Mr Jones
I've not yet seen the BMJ leader. GRO-C 10/3

Dr Steadman

cc: Dr Sibellas
Mr Anderson
Miss Stuart ✓ VI 3/2

(111)

HEPATITIS B VACCINE

VI 3/7
V/1

Thank you for your minute of 8 March about the Leader in the BMJ by Professor Zuckerman.

I confess that I am more relaxed than you are about this. Apart from the fact that he is, as you say, entitled to say what he likes, he is only expressing views which might reasonably have been made by anyone knowing something of the field, whether or not he or she were a member either of the Hepatitis Advisory Group or the JCVI or the WG. BMJ Leaders are expressions of opinion, not declarations of public policy, and we can take them or leave them as we feel inclined.

I agree that it would have been helpful if Professor Zuckerman had told us what he was about to say or had done his best to keep roughly to the proposals made by the WG but he has not represented himself as a member of that body nor is he suggesting that his views have any official backing.

We will soon be putting a submission to Ministers about the need to have a policy on the provision of vaccine, with which we would need to send an estimate of the financial consequences, and we could include the Zuckerman paper as part of the evidence, together with any comments on it that we felt appropriate.

I am not sure about your second point and perhaps Dr Sibellas, who is in regular touch with Professor Zuckerman, could ask him about it.

GRO-C

9 March 1982

T GEFFEN
C716 x GRO-C

Miss McCarthy. Please.
GRO-C
RS Anderson 11.3.82.

Please obtain copies of the minute + leader.

GRO-C 10/3/82.

Dr Geffen

HEPATITIS B VACCINE

You will have seen the attached BMJ Leader.

Though we cannot stop Professor Zuckerman saying what he likes when he likes, it is rather irrating to have the decisions of the Joint Working Group pre-empted publicly in this way. In fact some of his recommendations, such as the combined active and passive immunisation of infants born to carrier mothers, are not likely to be official policy. Dr Palakoff telephoned me this morning and was predictably very upset about this Leader.

The other section of the Leader dealing with new hepatitis vaccines naturally contains the expected reference to Zuckerman's own micellar antigen. However, he does make the surprising comment that safety tests and protective efficacy studies have been completed on this material. I am not aware that this is so, certainly nothing has been published along these lines. In fact I understood that the PHLS request for extra equipment for Malling's Laboratory at CAMR was in connection with just these studies.

Yet more to discuss on Thursday.

DR J STEADMAN
MED MCD
C.718 AFH x GRO-C

8 March 1982

cc Dr Sibellas

Regular Review

Priorities for immunisation against hepatitis B

ARIE J ZUCKERMAN

The development of a highly unusual vaccine against hepatitis B from the excess surface antigen protein coat of the virus obtained from the plasma of asymptomatic carriers is an ingenious solution by laboratory workers to an urgent need in preventive medicine. The non-infectious, surplus protein coat of the virus in the form of 22 nm spherical particles in the plasma of carriers, who number conservatively 200 000 000 worldwide, presents an attractive (if unusual) source of starting material for the preparation of a vaccine. Our inability to grow hepatitis B virus in tissue culture has hampered the development of conventional vaccines, but a series of studies conducted between 1970 and 1973 with the MS-2 strain of hepatitis B virus by Krugman and his colleagues[1–3] paved the way to the current exciting progress. These studies showed the relative efficacy of diluted serum containing hepatitis B virus and its associated antigens,[4] heated to 98°C for one minute, in preventing or modifying the infection in 70% of susceptible individuals after exposure to infective serum.

When pure, the preparations of 22 nm surface antigen particles are generally accepted to be free of nucleic acid and therefore non-infectious, but the fact that the starting material for the current vaccines is human plasma obtained from persons infected with hepatitis B virus means that extreme caution must be exercised to ensure their freedom from all harmful contaminating material.[5] Some concern has also been expressed about the possible induction of harmful immunological reactions to host components, including pre-existing structures of liver cells, which may be present in such vaccine preparations,[6 7] but reactions of this type have not been observed with the highly purified vaccines in the chimpanzees and individuals immunised so far.[8] The vaccine (Merck Heptavax B), recently licensed in the United States, undergoes a complex method of purification, inactivation, and safety testing over a period of 65 weeks to ensure removal of most, if not all, of the host material and any contaminating or extraneous material or viruses which may be present in large pools of plasma collected from carriers.[9] After safety testing in chimpanzees[10] to ensure freedom from residual live virus, and subsequently in healthy volunteers, the vaccine has undergone carefully conducted trials in the United States on protective efficacy and immunogenicity.[11–15] The results obtained appear to establish that the development of hepatitis B surface antibody is synonymous with protection against infection with hepatitis B virus.

Viral hepatitis is a major public health problem. Hepatitis A (infectious hepatitis) and hepatitis B (formerly referred to as serum hepatitis) are hyperendemic in countries with hot climates, in the developing countries, and also in some regions of Europe. The importance of hepatitis B cannot be exaggerated. Apart from the acute illness, which varies in severity, the infection may persist, especially in children infected perinatally or early in life. Furthermore, infection with hepatitis B virus may progress to chronic liver disease including chronic active hepatitis and cirrhosis, and hepatitis B virus is associated with primary liver cancer, one of the world's most common tumours.

