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Towards a hepatitis A vaccine. A review

By B. N. ANDERSON, A. G. COULEPIS and I. D. GUST
Virus Laboratory, Fairfield Infectious Diseases Hospital, Fairfield, Victoria 3078, *Australia*

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INTRODUCTION

Hepatitis A is an acute inflammation of the liver caused by a virus (HAV) whose morphology and physical characteristics resemble members of the enterovirus group. The disease, which is characterized by fever, malaise, anorexia, nausea, abdominal discomfort and jaundice has an average incubation period of 28–30 days and is spread from person to person by the faecal–oral route. Common-vehicle outbreaks have been reported following contamination of food or water and epidemics may occur in closed communities (institutions, day-care centres) or in the general community when there is a breakdown of environmental sanitation. Many infections, especially in children, are subclinical; the case fatility rate for patients requiring hospitalization is low and long-term sequelae are unknown.

The development of specific, sensitive tests for detecting past infections has resulted in an improved understanding of the natural history of this disease. Several different patterns of infection have been observed (Szmuness *et al.* 1977; Wong, Purcell & Rosen, 1979; Gust, 1982). In the most populous regions of the world, hepatitis A is endemic and most children are infected early in life. Under these circumstances clinical hepatitis is rare, except amongst visitors from developed countries, and epidemic disease is almost unknown. By contrast, hepatitis A is a major public health problem in some developing countries, where improved standards of hygiene and sanitation have resulted in a significant proportion of the population reaching adult life without having been exposed to the virus. Infections occurring at this stage of life, although relatively infrequent, are usually associated with clinical disease. Thus, paradoxically, in countries whose standards of hygiene are improving, the prevalence of hepatitis A infection gradually falls but this tends to be accompanied for a number of years by a steady increase in the prevalence of clinical disease. In highly developed countries such as Sweden and Japan, hepatitis A is almost unknown and the majority of patients with the disease acquire their infection while travelling overseas. However, with the increase in recreational travel, this has become a serious problem.

At present, the only means of preventing hepatitis A is the administration of gamma globulin prepared from pooled plasma collected from healthy adult donors, many of whom have previously been infected with HAV. While gamma globulin is both safe and effective it provides only temporary protection, and there is a clear need to develop a vaccine. Even in developed countries like the USA an average of 400 new cases of hepatitis A are reported each week (Centers for Disease Control,

1983), and the cost to society for hospital and medical care and lost production is enormous. Vaccines capable of controlling the disease are now feasible and should be available in the near future.

BACKGROUND TO DEVELOPMENT OF A HEPATITIS A VACCINE

Traditionally the requirements for the development of a viral vaccine have been an *in vitro* propagation system and sensitive, specific assays for both the virus and antibody directed against it. Attempts to cultivate hepatitis viruses during the 1950s and 1960s resulted in a large collection of 'candidate' viruses, none of which was shown to be the aetiological agent of hepatitis A. An important development occurred with the successful transmission of the disease to marmosets (Deinhardt *et al.* 1967; Mascoli *et al.* 1973) and chimpanzees (Maynard *et al.* 1975), which provided animal models for studies on infectivity and the pathogenesis of disease. The other major advance was the visualization of HAV virions in faecal specimens collected from patients with experimentally induced hepatitis A (Feinstone, Kapikian & Purcell, 1973). Using the technique of immune electron microscopy these findings were rapidly confirmed in patients with naturally acquired infections (Locarnini *et al.* 1974) and in patients involved in a common source outbreak (Gravelle *et al.* 1975). In subsequent years a wide range of diagnostic tests for the presence of HAV and total and class-specific antibody were developed (Miller *et al.* 1975; Provost *et al.* 1975; Purcell *et al.* 1976; Mathiesen *et al.* 1978; Locarnini *et al.* 1979, 1980), and these were used to define the period of maximum infectivity (Coulepis *et al.* 1980), and further define the epidemiology and natural history of the disease. Application of these assays to patients with post-transfusion hepatitis which was not hepatitis B led to the recognition of a third form of the disease which is now known as non-A, non-B hepatitis (Feinstone *et al.* 1981).

