The development of novel hepatitis B vaccines*

A. J. Zuckerman

Development of vaccines against viral hepatitis B has proceeded along four main lines. (1) Human plasma-derived vaccines are safe, effective and are now in general use. (2) Subunit polypeptide vaccines formulated in micelles have reached the stage of clinical trials. (3) Recombinant DNA vaccines have been produced in prokaryotic and eukaryotic cells, notably in yeast. The yeast-derived recombinant vaccines have proved safe and effective in extensive clinical trials, eliciting antibodies which in quantity and specificity are equal to those elicited by plasma-derived vaccine. DNA recombinant has also been applied to the development of hybrid and vaccinia virus vaccines which are capable of immunological “priming”, and other hybrid virus vaccines are under development. (4) Finally, chemical synthesis has succeeded in producing small peptides which include specific epitopes eliciting antibody responses in experimental animals. Such chemically synthesized preparations offer a prospect of ultimately producing multivalent synthetic vaccines against several viruses, bacteria and protozoa.

Infection with hepatitis B virus may progress to chronic liver disease such as chronic persistent and chronic active hepatitis, cirrhosis, and hepatocellular carcinoma. Persistent infection occurs in 5–15% of adults after acute infection and in over 90% if the infection was acquired early in life in some geographical areas. It is conservatively estimated that there are over 200 million carriers of hepatitis B virus worldwide (about 5% of the global population). Immunization against viral hepatitis B is therefore required for groups at high risk of infection according to epidemiological patterns, socioeconomic factors, cultural and sexual practices, and the environment. In addition, the high rates of infection and perinatal transmission of hepatitis B virus in some regions (Table 1) dictate the urgent need for protective immunization of susceptible women of childbearing age as well as infants, particularly infants born to carrier mothers, because it is the only practical way of interrupting transmission of the infection. Immunization must also be considered for

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1 Professor of Microbiology and Director of the Department of Microbiology and the WHO Collaborating Centre for Reference and Research on Viral Hepatitis, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, England. Requests for reprints should be sent to this address.
Table 1. Areas and features of hepatitis B prevalence

<table>
<thead>
<tr>
<th>Area</th>
<th>Low prevalence</th>
<th>Intermediate prevalence</th>
<th>High prevalence</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Northern, western and central Europe, Australia and North America</td>
<td>Eastern Europe, Mediterranean, USSR, and Central and South America</td>
<td>Parts of China, south-east Asia and tropical Africa</td>
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<tr>
<td>HBsAg</td>
<td>0.2–0.5%</td>
<td>2–7%</td>
<td>8–20%</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>4–8%</td>
<td>20–55%</td>
<td>70–95%</td>
</tr>
<tr>
<td>Neonatal infection</td>
<td>Rare</td>
<td>Frequent</td>
<td>Very frequent</td>
</tr>
<tr>
<td>Childhood infection</td>
<td>Infrequent</td>
<td>Frequent</td>
<td>Very frequent</td>
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</tbody>
</table>

persons living in certain tropical and non-tropical areas where the prevalence of hepatitis B infection is high, where 10–20% or more of the population may be carriers, and where hepatocellular carcinoma is common. Hepatocellular carcinoma is one of the ten most common tumours in the world, with over 250 000 new cases each year, and there is compelling evidence that hepatitis B virus is the cause in up to 80% of cases (1).

Current viral vaccines are usually prepared from the intact virus or from a portion of the virus such as the coat protein or its subunits after growth in vitro in cell culture. Such vaccines may contain contaminating antigenic components, such as proteins and other material derived from the virus, the host cell in which the virus has been grown or the cell medium, which are not relevant to protective immunity but could lead to undesirable side-effects.

HEPATITIS B VACCINE PREPARED FROM PLASMA

The failure to propagate hepatitis B virus in vitro has prevented the development of vaccines from virus grown in cell cultures. Attention has therefore been directed to the use of alternative sources of viral antigen for active immunization, including the use of hepatitis B surface antigen, the non-infectious surplus protein coat of the virus, after it has been purified from the plasma of asymptomatic human carriers and subsequently subjected to inactivation procedures. It is generally accepted that preparations of the 22-nm particles, when pure, are free of nucleic acid and therefore non-infectious, but the fact that the starting material is human plasma obtained from persons infected with hepatitis B virus means that extreme caution must be exercised to ensure that the antigen preparations are free of all harmful contaminating material, including host components. The highly purified and inactivated 22-nm particle vaccines have now been used for the immunization of several million individuals in many countries.

