, Assistant Registrar of the College, is designed to interest and inform physicians of all ages, as well as students of medicine and natural sciences.

This conference is in its eighth year and aims to cover aspects of research in science that affect present and future medical practice. The President emphasised that the Science and Medicine conference was special as it provides a forum for communication and discussion between scientists, clinicians, and students in training. The conference was attended by some 60 physicians and scientists, 20 invited junior hospital staff, and 60 invited students in medicine and science. Some of the students were sponsored by pharmaceutical companies and as the President said in her opening comments, ideas for increasing the numbers of bursaries available to students attending the conference would be welcome.

Better treatment through new biology? Prospects for the 1990s

Sir Colin Dollery (RPMS, London) laid to rest any doubts there might have been that the new biology was having little effect on current medical practice. With examples from receptor chemistry, transgenic mouse technology, tumour biology and basic immunology he demonstrated that the new advances were causing a revolution in therapeutics: the new biology had not only uncovered new cell enzymes and receptors, but was revealing new targets for drug development at such a rate that some drugs are at present looking for a disease to treat.

Successful drug therapy depends on having a suitable target and a compound to act on it. Cell receptors are proven examples of this type of target. One of the most successful strategies for their characterisation is based on the following scheme.

(a) The receptor of interest is purified by affinity chromatography using the receptor's natural ligand attached to a solid support.

(b) A partial amino-acid sequence is derived from the purified protein. This may only consist of a short sequence of, say, 10 or 20 amino acids.

(c) From the amino acid sequence, oligonucleotide probes (short DNA sequences) are designed to match the corresponding DNA sequence of the gene encoding this amino acid sequence.

(d) The oligonucleotide probe is used to screen, by molecular hybridisation, a cDNA library that represents all the mRNA molecules from a given tissue.

(e) Positive clones are then characterised to see if the correct gene has been identified. This last stage can take the form of DNA sequencing and prediction of structure and function from sequence data, testing tissues for expression of the gene, and *in vitro* expression of the gene product, to test its activity and ability to bind natural ligand.

This type of scheme has been used to clone, for example, genes for the many different isoforms of the receptor for γ-amino butyric acid (GABA). The receptor has three separate subunits, and different combinations of the many different subunit isoforms are possible. However, analysis of tissue expression by *in situ* hybridisation (a technique that localises the mRNA for a particular gene to distinct cells or cell populations) suggests that individual subunit isoforms are expressed in different parts of the brain and may therefore have different functions, particularly as the receptors display 1,000-fold variation in their sensitivity to GABA. Drug companies have not missed the potential for new drug development. For example, the peptide cholecystokinin (CCK) is found not only in the gastrointestinal tract, but like GABA is also a neurotransmitter in the brain. Using rapid automated screening systems, drug companies may screen up to 1,000 compounds per week in competitive binding assays. With this strategy Merck, Sharpe and Dohme identified a CCK receptor binding compound (the lead compound) and by structural modification of the lead compound found another agent, devazepide, with 1,000 times the binding affinity. This new CCK receptor ligand turns out to be a potentiator of opiate analgesia, with the advantage of having no sedative effects.

Sir Colin gave many other examples where 'the new biology' has led, or may lead, to new drug development. For example, granulocyte macrophage colony stimulating factor nearly halves the period of neutropenia after bone-marrow transplantation [1], and the recently cloned human interleukin-1 receptor antagonist [2] offers the prospect of novel treatment in rheumatoid arthritis. The development of a transgenic mouse model carrying the sickle haemoglobin mutation [3] allows the testing of new approaches for treatment of sickle cell disease. Finally, Sir Colin drew attention to research looking at DNA as a drug target, and described the discovery of a new anticancer drug campothecin, currently the strongest known inhibitor of experimental human cancer growth in nude mice. Campothecin was discovered on account of its inhibition of DNA topoisomerase, the enzyme responsible for 'untangling' DNA.

The optimistic tone of this talk was tempered by the speaker's caveat that new drugs often take ten or more years to develop and are massively expensive. He was

Rappoteur
TIM AITMAN, MRCP(UK), *Wellcome Research Fellow, Nuffield Department of Surgery, John Radcliffe Hospital, Oxford*

unsure who would pay for their development and use but felt that real advances for patients were in prospect.

Cell division and differentiation

To divide or differentiate: how does a cell decide?