The first successful *in vitro* propagation of HAV was announced in 1979 (Provost & Hilleman, 1979). A strain of HAV obtained from a Costa Rican patient with hepatitis A was passaged 31 times in marmosets and then successfully adapted to growth in explant cultures of marmoset liver cells and in a cloned line of fetal Rhesus kidney cells. Virus re-isolated after five passages in FRhK-6 cells was shown to have retained infectivity for marmosets. Subsequently, Flehmig (1980) propagated several strains of HAV in the FRhK-4 line of fetal rhesus monkey kidney cells, and Frosner and colleagues (Frosner *et al.* 1979) achieved serial propagation of HAV in a hepatoma cell line which was also infected with hepatitis B. Daemer and co-workers (1981) succeeded in isolating HAV in African green monkey kidney (AGMK) cells using virus obtained directly from human faecal specimens, as well as virus serially passaged in marmosets. One strain of HAV used in their study (HM 175) was obtained from the faeces of a patient with acute hepatitis A in Melbourne. The strain produces an intense immunofluorescence and has been passaged many times in cell cultures. At early passage levels HM 175 exhibited an eclipse phase of approximately one month before intracellular HAV antigen(s) became detectable. The eclipse phase shortened considerably upon further serial passage, a phenomenon observed by other authors (Frosner *et al.* 1979; Flehmig, 1980; Provost & Hilleman, 1979) with HAV of faecal origin. In each case HAV was not detected in the supernatant fluids, implying that viral antigen, at

least at early passage levels, is highly cell-associated. A number of laboratories have now reported the propagation of HAV in cell lines considered suitable for vaccine production, including human diploid lung fibroblasts (W 138, MRC 5) and human diploid embryo fibroblasts (HEF) (see Gauss-Muller, Frosner & Deinhardt, 1981; Provost *et al.* 1982).

In recent years the biochemical and biophysical characteristics of HAV have become sufficiently well documented to enable the formal classification of the virus as enterovirus type 72 within the family *Picornaviridae* (Coulepis *et al.* 1982; Gust *et al.* 1983). The progress of research with the hepatitis A virus has been relatively slow when compared with other picornaviruses such as poliovirus and foot and mouth disease virus (FMDV), but the qualitative requirements for the development of a vaccine appear to be present.

Inactivated vaccines

Provost & Hilleman (1978) prepared a crude inactivated hepatitis A vaccine by formalin treatment of virions purified from the livers of HAV-infected marmosets, and used this material to immunize marmosets and protect them against subsequent challenge with HAV. While demonstrating the feasibility of producing an inactivated hepatitis A vaccine the studies were of limited practical value because marmosets are an endangered species, and this type of preparation is too crude to be licensed for human use. At present cell culture does not offer an acceptable alternative for the production of viral antigen since most of the strains which have been isolated to date grow with relatively low efficiency. In general incubation periods are much longer, and virus yields two or three logs lower, than for other enteroviruses.

Live attenuated vaccines

Provost *et al.* (1982) inoculated marmosets with HAV variants which had been obtained after multiple passages in cell culture. Strains of HAV which had been passaged in FRhk-6 cells 25–30 times exhibited attenuation but retained the ability to induce anti-HAV. Lower passage level variants remained at least partially virulent for marmosets, while at higher passage levels the virus was found to be over-attenuated. The histopathological changes observed when attenuated variants were inoculated into marmosets were consistent with a lower level of viral replication compared with wild-type HAV. Marmosets vaccinated with suitably attenuated variants were resistant to infection with wild-type HAV, and careful study of liver tissue failed to reveal any evidence of viral replication in the protected animals. Similar results have been reported recently in chimpanzees (Feinstone *et al.* 1983; Provost *et al.* 1983). Preliminary trials to determine the safety, antigenicity and efficacy of selected attenuated strains of HAV in man are in progress (Provost, personal communication).

These strains do not replicate well in the gut and must be administered intramuscularly. The possibility of oral immunization needs to be explored further as this form of immunization results in gut immunity which is important in reducing the spread of infection in the community. Such a vaccine could perhaps be administered concurrently with Sabin oral polio vaccine.

Before the vaccines currently under test can be licensed a number of issues such

as the level of hepatic involvement and the stability and transmissibility of these strains need to be resolved, and sensitive, specific means of distinguishing wild from attenuated strains will need to be developed.

Alternative vaccines

An alternative approach to the development of viral vaccines involves the application of the relatively new disciplines of molecular biology and recombinant DNA technology. In the simplest case, a segment of viral DNA, or a complementary DNA (cDNA) copy of viral RNA, is ligated into a bacterial plasmid vector to form a molecular hybrid and introduced into a bacterial cell. All progeny of the original bacterial cell will carry identical (cloned) copies of the viral DNA or cDNA, which can be characterized further by restriction endonuclease mapping and nucleotide sequencing analyses. A variety of computer programs to facilitate these analyses are available, and most of these allow for the determination of the amino-acid sequences which would be expected to be encoded by either strand of the cloned DNA. Again, in the simplest case, the cloned DNA can be genetically 'engineered' to a position downstream from, and in appropriate reading frame to, a bacterial gene promoter such that the peptide specified by the cloned DNA is produced within the bacterium. The required peptide can then be purified and used for vaccine purposes. Of the many examples of the successful use of this approach, the most relevant to the production of a hepatitis A vaccine is the production of virion protein 3 (VP 3) of FMDV in *Escherichia coli* (Kleid *et al.* 1981), and its use to immunize animals and protect them against challenge by FMDV.

