THE LANCET

## Will the Real Hepatitis C Stand Up?

TO the casual observer, the claims and counter-claims with respect to the discovery of serological detection systems for non-A non-B (NANB) hepatitis must be confusing. Should the latest announcement[1,2] of yet another candidate be taken more seriously? The key to answering this question lies not only in an analysis of the specificity and reproducibility of the test results but also in an appreciation of the background to the work, since this discovery builds on a wealth of previous knowledge carefully assembled by clinical and basic research groups over many years.

The essential groundwork began in the 1970s, with the clinical recognition and description of cases of post-transfusion hepatitis which, on serological testing, were not due to hepatitis A or B infection. This long-incubation hepatitis (60 days) was notable for its mild, often subclinical, presentation but high rates of chronicity and progression to cirrhosis. A similar illness could also be transmitted by blood products such as clotting factors.[3] An important series of studies in chimpanzees clearly showed the presence of a transmissible agent in blood products and in serum from carrier blood donors.[4] The agent was sensitive to organic solvents, and less than 80 nm in diameter as assessed by filtration. Thus, even without conventional virological studies in vitro or knowledge of the genome, it was possible for Bradley[5] to make a calculated guess that the NANB agent could be a small togavirus-like envelope RNA virus.

Meanwhile, many groups, using the principles that had worked well for hepatitis A and B, were making unsuccessful attempts to design serological systems.[4] What was particularly frustrating was that the resulting "tests" often appeared to respond to something in suspect sera but were, on more rigorous investigation, either unreproducible or clearly non-specific,[6] or were detecting normal liver antigens whose production was stimulated by the infection.[7]

Houghton and colleagues,[1] working at the Chiron Corporation in California, adopted a new approach, consistent with Bradley's view of the virus. These researchers used large quantities of a well-characterised highly infectious chimpanzee plasma as a source of virus. The virus was concentrated into pellets by ultracentrifugation, the nucleic acid extracted, and cDNA synthesised from both RNA and DNA by reverse transcriptase. Cloning into the bacteriophage λgt11 provided a cDNA library that could be screened for expression of an antigen detected by serum from a patient with chronic NANB infection. After about a million clones had been screened, one phage-infected bacterial colony was found to be producing a protein that reacted with the patient's serum. The 155 base-pair insert in this clone was then cut out and used as a hybridisation probe to extract, from the original library, a larger (353 base-pairs) overlapping clone. Neither of the strands in this double-stranded cloned DNA hybridised to human or chimpanzee DNA, but one of the strands was homologous with a single-stranded RNA, containing up to 10 000 nucleotides, in the virus-rich pellet from the original chimpanzee serum. The cDNA also hybridised to RNA from infected, but not normal, chimpanzee liver. Examination of the nucleotide sequence in this and two other overlapping clones suggested a single continuous translational open reading frame; the next step was to insert this DNA sequence into a plasmid containing the human superoxide dismutase gene in order to express the open reading frame in a system that could generate large quantities of the resultant polypeptide. The fusion protein so produced, when expressed in bacteria, reacted on immunoblotting with serum from seven of eleven patients with NANB infection, but with none of ten control sera. The demonstration of seroconversion in four chimpanzees experimentally infected with the NANB innoculum, but not in seven with hepatitis A or B infection, provided further encouragement with respect to the specificity of this antigen/antibody system.

1. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A non-B viral hepatitis genome. *Science* 1989; **244:** 359–62.
2. Kuo G, Choo Q-L, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989; **244:** 362–64.
3 Dienstag JL. Non-A, non-B hepatitis, I: recognition, epidemiology, and clinical features. *Gastroenterology* 1983; **85:** 439–62.
4 Dienstag JL. Non-A, non-B hepatitis, II: experimental transmission, putative virus agents and markers, and prevention. *Gastroenterology* 1983; **25:** 743–68.
5. Bradley DW. The agents of non-A, non-B viral hepatitis. *J Virol Meth* 1985; **10:** 307–19
6. Suh DJ, White Y, Eddleston ALWF, et al. Specificity of an immunoprecipitin test for non-A, non-B hepatitis. *Lancet* 1981; i: 178–80.
7. Akatsuka T, Tohmatsu J-I, Abe K, et al. Non-A, non-B hepatitis related AN6520 Ag is a normal cellular protein mainly expressed in liver, II. *J Med Virol* 1986; **20:** 43–56