Professor R. A. Laskey (Wellcome CRC Institute, Cambridge) contrasted the initial growth of amphibian embryo cells displaying rapid proliferation and little differentiation or cell growth with the growth of the human embryo, which at the one cell stage shows diverse gene transcription and considerable increase in size, demonstrating advanced differentiation at this stage. Amphibian embryos show little increase in size or gene transcription until the 4,000 cell stage. These are clearly extreme cases of cells primarily devoting their efforts toward either replication or differentiation. Intermediate between these extremes are countless examples of committed stem cells, for example the bone marrow haemopoietic stem cells, which are responsible for tissue renewal.

The cellular mechanisms for replication and differentiation are turning out to be remarkably similar. A wide range of growth factors and their receptors act together to activate their corresponding second messenger. The net effect of many of these signals is to activate transcription factors, thereby altering gene expression and determining the cell's fate. For example, the proto-oncogenes *c-fos* and *c-jun* that are induced very early in cell replication dimerise (by secondary structures known as 'leucine zippers') to form the transcription factor AP-1 resulting in activation of many cellular genes [4]. The *c-myc* and *c-myb* genes are thereby activated, effecting further gene transcription and leading to cell replication; like many oncogenes, *c-fos* also downregulates its own production. This leads to discrete spikes of *c-fos* activity at the required stage of the cell cycle.

The *c-myb* gene also appears to have a specific function in differentiating haemopoietic cells. Originally defined by its homologue in the transforming avian **my**elo**b**lastosis virus *v-myb*, this cellular oncogene is expressed in all committed haemopoietic cells, but not in terminally differentiated haemopoietic cells. It is also expressed at high levels in some haemopoietic tumours. Intriguingly, another related cellular oncogene, *a-myb*, is expressed in amphibian testis and in this organ expression is limited to developing spermatogonia. These findings support the view that the *myb* family has a widespread role in cellular differentiation.

In contrast, other cellular proteins have an inhibitory influence. The RB1 and p53 genes are such examples. The RB1 gene is mutated in the hereditary form of retinoblastoma and in its non-mutated form may repress transcription of *c-fos*. The gene p53 acts independently to suppress replication, but surprisingly both can be suppressed by certain strains (E6 and E7) of papilloma virus leading to cell replication.

Professor Laskey thus built up the concept of a growth control network, integrating positive and negative regulators of replication and differentiation ultimately to determine cell behaviour.

Gene activation in the frog mesoderm

Dr N. Hopwood (Wellcome CRC Institute, Cambridge) reminded us how important the mesoderm is in embryonic development and described his very elegant studies that have identified some of the factors leading to the earliest stages of muscle differentiation [5]. The mesoderm is the middle germ-cell layer and gives rise to the muscles, connective tissue, skeleton, vascular system, and most of the genitourinary system of the adult vertebrate. The mesoderm also initiates development of the nervous system and is required for differentiation of the digestive system. An understanding of mesoderm differentiation factors will therefore have major implications on our understanding of early embryogenesis.

Research in this field has been revolutionised by the cloning of MyoD, a gene expressed in myoblasts (in the mouse) but not fibroblasts. MyoD is a DNA binding protein of the helix-loop-helix family and in cell culture its gene product converts fibroblasts (uncommitted cells) into myoblasts (committed muscle cells). (To show that MyoD is active physiologically, Dr Hopwood looked at the expression of MyoD compared to other muscle specific proteins, for example myosin and actin. MyoD mRNA was found to accumulate two hours before muscle specific actin and the MyoD was localised to the same cells as those that were developing into skeletal muscle.) Myogenesis proceeds in an antero-posterior direction: MyoD expression parallels this time course. As final evidence for the role of MyoD, Dr Hopwood microinjected *Xenopus* embryos with MyoD mRNA (which is subsequently translated into mature MyoD protein) and found muscle-specific actin expressed in animal-cap cells, a site where this gene is not normally expressed (see Fig. 1).

The importance of MyoD was emphasised by the finding that another mesoderm-inducing gene, Xtwi, is expressed in much of the mesoderm, but not in MyoD producing cells. The fact that MyoD is expressed in the mesoderm before Xtwi suggests that the decision to differentiate into muscle cells is a very early step in mesodermal differentiation. Dr Hopwood said that the next step was to find the stimuli for MyoD expression itself.

Regulating gene expression

Dr S. Jackson (Wellcome CRC Institute, Cambridge) emphasised the primacy of transcriptional control over later regulatory mechanisms in gene expression.