The development and safety of hepatitis B vaccines derived from plasma and consisting of surface antigen purified by several physical and biological procedures and inactivated by at least two different methods has been reviewed recently (2). Furthermore, the currently licensed vaccines which meet the WHO requirements of 1981 and 1983 have been shown to be safe and effective, and have not been associated with risk of transmission of the human immunodeficiency virus or any other infectious agent (3, 4).

Indications for immunization against viral hepatitis B

The current indications for the use of hepatitis B vaccines in low prevalence areas (Tables 1 and 2) are summarized below. In intermediate and high prevalence regions, immunization is required for infants.
Table 2. Recommendations for immunization with hepatitis B vaccine according to prevalence

<table>
<thead>
<tr>
<th>Low prevalence areas</th>
<th>Intermediate and high prevalence areas</th>
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<tbody>
<tr>
<td>Pre-exposure</td>
<td>All infants</td>
</tr>
<tr>
<td>Health care personnel</td>
<td></td>
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<tr>
<td>Dialysis patients</td>
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<tr>
<td>Patients and staff in institutions</td>
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<tr>
<td>Drug addicts</td>
<td></td>
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<tr>
<td>Male homosexuals</td>
<td></td>
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<tr>
<td>Prostitutes</td>
<td></td>
</tr>
<tr>
<td>Rescue service personnel</td>
<td></td>
</tr>
<tr>
<td>Post-exposure</td>
<td>Infants of HBsAg-positive mothers</td>
</tr>
<tr>
<td>Accidental inoculation*</td>
<td></td>
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<tr>
<td>Infants of HBsAg-positive mothers*</td>
<td></td>
</tr>
<tr>
<td>Sexual contacts of acute cases*</td>
<td></td>
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<tr>
<td>and carriers</td>
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* Hepatitis B immunoglobulin should be given intramuscularly at a contralateral site.

1. Health care personnel in frequent contact with blood or needles, particularly those caring for patients, over a period of time, in residential institutions for the mentally handicapped where there is a known high risk of hepatitis; those who give treatment to known carriers of hepatitis B infection; those working in haemodialysis, haemophilia, and other centres and treating patients with blood or blood products; laboratory workers regularly exposed to increased risk from infected material; health workers seconded to areas of the world where there is a high prevalence of hepatitis B infection, where they are directly involved in patient care; and dentists and auxiliary dental personnel with direct patient contact.

2. Patients, particularly those who are admitted for the first time to residential institutions for the mentally handicapped where there is a known high incidence of hepatitis B; those who are being treated by maintenance haemodialysis or are frequently receiving blood or blood products; and those who, before major surgery, are likely to require a large number of blood transfusions and/or treatment with blood products.

3. Contacts of patients with hepatitis B, particularly the spouses and other sexual contacts of patients with acute hepatitis B or carriers of hepatitis B virus, and other family members in close contact.

4. Other indications for immunization:

— Infants born to mothers who are persistent carriers of hepatitis B surface antigen (HBsAg) or are HBsAg positive as a result of recent infection, particularly if hepatitis B e antigen is detectable, or HBV-positive mothers without antibody to e antigen (anti-e); the optimum timing for immunization in conjunction with the administration of hepatitis B immunoglobulin at a contralateral site is immediately at birth or within 12 hours.

— Health care workers who are accidentally pricked with needles used for patients with hepatitis B. The vaccine may be used alone or in combination with hepatitis B immunoglobulin as an alternative to passive immunization with hepatitis B immunoglobulin only; studies on the efficacy of these different schedules of immunization are nearing completion.

5. Immediate protection: whenever immediate protection is required (e.g., for infants born to HBsAg-positive mothers (see above), or following transfer of an individual into a “high risk” setting or after accidental inoculation), active immunization with the vaccine should be combined with simultaneous administration of hepatitis B immunoglobulin at a different site. It has been shown that passive immunization with up to 3 ml (200
international units of anti-HBs per ml) of hepatitis B immunoglobulin does not interfere with an active immune response. A single dose of hepatitis B immunoglobulin (usually 3 ml for adults; 1–2 ml for the newborn) is sufficient for healthy individuals. If infection has already occurred at the time of the first immunization, virus multiplication is unlikely to be inhibited completely, but severe illness and, most importantly, the development of the carrier state may be prevented in many individuals, particularly in infants born to carrier mothers.

6. The immune response to the current hepatitis B vaccines is poorer in immunocompromised patients and the elderly. For example, only about 60% of patients undergoing treatment by maintenance haemodialysis develop anti-HBs. It is suggested therefore that patients with chronic renal damage should be immunized as soon as it appears likely that they will ultimately require treatment by maintenance haemodialysis or will receive a renal transplant. Consideration should be given to the use of blood from healthy immunized donors with high titres of anti-HBs for the routine haemodialysis of patients who respond poorly to immunization against hepatitis B.

