Hepatitis—how far down the alphabet?

The past three decades have witnessed an explosion in knowledge of viral hepatitis, a major public health problem throughout the world affecting several hundreds of millions of people. Viral hepatitis is a cause of considerable morbidity and mortality in the human population both from acute infection and chronic sequelae which include, with hepatitis B and hepatitis C infection, chronic active hepatitis, cirrhosis and primary liver cancer.

The hepatitis viruses include a range of unrelated and often unusual human pathogens:

**Hepatitis A virus** (HAV), a small non-enveloped symmetrical RNA virus which shares many of the characteristics of the picornavirus family. This virus has been classified as hepatovirus within the hepacivirus genus and is the cause of infectious or epidemic hepatitis transmitted by the faecal–oral route.

**Hepatitis B virus** (HBV), a member of the hepadnavirus group, double stranded DNA viruses which replicate by reverse transcription. Hepatitis B virus is endemic in the human population and hyperendemic in many parts of the world, and is transmitted essentially by blood to blood contact and by the sexual route. Mutations of the surface coat protein of the virus and of the core and other proteins have been identified in recent years. Natural hepadnavirus infections also occur in other mammals including woodchucks, beechy ground squirrels and ducks.

**Hepatitis C virus** (HCV), an enveloped single stranded RNA virus which seems to be distantly related (possibly in its evolution) to flaviviruses, although hepatitis C is not transmitted by arthropod vectors. Seroprevalence studies confirmed the importance of the parenteral route of transmission and transmission by blood and blood products, but in as many as 50% of patients the origin of the infection has not been identified. Several genotypes have been described. Infection with this virus is common in many countries, and it is associated with chronic liver disease and also with primary liver cancer at least in some countries.

**Hepatitis D virus** (HDV) is an unusual single stranded circular RNA virus with a number of similarities to certain plant viral satellites and viroids. This virus requires hepadnavirus helper functions for propagation in hepatocytes, and is an important cause of acute and severe chronic liver damage in some regions of the world. The modes of transmission are similar to the parenteral transmission of hepatitis B.

**Hepatitis E virus** (HEV) is an enterically transmitted, non-enveloped, single stranded RNA virus, which shares many biophysical and biochemical features with caliciviruses. Hepatitis E virus is an important cause of large epidemics of acute hepatitis in the subcontinent of India, Central and South-East Asia, the Middle East, parts of Africa and elsewhere; and this virus is responsible for high mortality during the third trimester of pregnancy.

**Hepatitis F virus** Virus-like particles (referred to by some as candidate hepatitis F virus) were identified by electron microscopy in the livers and grafts with haemorrhagic necrosis in a subset of British patients with sporadic fulminant hepatitis in whom liver failure recurred about seven days after grafting. However, subsequent intensive search by advanced molecular techniques has failed to identify a candidate viral agent. This slot in the hepatitis alphabet therefore remains vacant.

Over the years, evidence became available for additional hepatitis viruses, the so-called non-A–E hepatitis viruses. The evidence was based on the observation of short and long incubation periods in post-transfusion hepatitis and in experimental transmission studies; multiple bouts of hepatitis in the same patient; chronic hepatitis not caused by HBV, HCV or HDV; chloroform resistant non-ABC virus, and cross-challenge experiments in susceptible primates. The search for new hepatitis viruses was resumed.

**The GB hepatitis viruses and hepatitis G virus.** About 30 years ago, a series of transmission studies of human viral hepatitis were initiated in small South American tamarins or marmosets, which were chosen because of their very limited contact with humans, implying that they were unlikely to have been infected with human viruses.

A serum obtained on the third day of jaundice from a young surgeon (GB) with hepatitis, induced hepatitis in each of four inoculated marmosets and was passaged serially in these animals. These important observations remained controversial until the application recently of modern molecular virological techniques. Preliminary results indicated the identification of two independent viruses, GBV-A and GBV-B, in the infectious plasma of tamarins inoculated with GB.