More commonly, stable expression of cloned genes cannot be obtained, or maintained, in bacteria, necessitating the re-cloning of the DNA into other host/vector systems. This approach is often necessary if the final antigenic products embody post-translational modifications such as specific intra-chain polypeptide processing, or glycosylation which does not occur in bacteria. Racaniello & Baltimore (1981) found that a complete copy of the poliovirus genome cloned as cDNA in the plasmid vector pBR 322 was infectious when transfected into eukaryotic cells in culture, yielding intact, infectious poliovirus virions. This result has important implications for the development of vaccines against picornaviruses such as HAV as it renders feasible the generation of attenuated viral strains by genetic engineering of cDNA *in vitro*. Further, if one could determine why HAV grows so poorly *in vitro* compared with its picornaviral counterparts, it might be possible to alter, or substitute, the relevant DNA segments *in vitro* and – with appropriate safeguards and caution – re-introduce the molecular hybrids into tissue culture cells and generate higher-yielding chimaeric strains.

Chimaeric vaccines

An alternative approach to vaccine development is to splice DNA coding for the antigenic determinants of HAV into cDNA copies of existing attenuated poliovirus variants to generate polyvalent chimaeric strains. Examples of the use of a viral genome to carry a DNA segment encoding an antigen of a different virus have been reported by Smith and his colleagues (Smith, Mackett & Moss, 1983*a*), who inserted DNA encoding the hepatitis B virus surface antigen (HBsAg) into the DNA genome of vaccinia virus. The HBsAg was subsequently expressed to a level of 2·6 μg per

5 million cells infected with the variant virus and rabbits inoculated with the live variants developed antibodies to HBsAg within nine days. Smith, Mackett & Moss (1983*b*) together with Panicali *et al.* (1983) have extended this work by demonstrating, in animals, that vaccinia virus variants carrying the influenza virus haemagglutinin gene are as effective as live attenuated vaccines in prevention of infection. Smith & Moss (1983) have estimated that the vaccinia virus genome can accommodate up to 25000 base pairs of foreign DNA (almost 20-fold more than the length of HBsAg DNA), raising the possibility of constructing infectious polyvalent vaccines against a number of different pathogenic agents.

Ticehurst *et al.* (1983) and Von der Helm & Deinhardt (1984) have published accounts of cloning HAV cDNA into plasmid vectors in *E. coli*, the first step in the development of recombinant DNA-derived HAV vaccines. In the latter case more than 99% of the HAV genome was represented amongst a series of overlapping cloned DNA fragments, and some preliminary nucleotide sequence information was reported. The sequence information has now been extended to 3119 bases (Baroudy *et al.* 1984). To date, there have been no confirmed reports of stable expression of antigens from cloned HAV fragments, although this is just a matter of time.

Polypeptide vaccines

A relatively new approach to the development of viral vaccines is to make use of the peptide sequences predicted from the nucleotide sequences determined from the cloned viral DNA or cDNA. Peptides likely to be antigenic can be chemically synthesized, coupled to carriers mixed with adjuvants, and tested in animals to determine whether they are immunogenic, and if the immune response is virus-neutralizing, or protective. This approach was employed successfully by Bittle *et al.* (1982), who synthesized peptides of the VP1 protein of FMDV and used this material to stimulate a neutralizing antibody response in animals. Linemeyer *et al.* (1984) have used DNA sequence data from HAV cDNA clones to compare predicted amino-acid sequences with known sequences of cyanogen bromide-cleaved fragments of VP 1 and VP 3 of HAV to locate the cDNA sequences coding for these structural proteins. In the near future, it should be possible to use this data to define and synthesize a series of oligopeptides which stimulate antibody to major antigenic site(s) of HAV.

There are several advantages in the use of polypeptide vaccines, including the low cost of synthesis, the complete absence of adventitious agents, and the relative ease of varying the antigenic content. The only disadvantages which are currently recognized are the need to administer several injections in a vaccination programme, and the absence of a local immune response.

CONCLUSION

A variety of new approaches to vaccine development have been applied to picornaviruses and are being used with HAV. It seems likely that expression of antigen(s) in eukaryotic or prokaryotic cells will be achieved soon, that immunogenic peptides will also be produced and that one or more attenuated strains of virus will become available, bringing control of the disease within reach.

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