Transcription factors are DNA binding proteins that

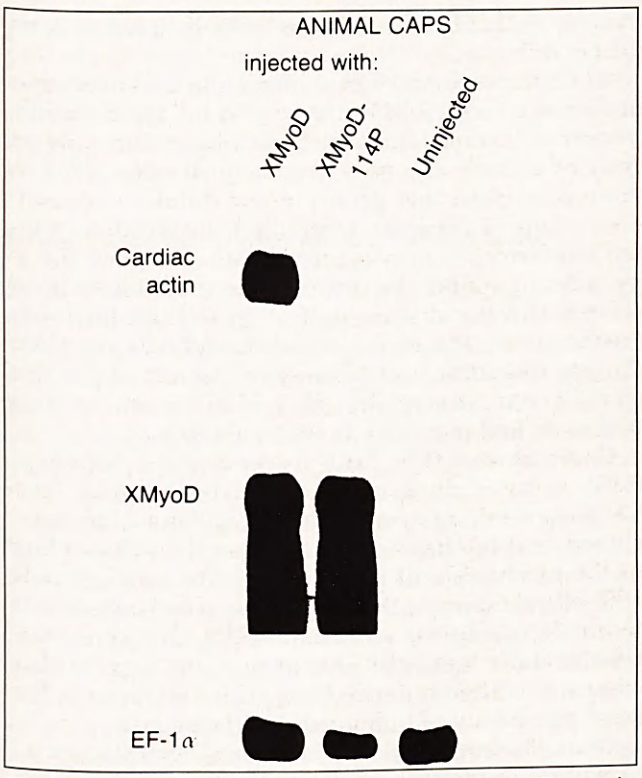

Fig. 1. *Xenopus MyoD (XMyoD) activates the cardiac actin gene in animal cap cells [5].* Enopus embryos were injected with either XMyoD mRNA or mutated XMyoD mRNA (XMyoD-14P). Animal caps were subsequently removed and cultured beyond the time of normal activation of the cardiac actin gene in primitive muscle. Northern blotting of cultured animal cap cells shows XMyoD mRNA in all injected cells and muscle-specific actin mRNA only in cells injected with non-mutated XMyoD mRNA. Rehybridising the Northern blot with the probe EF-1a shows that the samples contain similar amounts of total RNA. (Reproduced with permission from Hopwood 1990 from Ref. 5. © ICSU Press).

influence the rate of gene transcription. They fall into two main categories: those binding DNA sequences close to the site for initiation of transcription (at the 'promoter'), and those acting on DNA from 1,000 to 30,000 base pairs away from the transcription initiation site, at the 'enhancer'. While the promoter is always immediately upstream (or 5') of the start site, enhancer sites can be either upstream or downstream. It is the sequence specificity of the DNA sequence to which transcription factors bind that determines the transcriptional activity of a given gene to be regulated.

Transcription factor activity can be assessed *in vivo* by measuring mRNA and protein levels in cells and tissues. The ubiquitous nature of Sp1 with its action on many genes might lead to the conclusion that Sp1 is expressed uniformly in all cell types. This turns out not to be the case, with 100-fold variation in mRNA levels in different cell types [6]. Immunofluorescence studies showed that at birth all mouse liver cells express high levels, but that by 23 days only certain subpopulations do so. This suggests a (currently undefined) role for Sp1 in tissue differentiation and is supported by the pattern of Sp1 expression in other tissues.

Sp1 acts as a promoter for viral genes as well as eukaryotic genes and was originally defined as a transcription factor for the simian virus 40 (SV40) transcription. While Sp1 is normally present in limiting amounts in cells, it is fascinating that SV40 infection causes a 10-fold increase in Sp1 protein levels. The fact that SV40 also phosphorylates Sp1 has led to the observation that Sp1 becomes phosphorylated on binding DNA, which may represent a novel mechanism for regulating transcription initiation [7].

Dr Jackson finally described experiments which showed that Sp1 acts not only on promoter elements, but can also act at more remote enhancer sites provided that an Sp1 molecule has previously bound the promoter site [8]. Electron microscopy showed that Sp1 molecules at the enhancer can interact directly with Sp1 molecules at the promoter by forming a DNA loop. These experiments support the view that enhancer binding proteins may influence gene transcription by direct contact with proteins binding the promoter.