Higher yields of the fusion protein (containing 363 viral aminoacids) were obtained from recombinant yeast cultures and used to coat microtitre wells. These could then form the basis of a radioimmunoassay for antibody in test sera.[2] Initial tests were conducted blind, with a well-characterised panel of NANB sera that have proved to be the undoing of several previous candidate test system.[8] The new test cleared this first hurdle with ease. Of seven NANB serum samples shown to be infectious in chimpanzees, six gave clearly positive results in the assay and the one negative sample came from an individual in the acute phase of post-transfusion NANB hepatitis. Seven non-infectious control sera were all negative. In serial samples from ten well-characterised cases of post-transfusion NANB hepatitis, antibody appeared about 6 months after the transfusion in all but one, and the assay was positive in 71% of a further twenty-four patients with post-transfusion NANB hepatitis. It has always been unclear whether community-acquired NANB hepatitis without an apparent source was due to the same agent as that causing post-transfusion NANB infection, and it is of some interest that 58% of fifty-nine cases of the sporadic type were also positive at some stage after the clinical onset of acute hepatitis.

In this issue we publish four series of results with the new test system. The Spanish (p 294) and Dutch (p 297) workers used the prototype radio-immunoassay originally developed by the Chiron researchers whereas the two German groups who report their data in our correspondence columns (p 324) used the second generation enzyme-linked immunosorbent assay marketed by Ortho (the antigen is the same for both assays). In general, the results support the sensitivity and specificity of the test system, and underline both the urgency of making the test system available for blood donor screening, and the importance of despositing the sequence of the viral genome in the GenBank database where it would be available to the wider scientific community.

There are many questions yet to be settled. How many other agents are involved? Is there a short-incubation agent? What is the cause of the negative cases of sporadic NANB in the community? Are any of these related to the enterically transmitted NANB virus so common in the Indian subcontinent?[3] Will the assay help to select patients for interferon treatment? Meanwhile, this new test system represents a clinically important advance in the detection of one of the causal agents of NANB hepatitis, which has the characteristics of either a togavirus or a flavivirus. It would be logical to confer the title of hepatitis C on the newcomer.

Finally, it is worth re-emphasising that the complex molecular engineering, although almost routine as far as the technology is concerned, could not have succeeded and would probably never have been conceived without the detailed clinical groundwork and chimpanzee studies that established the nature of the disease, broadly defined the agents involved, and provided the essential sera. The hepatitis C story is an excellent example of the need for a broad approach to research funding. The flash of the diamond may catch the eye but the setting is often the key to the beauty of the display.

8. Alter HJ, Purcell RH, Feinstone SM, Tegtmeier GE. Non-A, non-B hepatitis: its relationship to cytomegalovirus, to chronic hepatitis, and to direct and indirect test methods. In: Szmuness W, Alter HJ, Maynard JE, eds. Viral hepatitis—1981 International Symposium. Philadelphia: Franklin Institute Press, 1982: 279–94.

## Bitter Reinstatement

AT a major London teaching hospital the division of obstetrics and gynaecology is chaired by an otorhinolaryngologist; four years ago at that same hospital a consultant obstetrician of eight years' standing was suspended without warning. In the interim the consultant, whose professional competence had been challenged, was reinstated after a long and expensive inquiry, an advisory panel made recommendations about the establishment of professional working arrangements in light of the inquiry's findings, and a senior figure at the Royal College of Obstetricians and Gynaecologists was called upon to pronounce because the recommendations of the panel were not being implemented. Many of our readers will instantly perceive that we are talking about the London Hospital and Mrs Wendy Savage. Why should we wish to revive the subject? Unfortunately, this miserable saga shows no signs of ending satisfactorily. When the disciplinary inquiry headed by Mr Christopher Beaumont QC made the final part of its report public almost exactly three years ago we commented that "it would be naïve to assume that all will now be harmony and light".[1] Anything but!

The advisory panel established after the Beaumont inquiry (the first such inquiry to be held in public) was chaired by Dame Alison Munro (chairman, Chichester Health Authority) and reported in October, 1986. The panel suggested several modifications to the working arrangements and organisation of the Division of Obstetrics and Gynaecology at the London Hospital. In all, there were thirteen recommendations. Members of the panel seem to have been aware of the depths to which interprofessional relationships had sunk because one of the recommendations, number eleven, stated "If difficulties of interpretation of these working arrangements should arise they should in the first instance be discussed by the Division incorporating Obstetrics and Gynaecology. If they are unable to be resolved, the matter should be referred to the Chairman of the Hospital Medical Council who, in consultation with the Dean, will convene a small

1. Editorial. Attitudes to obstetric care. *Lancet* 1986; ii: 257–58.