7. Other groups at risk of hepatitis B include individuals who frequently change sexual partners, particularly promiscuous male homosexuals and prostitutes; narcotic and intravenous drug abusers; and staff at reception centres for refugees and immigrants from areas of the world where viral hepatitis B is very common, such as south-east Asia. Although they are at “lower risk”, consideration should also be given to long-term prisoners and staff of custodial institutions, ambulance and rescue services, and selected police personnel.

SUBUNIT POLYPEPTIDE VACCINES

Hepatitis B polypeptide vaccines containing specific hepatitis B antigenic determinants of the major non-glycosylated polypeptide I of the surface antigen (with a relative molecular mass \(M_r\) of 22–24 000) and its glycosylated polypeptide (with an \(M_r\) in the range of 28–30 000) have been prepared. The individual polypeptides of the surface antigen are immunogenic, and the purified \(M_r\) 25 000 (designated as p25) and \(M_r\) 30 000 (gp30) polypeptides are effective antigens. The subunit polypeptide vaccines have been tested for safety, immunogenicity and protective efficacy in susceptible chimpanzees (5–7). Such polypeptide vaccines can readily be defined chemically and have an added margin of safety since these do not contain contaminating virus or host proteins.

The purification of viral coat peptide subunits in large quantities presents several problems with viruses possessing a lipoprotein envelope, where the immunogenic components are integral membrane proteins that are highly hydrophobic, insoluble in aqueous media and require drastic treatment with detergents. The extraction of the antigenic polypeptides with the non-ionic detergent Triton X-100 resolved one of the problems.

However, polypeptides in monomeric solution in high concentrations of detergent are not a suitable form of vaccine. Consequently, a method of detergent removal which allows membrane polypeptides to reassociate into water-soluble protein micelles was developed at the London School of Hygiene and Tropical Medicine. Protein micelles are aggregates of polypeptides arranged so that the hydrophobic regions are sequestered in the interior of the particles with the hydrophilic residues on the surface so that the resulting particulate forms are water soluble. Comparison of the immunogenicity of hepatitis B micelles with the 22-nm particle vaccine in a mouse potency assay showed that the micelles elicited a more vigorous protective surface antibody response than the intact 22-nm spherical antigen particles at all dose levels tested.
Safety tests and protective efficacy studies of the micelle vaccine in non-human primates have been completed and clinical trials are in progress (8). More recently, polypeptide micelles have also been prepared from hepatitis B surface antigen protein expressed by recombinant DNA technology in yeast and in mammalian cells.

RECOMBINANT DNA VACCINES

Progress in molecular genetics and nucleic acid chemistry led to the identification and analysis of genes coding for biologically active substances, and permitted the transfer of genetic material within and between organisms and the expression of proteins under controlled conditions. Recombinant DNA techniques have been used for expressing hepatitis B surface antigen and core antigen in prokaryotic cells (Escherichia coli and Bacillus subtilis) and in eukaryotic cells, such as mutant mouse LM cells, HeLa cells, COS cells, CHO cells and yeast cells (Saccharomyces cerevisiae).

Recombinant yeast hepatitis B vaccines are undergoing extensive evaluation by clinical trials. The results to date indicate that this vaccine is safe, antigenic, and free from side-effects (apart from minor local reactions in a proportion of recipients). The immunogenicity, using a dose of 10 μg/ml as compared with 20 μg/ml of the plasma-derived vaccine, is similar to that of the plasma vaccine, and indeed a lower dose may be as effective. The antigen expressed in yeast has also been prepared in micellar form. The yeast-derived polypeptide micelles were found to be considerably more antigenic with enhanced immunogenic potency.

Recombinant DNA studies have also established the organization of the hepatitis B viral genome and the nucleotide sequence. The genome contains four major polypeptide "reading frames". The "S" gene codes for an M, 25 000 protein, which resembles closely in predicted sequence the non-glycosylated major hepatitis B surface antigen polypeptide (p25) identified by electrophoretic analysis of 22-nm antigen particles purified from plasma of infected individuals. Another "reading frame", the "C" gene, codes for the M, 21 000 viral core polypeptide. The third "reading frame", the putative polymerase "P" gene, overlaps the "S" gene, and the fourth "reading frame" is designated as "X".

