Conference report

The Association of Physicians of Great Britain and Northern Ireland, Oxford, 14 to 15 April 1989

It is evident that recombinant DNA technology will prove to be one of the most important influences on medical research and practice into the 21st century. Chaired by Sir David Weatherall, the emphasis at the 83rd Annual Meeting of the Association of Physicians of Great Britain and Northern Ireland was on the wide range of applications of this technology across the field of clinical medicine. This included the recognition of the presence of incorporated retroviruses, identification of the peptide sequence of antigens in autoimmune diseases, discovery of closely linked RFLPs for gene mapping and tracking, and, through sequencing of DNA, the development of mutation specific DNA probes for use as accurate diagnostic tools.

Amplification of DNA by polymerase chain reaction (PCR) has enabled study of DNA sequences in only single or few copies and had played a key role in several of the studies presented. Phillips (Oxford) showed how incorporated retrovirus HTLV-1 could be detected by PCR in DNA from patients with tropical spastic paraparesis. Sequence analysis of the incorporated viral genome had shown considerable variation both between and within affected subjects, highlighting the potential ability of viruses to respond to evolutionary selective pressures. Similar studies in multiple sclerosis had failed to confirm the presence of retrovirus in DNA extracted from peripheral blood leucocytes, but studies of DNA extracted from neuronal cells are now indicated.

By amplification of the cDNA reverse transcript of mRNA sequences, PCR had also enabled identification of the heart and kidney as the sites of production of the vasoconstrictor peptide, endothelin (Brown: Cambridge). By the same technique Maxwell (Belfast) had shown that erythropoietin production is localised in the tubular cells of the renal cortex. In Mendelian disorders, amplification of the cDNA reverse transcript of gene specific mRNA may perhaps also provide the key to routine characterisation of family specific point mutations.

In primary biliary cirrhosis (which is characterised by antimitochondrial antibody), Bassendine (Newcastle) had used DNA cloning techniques to show that the antigenic protein is part of the pyruvate dehydrogenase complex located in liver cell mitochondria, and that the lipoyl domain of the E2 complex acts as the antigenic determinant (the epitope). Sequencing of the encoding DNA will enable clarification of the amino acid sequence of the epitope and facilitate development of drugs to block the specific immune response. Sequencing will also help test the assumption that autoimmune disease results from an abnormal response to normal antigens rather than vice versa. Similar studies of thyroid autoantibodies (Banga: King’s College) had characterised the epitope of thyroid peroxidase responsible for some autoimmune thyroid disease.

In some conditions the use of indirect (linked) DNA probes is now able to alter medical and surgical management. Opening the meeting, Sir Walter Bodmer (Imperial Cancer Research Fund) discussed the mapping of the gene for familial adenomatous polyposis coli (FAP) to chromosome 5q, and showed that the probe RC057 is close enough ($Z_{max}=4.0$ at $\theta=0.02$) and sufficiently informative (two alleles with frequencies of 0.5) to allow accurate predictive testing. FAP is the genetic condition which, par excellence, should now be managed on a register basis. Similar reasoning may be applied to familial hypercholesterolaemia (FH), which has tended to be the Cinderella of those genetic diseases suitable for a register approach. The results of trials with a cholesterol lowering agent in non-familial hypercholesterolaemia were presented (Maher: Hammersmith) and by emphasising the therapeutic options available, highlighted a common disorder where a register approach could have considerable benefit.

Some 20 to 25% of non-familial colorectal cancers show loss of heterozygosity for DNA markers on chromosome 5q. Although the Knudson two hit hypothesis may be of general application in cancers,
whereby there can be dominant germline inheritance for a first hit, with recessive inheritance in somatic cells for the full cancer syndrome. Bodmer discussed how DNA probes have implicated several different loci in the pathogenesis of colorectal and other cancers.

Molecular genetic analysis is showing, however, that other conditions previously considered to be multifactorial may result from the principal influence of a single dominant gene with reduced penetrance. In a study of families with atopy, Cookson (Oxford) identified several with apparent dominant inheritance and 90% penetrance in obligate heterozygotes. Raised IgE levels were used to indicate affected status in cases of clinical doubt. Linkage analysis gave a maximum lod score of Z=5.6 at a recombination fraction (θ) of 0.1 with the DNA probe pλMS.51 mapping to 11q12–q13.

The clinical use of indirect probes is limited by recombination and by the requirement for absolute accuracy in differential clinical diagnosis. The value of direct DNA probes was emphasised in a presentation on the use of dystrophin cDNA probes to establish a diagnosis of Becker or Duchenne muscular dystrophy in several males referred for genetic counselling with a diagnosis of ‘spinal muscular atrophy’ or of other limb-girdle syndromes (Lunt: Manchester). Sir John Walton proposed that the problems of classification were equivalent to a pendulum which had previously been swung too far in the direction of a ‘neurogenic’ interpretation of biopsy and EMG findings, and which was now swinging back. Although diagnostic confirmation currently rests on the finding of gene deletion, one can anticipate the development of techniques to confirm diagnoses through identification of point mutations. The use of PCR to amplify the site of mRNA mismatch compared with normal mRNA, and the subsequent sequencing of mutation specific cDNA, is already a feasible method in some genetic disorders. Cox (Hammersmith) showed how this approach enabled characterisation of mutations in the muscle aldolase B gene in cases of hereditary fructose intolerance, thereby also enabling absolute confirmation of the diagnosis. Recombinant DNA technology is therefore already advanced enough to develop accurate diagnostic probes based on recognition of point mutations within individual genes. Although these will become the diagnostic tests of the future, the comment that in hereditary fructose intolerance aversion to sugar is a simple clue to the diagnosis emphasises that the use of clinical diagnostic skills should always remain the fundamental approach to assessment of patients.

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