The human genome project

Professor E. Southern (Department of Biochemistry, Oxford) introduced the session by commenting that many people may consider the human genome project to be a rather sterile exercise. In fact, the community of researchers in the project, as well as providing much sequence data, also contribute to many other projects. For example, by exploiting the rich resource of polymorphism in the genome, genes for rodent models of insulin-dependent diabetes mellitus, epilepsy, and hypertension have recently been mapped [9–12]. The mapping of such loci becomes much easier as the genome project progresses. In Europe, the aim of sequencing the whole genome has given way to mapping and sequencing the small proportion of the genome that encodes protein products, the cDNA sequences. This work is progressing rapidly, as is the goal of ordering the whole of the genome in a map of overlapping contiguous cloned DNA segments, or 'contigs' as they are known.

Professor J. M. Connor (Duncan Guthrie Institute of Medical Genetics, Glasgow) asked what changes have occurred that are relevant to patients? The most obvious answer is in risk assessment for inherited disease. For example, Duchenne muscular dystrophy (DMD), the commonest lethal X-linked disorder, affects 1/3,000 live male births. As recently as five years ago, prediction of DMD was limited to the rather unreliable detection of the mother's carrier status by

measuring creatine kinase levels and determination of the sex of the fetus. This frequently led to the abortion of any male fetus in at-risk pregnancies, although only 50% of such fetuses would be affected. Following the finding of linked genetic markers, the gene for Duchenne (and Becker) dystrophy was cloned, this process being known as 'positional cloning' (formerly called 'reverse genetics').

The cloning of the DMD gene now makes possible the rapid determination of disease and carrier status. At one time this would have taken about a week from sample collection to result and counselling. Four years ago, the ability to amplify a small area of the genome by the polymerase chain reaction (PCR) made it possible to reduce this time to a matter of hours. The technique is now sufficiently developed to allow multiple areas of the gene to be tested simultaneously for deletions.

In cystic fibrosis (CF), the disease gene has also been cloned; surprisingly, 70% of cases in the UK possess the same three base pair deletion. Characterisation of this and other CF mutations has raised the feasibility of population screening for this common disorder [13]. Linked DNA markers or the cloned disease gene itself enable accurate risk assessment to be made in many diseases with Mendelian inheritance, including Huntington's chorea, polyposis coli, sickle cell disease, thalassaemia, adult polycystic kidney disease, and many others.

Most people consider that these advances help them to make a decision, and at present 85% of families with the diagnosis of a serious recessive disorder do opt for termination of pregnancy. However, counselling must always be undertaken before collection of specimens, and it must be realised that the technology is open to potential abuse. In this category would be included attempts to improve normal human traits or use of genetic tests for insurance purposes.

Gene therapy

Professor R. Williamson (St Mary's Hospital Medical School, London) opened his talk with the incisive comment that while gene markers can help to make a diagnosis, they are of no use to individuals who already have the disease. Accordingly, in the last two or three years gene therapy has become part of the immediate agenda in clinical practice, and much of the original scepticism has now diminished.

Gene therapy may be defined as the introduction of a normal copy of a gene to correct the pathological effects of the mutated version of that gene. In theory, this could include somatic and germ line therapy, but germ line therapy is illegal in Europe, therefore in practice only somatic cell gene therapy is considered. Nevertheless, Professor Williamson questioned whether, in this extremely controversial field, if the technology existed safely to correct a germ line defect causing cystic fibrosis, there were really good reasons for not doing so.

While the division of genetic disease into dominant or recessive conditions is not very useful, the classically 'recessive' conditions are those where one normal copy of a disease gene will prevent disease. It is in these conditions that gene therapy should be considered. Many of them are severe, lethal conditions without satisfactory alternative treatments. For gene therapy to be an option, the disease gene should have been cloned and the disease pathology well understood. Furthermore, the major cellular candidate for gene therapy should be accessible—not only for easy access to deliver treatment, but also, if necessary, to stop treatment and return to the *status quo ante*.

Cystic fibrosis (CF), with its pulmonary pathology, fulfils many of these criteria. The disease gene—the CF transmembrane conductance regulator—has been cloned, and this has led to an increased understanding of the mechanism of pathology. Furthermore, if only 10% of pulmonary epithelial cells in a cell culture can be made to express a normal CFTR, the whole cell layer assumes a normal phenotype. This suggests that clinical benefit may derive from gene therapy to only a small population of pulmonary epithelial cells.