The priorities for immunisation against hepatitis B are not the same for each geographical region or country, for the needs are dictated by differing epidemiological patterns, socioeconomic factors, cultural and sexual practices, and by the environment.

Groups at high risk of infection include patients requiring multiple transfusions; patients with natural or acquired immune deficiency and patients with malignant disease; patients and staff of haemodialysis, transplant, and oncology units; and residents and staff of institutions for the mentally handicapped. Viral hepatitis is an occupational hazard among health care and laboratory personnel (though the risk of infection has been exaggerated). High rates of infection occur in homosexual men, drug addicts, and prostitutes. Perinatal transmission of hepatitis B from carrier mothers to their infants occurs frequently in some regions, and if acute infection is contracted during the last two trimesters of pregnancy or within two months of delivery protective immunisation of susceptible women of childbearing age and their newborn infants may well be the only practical way of interrupting transmission of the infection. Immunisation must also be considered for the whole population of certain tropical and non-tropical areas where the prevalence of hepatitis B infection is high, the carrier state may reach 10-20% of the population, and primary liver cancer is common.[10]

Priorities for immunisation against hepatitis B in Britain would include current high risk groups in the Health Service such as the medical and laboratory staff of hepatitis reference centres and staff engaged in the development and production of hepatitis B vaccine; staff of liver units and gastrointestinal units with an interest in the liver; staff of surgical intensive care units; dental surgeons, dental nurses, and ancillary staff of units where dental care is provided for known hepatitis B carriers; patients with natural or acquired immune deficiencies and staff of units caring for these patients, including transplant and oncology units; and direct family and medical contacts of known carriers treated by maintenance haemodialysis. Active immunisation is also required for medical, nursing, ancillary, and teaching staff of patients resident in institutions for the mentally handicapped; for patients who do not have serological markers of infection with hepatitis B or immunity to this virus; as well as for close family contacts of known mentally

handicapped carriers. Other groups include the immediate family contacts of patients and staff in close contact with patients requiring frequent administration of blood and coagulation factors such as patients with haemophilia[16] and thalassaemia.[17] Male homosexuals are another group at high-risk of hepatitis B and an important reservoir for transmission of infection because of their considerable promiscuity. Vaccine is also recommended for spouses and sexual contacts of patients with acute hepatitis B and of carriers of the virus and *e* antigen.

Combined passive immunisation with hepatitis B immunoglobulin and vaccine would be recommended for infants born to carrier mothers with *e* antigen and to mothers who contract acute hepatitis during the last two trimesters of pregnancy or within two months of delivery, and their midwives. Combined prophylaxis would also be required for a single acute exposure to hepatitis B virus, such as when blood known or strongly suspected to contain the virus is accidentally inoculated, ingested by mouth, or splashed on to mucous membranes. This category includes ambulance attendants and police officers,[18] who frequently suffer accidental penetration by a needle or massive exposure to blood.

Apparent glaring omissions for these recommendations for high-risk groups include the staff and patients of maintenance haemodialysis units, but at present in Britain a strict code of practice and measures for the control of cross-infection have contained the problem of hepatitis B. Indeed, hepatitis B as an occupational hazard has been largely prevented by environmental hygienic measures.[5 19] Nevertheless, the list for active immunisation may readily be extended to all medical, dental, nursing, and laboratory personnel, to staff and inmates of custodial institutions, and others. The need for active immunisation, however, is principally dictated by epidemiological data, and in the immediate future it will also be governed by the availability of the vaccine and its very high cost.

Indeed, this type of the 22 nm particle hepatitis B vaccine has several disadvantages. Firstly, pooled plasma with high titre of hepatitis B surface antigen (often *e* antigen-positive) is required in large quantity from persistent carriers, and each carrier donor cannot be characterised on an individual basis. Secondly, supply may be difficult to secure in the long term. Thirdly, facilities for containment of live virus are required for production. Fourthly, the manufacturing process is lengthy, extending over 65 weeks, during which possible extraneous/adventitious agents and other contaminants must be removed. Fifthly, manufacture is expensive; and, lastly, strict safety testing of the vaccine is required including, at least for the present, tests for residual infectivity of hepatitis B virus in susceptible chimpanzees.[10]

"Second generation" hepatitis B vaccines have therefore been developed, and other sources of antigen are being sought. Hepatitis B polypeptide vaccines containing hepatitis-B specific antigenic determinants associated with a non-glycosylated polypeptide with a molecular weight in the range of 22 000-24 000 and a glycosylated polypeptide with a molecular weight in the range of 26 000-29 000 have been prepared and tested for safety, immunogenicity, and protective efficacy in susceptible chimpanzees.[20 21]