GBV-A does not replicate in the liver of tamarins whereas GBV-B causes hepatitis. Cross-challenge experiments showed that infection with the original infectious tamarin inoculum conferred protection from reinfection with GBV-B but not GBV-A. A third virus, GBV-C, was isolated subsequently from a human specimen which was immunoreactive with a GBV-B protein. GBV-C RNA was found in several patients with clinical hepatitis, and shown to have substantial sequence identity with GBV-A.

A series of studies including phylogetic analysis of genomic sequences showed that GBV-A, B, and C are not genotypes of hepatitis C virus, and that GBV-A and GBV-C are closely related. GBV-A/C and GBV-B and the hepatitis C viruses are members of distinct viral groups. The organisation of the genes of the GBV-A, B, and C genomes shows that they are related to other positive strand RNA viruses with local regions of sequence identity with various flaviviruses. The three GB viruses and HCV share only limited overall amino acid sequence identity.

Serological reagents were prepared with recombinant antigens and testing for antibodies and by RT-PCR for specific RNA was carried out in groups of patients, blood donors and other selected individuals, patients with non-A, B, C, D, E hepatitis, multitransfused patients, intravenous drug addicts, and other populations with a high incidence of viral hepatitis. Preliminary studies indicated the presence of antibody to each of the GB viruses in 3% to as many as 14%. GBV-A and GBV-B were identified in tamarins and are probably not human viruses.

The development and availability of specific diagnostic reagents will establish the epidemiology of these newly identified viruses, their pathogenic significance in humans and their clinical and public health importance. It should be noted that the virus described more recently as hepatitis
A rational approach to immunohistochemical analysis of malignant lymphomas on paraffin wax sections

The recently proposed Revised European-American Lymphoma (REAL) classification is the first to regard immunophenotyping as an integral part of the process of diagnosing lymphoid tumours, as it permits more objective interpretation of the histological pattern. The usefulness of this approach has been confirmed in the course of a validation study on the REAL classification sponsored by the National Cancer Institute. This validation study showed that the rate of diagnostic consensus for several categories of malignant lymphomas (for example, diffuse large B cell, peripheral T cell unspecified, anaplastic large cell lymphomas) is notably increased when morphology and phenotypic findings are considered together (Müller-Hermelink H-K, personal communication). Furthermore, immunohistochemistry provides valuable prognostic and therapeutic information, and contributes to the better understanding of the histogenesis and pathogenesis of lymphoid neoplasms. In the light of its practical impact, immunohistochemical analysis of malignant lymphomas deserves further attention. In their paper in this issue (p16), Singh and Wright look at the possibility of distinguishing between B-small lymphocytic lymphoma and mantle cell lymphoma via immunohistochemical analysis of routinely processed paraffin wax sections, prompting the following general remarks.

Sample processing is a basic step. Wherever possible, part of the sample should be kept frozen in order to optimise preservation of both molecules and nucleic acids. However, as most laboratories are not equipped for cryo-preservation, immunophenotypic analysis of paraffin wax sections is the next logical step.

Fixation is a crucial step for routine samples: we recommend incubation in 10% buffered formalin at room temperature for six (gastric biopsy specimens) to 24 (2–3 µm thick sections of lymph node or spleen) hours. This ensures homogeneous fixation of all the parts of the specimen. For bone marrow needle biopsy specimens, we recommend fixation in B5 for two and a half hours, followed by a 30 minute wash in 70% alcohol and decalcification in DECAL for two and half hours. All of these procedures result in optimal preservation of cytological details and homogeneous immunostaining of different parts of the same specimen.

Other important points are (1) the method chosen for antigen retrieval; (2) the sensitivity of the detection system; and (3) the use of semi/automated immunohistochemical techniques. With regard to antigen retrieval, the introduction of heat-based techniques has greatly improved the possibility of detecting epitopes masked by fixation: however, different approaches have been proposed in the literature, with varying efficacy (for a comprehensive review see Cattoretti et al.). We recently compared the main variables in antigen retrieval techniques: different buffers; proteolytic enzymes versus heat-based antigen retrieval; and microwaving versus pressure cooking (Pileri et al., manuscript submitted). The aim of our study was to optimise antigen retrieval at our centre, which routinely receives heterogeneously treated material for consultation.