Gene therapy could take two forms: cell therapy to provide a framework of normal cells, or adenovirus mediated transfer of the normal gene to existing lung epithelium. Viral vectors traditionally used for gene transfer are the RNA tumour viruses or retroviruses. These seemingly unattractive vectors are made safe by making them unable to replicate, and they are also made to require the presence of helper virus to package them into their infective form. The transferred gene may come to be integrated anywhere in the host genome, and this causes concern over possible damage to the host gene at the site of integration. Perhaps more attractive is gene transfer to epithelial cells *in situ* in the lung, by inhaling the normal gene incorporated into adenoviruses which have natural affinity for lung epithelium and low pathogenicity [14]. The relative merits of these systems have yet to be worked out, and may be superseded by gene transfer on human artificial chromosomes when this technique becomes established.

The more severe the condition, the greater is the opportunity for success with gene therapy. Thus, to date, gene therapy has been successfully tried in adenosine deaminase (ADA) deficiency where death usually occurs in the first year of life. In this condition ADA gene transfer to lymphocytes has already been shown to restore nearly normal immune function. Similarly, protocols now exist for treating hypercholesterolaemia in homozygous low-density lipoprotein receptor deficiency.

Professor Williamson closed with a discussion of gene therapy in cancer. Although most cancers were caused by four or five independent mutations, one of these might be a predisposing one and correcting this

predisposition might be of benefit. However, all the cells at risk would need to be targeted, and this would require considerably more efficient gene transfer procedures than are presently available. Current efforts are directed to antitumour targeting by delivery of noxious agents through gene therapy.

Genetic imprinting: what Mendel did not know

Dr S. Malcolm (Institute of Child Health, London) described the genetics of two rare examples of non-Mendelian inherited disorders: Angelman syndrome and Prader-Willi syndrome. The frequency of uniparental disomy and genetic imprinting, the genetic mechanisms responsible for these conditions, is unknown, but now that they have been recognised they may well turn out to be widespread.

Angelman syndrome (AS) was formerly known as 'happy puppet syndrome' due to the characteristic ataxia and inappropriate laughter; other features of AS are developmental delay (particularly verbal communication) and subtly dysmorphic craniofacial features. Prader-Willi syndrome (PWS) has a very different phenotype with obesity, sexual infantilism, and developmental delay, but both this condition and PWS are associated with deletions on chromosome 15 at 15q11–13. The unusual feature of their inheritance is that for AS, out of 26 cases analysed, the origin of the deletion was in all cases due to a deletion in the maternally inherited chromosome. Furthermore, in two AS patients without discernible chromosome deletions, the patients were found to have uniparental paternal disomy—ie each patient had inherited both his chromosomes 15 from his father [15] (see Fig. 2). Despite this, the cases were phenotypically identical to cases with deletions. In PWS, the reciprocal is true, with deletions occurring exclusively in the paternal chromosome and most of the remainder showing uniparental maternal disomy. In the patients with deletions, the phenotype may thus arise because of inactivation of the normal remaining genes from either the father (AS) or the mother (PWS). This event is known as genetic imprinting, and has also been described in Beckwith-Wiedemann syndrome.

Dr Malcolm gave other examples of non-Mendelian inheritance, such as mitochondrial gene mutations in mitochondrial encephalopathies, and gene duplications in some cases of Charcot-Marie-Tooth disease. If these mechanisms are not recognised, they may give rise to errors in genetic linkage studies for these diseases.