Such vaccines are better defined chemically and have an added margin of safety, since they would be even less likely to contain infectious virus or contaminating host proteins.[5] Several studies have shown that individual polypeptides of the surface antigen are immunogenic, but, more particularly, that purified 23 000 and 28 000 molecular weight polypeptides were effective antigens. The major difficulty of obtaining sufficient quantities of the peptides in pure form has recently been overcome by the extraction of the antigenic polypeptides with the non-ionic detergent Triton X-100,[22] and a method of detergent removal has been developed which allows membrane polypeptides to reassociate into water-soluble protein micelles. Protein micelles are aggregates of polypeptides arranged so that the hydrophobic regions are sequestered in the interior of the particles with the hydrophilic residue on the surface, so that the resulting particulate forms are water soluble, and a micelle vaccine has been developed at the London School of Hygiene and Tropical Medicine. Comparison of the immunogenicity of the micelles with the 22 nm particle vaccine in a mouse potency assay showed that the micelles elicited a more vigorous surface antibody response than the intact particles at all dose levels tested.[20] Safety tests and protective efficacy studies have been completed, and clinical trials of the micelle vaccine should be starting shortly.

The rapidly progressing discipline of recombinant DNA technology offers particularly attractive sources of antigenic material—for example, from prokaryotic cells expressing hepatitis B surface antigen proteins as a result of cloning of hepatitis B viral DNA. The production of small amounts of surface antigen has been reported by several groups, and appreciable amounts of antigen have also been obtained.[23] Expression of glycosylated hepatitis B surface antigen in yeast cells has recently been reported, and this represents potentially a most important development for large scale in vitro production of an immunogen. Expression of hepatitis B proteins in eukaryotic cells has been achieved in mutant mouse LM cells and in HeLa cells, but sources such as transformed heterotransplantable producer cell lines have not yet been licensed for the production of vaccine.

Perhaps the most exciting prospect is the development of synthetic vaccines. These offer many advantages in attaining the ultimate goal of chemically synthesised multivalent vaccines to replace many current bacterial and viral vaccines, which often contain many irrelevant microbial antigenic determinants, proteins, and other material that contaminate the essential immunogen and which may lead to untoward side effects.[24] Possible approaches to the development of chemically synthesised hepatitis B vaccines were described a few years ago by several groups of investigators, and recent progress suggests that synthetic peptide vaccines are now within reach.[25]

An 892 base pair region along the DNA strand of hepatitis B virus with the *adw* determinants has been identified by using cloned DNA fragments, and the full sequence of the 226 amino-acids coding for the 23 000 to 25 000 molecular weight polypeptide of hepatitis B surface antigen was subsequently predicted.[26] The corresponding sequence for the *ayw* subtype indicated a variation of 16 amino-acids.[27] By employing a computer programme which has been used to predict the internal and external residues of proteins with known structure, 13 peptides were chemically synthesised corresponding to amino-acid sequences predicted from the nucleotide sequence for hepatitis B surface antigen.[28] Seven out of the 13 free or protein carrier-linked synthetic peptides elicited an anti-peptide response in rabbits. When used, the carrier protein was Keyhole limpet haemocyanin in complete and subsequently incomplete Freund's adjuvant. Antisera against four out of the six soluble peptides, ranging from 10 to 34 amino-acid residues, reacted with the native antigen and also precipitated the 23 000 and 28 000 molecular weight major polypeptides of hepatitis B surface antigen. A computerised analysis of the

amino-acid sequences of the surface antigen protein was also used by Hopp to predict an antigenic site determinant.[29] An amino-acid sequence of residues 138-149 was synthesised and examined for its ability to bind antibodies to a mixture of the *ad* and *ay* subtypes of hepatitis B virus. Though the peptide bound only 9% of the antibodies, the maximum binding capacity was not reached. A similar technique was employed more recently by Dreesman *et al*[30] to predict two hydrophilic regions of the surface antigen molecule. Two cyclic peptides containing disulphide bonds in the region between the amino-acid sequences 117 and 137 were synthesised. The two synthetic peptides with sequences 117-137 and 122-137 were incorporated into several adjuvants, including Freund's complete adjuvant, alum, and multilamellar liposomes with and without muramyl dipeptide. Hepatitis B surface antibody was induced seven to 14 days after inoculation in roughly half of the mice in each group and in four or five of six mice when the immunising preparation of the 117-137 peptide was emulsified with Freund's complete adjuvant. On day 21 the peak titres of antibody decreased in most groups of mice. The antibody response was also elicited in mice after a single injection without covalent linkage to a carrier protein.

Synthetic peptides may be employed, therefore, in due course as vaccines, though mixtures of more than one of the peptides may be required. Of the many questions which remain to be answered, the critical issues are whether antibodies induced by synthetic immunogens will be protective and whether protective immunity will persist. One of the proteins and some of the adjuvants which had been linked to the synthetic molecules cannot be used in man, and acceptable and safe material needs to be found for covalent linkage. Clearly we are entering the era of antigen and antibody engineering, and the prospect of multivalent synthetic vaccines against a variety of microbial agents may well be within reach.

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