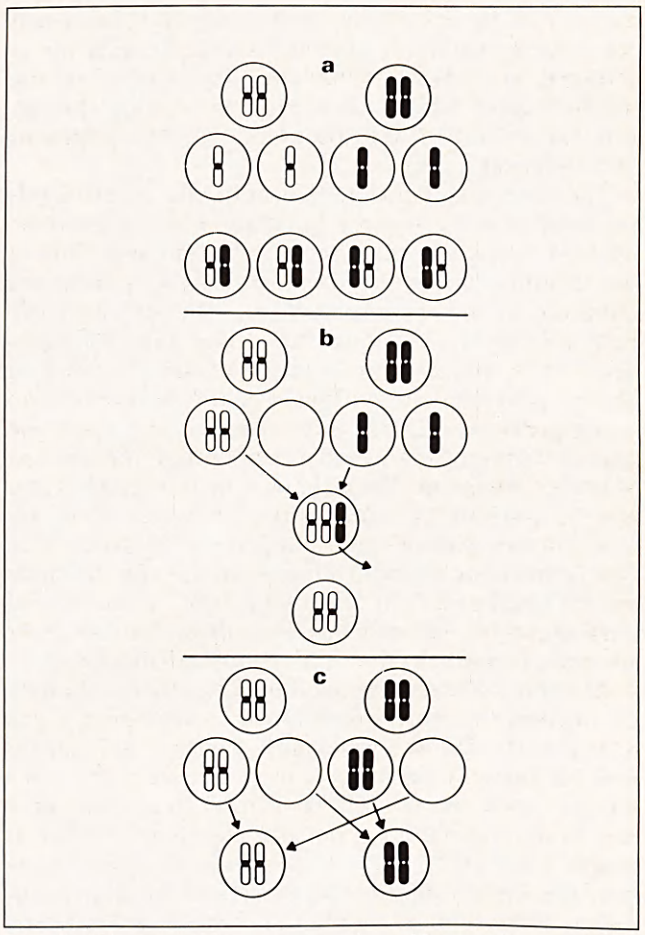

Fig. 2. *Diagrammatic representation of how uniparental disomy may arise.*
a=normal haploid gamete formation in which offspring inherit one of a pair of chromosomes from each parent.
b=non-disjunction with a trisomic conceptus which then loses a chromosome to become an embryo with uniparental disomy.
c=uniparental disomy from fertilisation of a disomic and a nullisomic gamete.
(Reproduced with permission from the *Lancet* from Ref. 15)

New methods in medicine

Human monoclonal antibodies

Two speakers from the University of Cambridge described new methods for *in vitro* synthesis of high affinity human monoclonal antibodies. Such antibodies might replace antitetanus, anti-Rhesus group D, and other antisera. These antisera are currently collected either from immune donors, who are few in number, or from immunised animals, where treatment using their sera leads to the risk of hypersensitivity to foreign proteins. Furthermore, the availability of human monoclonal antibodies would remove the need to vaccinate animals to produce these antisera. Other potential applications are in the treatment of autoimmune disease and transplant rejection.

Dr E. Gherardi (ICRF Laboratory, Cambridge) recalled the impact of mouse monoclonal antibodies

synthesised by hybridoma technology. But human B cell hybridomas have proved technically difficult to produce, and attention has therefore shifted to the production of human monoclonals by gene cloning and expression in heterologous expression systems such as bacteria [16].

The diversity of the human antibody repertoire is achieved by somatic recombination events in genomic DNA—the splicing together of different segments of the immunoglobulin genes—followed by targeted mutation of the recombined genes to increase antibody affinity. These two mechanisms account for a possible 10^{10} combinations of antibody sequences. The nucleic acid sequence of many of the different gene segments is now known and from the sequence the final structure of the variable antigen binding site can often be predicted. These known gene segments can now be combined *in vitro* for the synthesis of libraries of antibodies with designer antigen binding sites. This type of synthetic antibody library can use any combination of heavy and light chain segments, unlike natural libraries, which will only consist of those combinations surviving clonal selection into mature B lymphocytes.

A second strategy for producing synthetic libraries of cloned human monoclonal antibodies was described by **Dr W. Ouwehand** (Division of Transfusion Medicine, Cambridge). In this strategy the polymerase chain reaction (PCR) is used to amplify separate immunoglobulin gene segments from mature B lymphocytes [17]. The resulting PCR products represent the unrecombined repertoire of antibody segments. When joined together and expressed in bacteria the number of gene combinations exceeds those normally found in the mature human repertoire. The problem then is to find the bacteria expressing the required antibody. This has been achieved by using the target antigen to screen the library of such bacteria for those producing high affinity antibodies. So far successful cloning of monoclonal antibodies to the blood group B antigen has been accomplished with this technique. Study of the gene segments that make up these antibodies has revealed a surprisingly high rate of somatic mutation in the antigen binding domain. It is clear that this technology will not only provide high affinity monoclonal antibodies against antigens of biological and pharmacological interest, but also stands to unravel some remaining secrets in the mechanism of generation of antibody diversity.

Confocal fluorescence scanning microscopy

Under this apparently mundane title, **Dr G. Evan** (ICRF Laboratories, London) attempted to convince the conference why it was worth spending £40,000 on a new microscope.

The principle of confocal fluorescence microscopy lies in a laser light source, emitted through a pinhole and focused to a single point on the biological section. An image is built up by computerised scanning of the specimen, to derive a real time image in digital form. The resolution achieved is astonishing for a light microscope: cytoskeletal elements in single cells can be visualised and the image is always in focus, because the picture derives from a single point. Furthermore, three-dimensional images can be built up by comparing image data from more than one focal plane. Dr Evan foresees applications in diverse fields such as the biology of dysplastic and neoplastic tissues, and analysis of chromosomal abnormalities.

The limitations appear to lie not so much in the potential application of the technique as in its cost and demand for training and computer time. One image may use up to ten megabytes of computer storage space, while a stacking, three-dimensional image may use one gigabyte and take three or four days to analyse. The technique will clearly await advances in computer technology before its use can become widespread, but in the meantime, advances in the hands of the privileged few seem likely.

Old and new concepts of antibiotics

History of penicillins and cephalosporins

Fifty years ago the world learned that penicillin could cure dangerous infections in man. To mark this anniversary, **Sir Edward Abraham FRS** (Sir William Dunn School of Pathology, Oxford) gave his personal view of how the discovery came about and how it evolved to clinical utility. At this conference, with its emphasis on how scientific advance can affect clinical practice, this talk gave a refreshing historical perspective on scientific progress from one who was centrally involved in one of the greatest discoveries this century.

Sir Edward began by acknowledging that although Alexander Fleming was the first person associated with penicillin, its organism of origin, *Penicillium*, may well have been cultured on bacterial plates at the end of the 19th century. Fleming's achievements included the discovery of its activity against Gram positive organisms and of its non-toxicity, *in vitro* to leukocytes and *in vivo* to rabbits. However, Fleming concluded that while it might be used as an antiseptic, the trouble of making it was not worthwhile, owing to its lability in solution. To his credit, Fleming saved the original culture, enabling penicillin research to be continued by his colleagues.

Ten years later, Sir Edward Abraham came to work on penicillin in Florey's department of pathology in Oxford. Following initial encouraging experiments in mice, a crude 2% extract of penicillin was first given to a patient in 1941. In the early days such was the scarcity of the new drug that it was repurified from patients' urine to be re-used. In 1943, Abraham and Chain suggested the four member β-lactam ring as the structural basis of penicillin, and in 1945 this was proved to be correct by X-ray crystallography. By 1945, large-scale production in deep fermentation wells had begun

using improved strains of *Penicillium* that gave a 40,000-fold greater yield than Fleming's original strain.

As early as the 1950s, however, strains of *Staphylococcus aureus* emerged, particularly in hospitals, that were resistant to penicillin. They were due to bacterial production of penicillinase that degraded the β-lactam ring. The discovery of a new lactam antibiotic, cephalosporin N, by Abraham and Newton in 1953 [18] gave a partial solution to the problem of penicillin resistance and gave rise to the new family of cephalosporin antibiotics.

Molecular and clinical aspects of β-lactamases

Dr S. G. Amyes (University of Edinburgh Medical School) explained that β-lactamases are enzymes that confer resistance to antibiotics by hydrolysis of the β-lactam ring of penicillins and cephalosporins. They may be encoded by genes either on the bacterial chromosome or on resistance plasmids or 'minichromosomes' that can be passed from one bacterium to another.

The susceptibility of both penicillins and cephalosporins to β-lactamase has been overcome by modifying the original antibiotic to stabilise the β-lactam ring. Examples of these new derivative antibiotics are oxacillin and carbenicillin (new penicillins), and cefuroxime and cefotaxime (second and third generation cephalosporins). However, successive new antibiotics have met with further new mechanisms of drug resistance. The molecular evolution of plasmid encoded β-lactamases is one such mechanism.

Plasmid encoded β-lactamases were first described in 1974, and they are now classified according to their molecular size, activity against different penicillins and cephalosporins, and the DNA sequence of the genes that encode them. The earliest described plasmid encoded β-lactamase, TEM-1 in 1974, is still the commonest cause of plasmid mediated drug resistance, accounting for 40–50% of all β-lactamases in several recent surveys [19]. TEM-2 differs from TEM-1 by only one amino acid, and has very similar enzymatic properties. In the last three years, however, several new β-lactamases have been found, TEM-3 to TEM-14. Some of these have an extended range of activity and are able to hydrolyse the third generation cephalosporins, conferring some associated drug resistance. Most of them have been isolated only in hospital infections, but the imminent availability of third generation cephalosporins as oral agents suggests that these β-lactamases may become more widespread in the near future. Perhaps the latest evolution in β-lactamases is the finding of a new plasmid β-lactamase, BIL-1, unrelated to TEM-1. This β-lactamase confers not only cephalosporin resistance, but is also the first to show resistance to the β-lactamase inhibitor clavulanic acid [20]. This demonstrates the resourcefulness of these carriers of antibiotic resistance and spells trouble for the future use of these classes of drugs.

Other mechanisms of resistance to penicillin

Penicillins work by covalent binding to bacterial transpeptidases that are responsible for cross linking bacterial wall proteoglycans. **Dr P. E. Reynolds** (University of Cambridge Medical School) reminded us that these cross links maintain the integrity of the bacterial cell wall and prevent osmotic damage. *E. coli* possesses seven cell wall proteins to which penicillins bind. Each of them has distinct functions that have been characterised by the isolation of bacterial mutants for each of these penicillin binding proteins (PBPs). The PBPs appear to be structurally related to each other, particularly in their penicillin binding sites [21].

Intriguingly, PBPs may mutate to produce penicillin resistance, and where this has been analysed, the PBP is found to have incorporated a new segment of amino acid sequence in only one part of the protein, suggesting mutation by transformation and genetic recombination. This mechanism appears to be responsible for inducing methicillin resistance in some cases of methicillin resistant *Staph. aureus* (MRSA). Hopefully, when the structure of this new mutant gene has been cloned, new clues will be obtained to try once again to outwit the 'crafty coccus'.

Acknowledgements

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Application of immunological techniques to disease

'Application of immunological techniques to disease' was the title of a conference held at the Royal College of Physicians on 27 September 1991. The conference planned by Dr Carol Seymour FRCP, dealt specifically with the application of immunological techniques to understanding and controlling disease.

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An Overview

Professor N. A. Mitchison (University College, London) gave an overview of immunology in much the same sense that an experienced alpinist atop a savage peak might make sense of the tumbled landscape for a party of terrified novices. His review recalled Peter Medawar's well-known dictum that more knowledge ultimately leads to simplicity and clarity. Immune recognition and the resulting immune reactions are all about eliminating invading microbes and minimising immune-mediated damage to the infected host. The important point is that these reactions take place in a tightly controlled micro-environment. The boundaries of the reaction are defined by many now well–understood mechanisms.

Chief among these are methods for confining interactions between immunogenic peptides and T cells. Peptides are processed by antigen-presenting cells and presented in the strict confines of class II HLA molecules, geometrically spaced on the cell surface. Only those T cells with the correct antigen receptor can bind a given peptide, and there is an array of adhesion molecules to ensure that the reacting T cells only engage relevant cells. Selective V gene usage in T and B cells governs the specificity of the response. The resulting limitation of the reaction ensures that cytokines are only generated locally or, at least, that systemic leakage is contained. Indeed, the idea that immune regulation depends on the creation of barriers to immune reactions is, at least in man, more attractive than earlier concepts of suppressor T cells.

This thesis has obvious implications for clinicians faced with immune-mediated inflammatory diseases in which there is prominent local and systemic generation of an array of cytokines. A disease such as rheumatoid arthritis is an obvious example of a process which might result from the successive breakdown of constraints on cytokine generation.

A fundamentally important problem in immunology is to understand peptide recognition by T cells. Among the most dramatic recent advances in immunology has been the elucidation of the structure of peptide binding sites on class I, and to a lesser extent class II, HLA molecules. It is now clear that T lymphocytes recognise processed oligopeptides in the binding grooves of these molecules. In addition to the physiological interest in solving this aspect of immune recognition, variations in peptide binding may help to explain disease associations with the inheritance of certain class I and II HLA molecules.

What the immune system sees

This subject was discussed by **Dr J. J. Skehel** (National Institute for Medical Research, London). Our knowledge of antigen recognition by antibodies has stressed the importance of quaternary structure and not just peptide sequences in determining the location of antibody-binding sites on complex protein molecules. Information is increasingly about the sequences of the oligopeptides which occupy the peptide binding groove of the HLA B27 and other class I molecules. These are usually oligopeptides of nine amino acids with apparent restrictions on the residues in certain positions which permit occupancy. Many points about the peptides engaging the binding groove are still