A contiguous upstream region of the open "reading" frame for the S region is termed the pre-surface or pre-S region. An M, 30–35 000 protein is partially encoded by the pre-S gene and mediates the binding or interaction of hepatitis B surface antigen and polyalbumin.

Studies with monoclonal antibodies, synthetic peptides with sequences predicted from the nucleotide sequence of the pre-S region, and on pre-S polypeptide fusion products expressed in E. coli, established that the M, 30–35 000 protein moieties of HBsAg components correspond to the following sequences: the "middle" protein representing S-protein with 55 additional amino acids at the N-terminal encoded by the pre-S region (amino acid residues pre-S (120–174)), and the "large" protein consisting of the "middle" protein with an additional 108–119 N-terminal amino acids (depending on the antigenic subtype), amino acid residues pre-S (1–119) or pre-S (12–119), and containing all amino acids encoded by the pre-S + S regions.

The following are some of the results of studies on the antigenicity, immunogenicity and biological role of pre-S sequences of the middle and large proteins: antisera prepared by immunization of rabbits with hepatitis B virus (HBV) recognized at high dilution synthetic peptides spanning the pre-S sequence. The highest dilution endpoints were observed with peptides from the N-terminal portions of the middle and large proteins. These peptides were also the most efficient in eliciting antibodies reacting with HBV.

Rabbit antisera also reacted with a fusion protein expressed in E. coli containing the sequence of 55 amino acids of the middle protein. Studies on the genetic restriction of the
immune response in inbred strains of mice to the S-protein and to synthetic peptides corresponding to portions of the pre-S sequence indicate that antibody responses to S and pre-S determinants are regulated by distinct genes. Pre-immunization with the synthetic peptide pre-S (120–145) of a strain of mice non-responsive to S-protein resulted in an enhanced response to S-protein after subsequent immunization with HBsAg particles containing both S-protein and pre-S sequences. Thus, non-responsiveness to the S-protein in mice was circumvented by the presence of pre-S sequences in HBsAg. The presence of pre-S sequences in HBsAg particles enhanced the antibody response to S-protein (9, 10).

The addition of pre-S gene products to hepatitis B vaccines may increase their immunogenicity, although the role of pre-S antigens and antibodies in the pathogenesis of hepatitis B has not been clearly established.

Hybrid vaccinia virus vaccines

Potential live vaccines using recombinant vaccinia viruses have been constructed for hepatitis B, and also for herpes simplex, rabies and other viruses. Foreign viral DNA is introduced into the vaccinia DNA by construction of chimeric genes. This is accomplished by homologous recombination in cells since the large size of the genome of vaccinia virus (185 000 base pairs) precludes in vitro gene insertion. A chimeric gene consisting of vaccinia virus promoter sequences ligated to the coding sequence for the desired foreign protein is flanked by vaccinia virus DNA in a plasmid vector. The hepatitis B surface antigen made by vaccinia virus recombinants was similar or identical in its polypeptide composition, buoyant density, sedimentation rate, and antigenicity to material obtained from the plasma of hepatitis B carriers (11).

The recombined vaccinia virus containing hepatitis B surface antigen coding sequences was used to vaccinate rabbits with the production of typical vaccinia lesions in the skin and high titres of hepatitis B surface antibody in the circulation. Preliminary studies in chimpanzees indicated the feasibility of using a recombinant vaccinia virus. The vaccinated chimpanzees had a secondary antibody response when challenged intravenously with live hepatitis B of a heterologous subtype with a mild inapparent infection characterized by seroconversion to surface antibody and hepatitis B anti-core. Although the chimpanzees had little or no circulating surface antibody after vaccination when the recombinant vaccinia was growing in the skin, they were immunologically 'primed'. As a result, the chimpanzees had a brisk and sustained antibody response, presumably due to newly synthesized surface antigen after challenge with live hepatitis B virus. IgG surface antibody and the anti-\( \alpha \) responses were detected early, which is consistent with the anamnestic nature of the response.

However, at present there are no accepted laboratory markers of attenuation or of virulence of vaccinia virus for man, either in the host directly inoculated with the virus or after several passages in the same species. Alterations in the genome of vaccinia virus, which are concomitant with the selection of recombinants, may alter the virulence of the virus. It has been noted, however, that interruption of the thymidine kinase gene of the virus by insertion of foreign DNA reduced its virulence for mice. Nevertheless, changes in host range or tissue tropism of vaccinia viruses may occur as a result of their genetic modification and these could be caused by changes in the virus envelope as a result of the incorporation of gene products of the foreign viral genes inserted into the vaccinia virus.

The advantages of vaccinia virus recombinant as a vaccine include low cost, ease of administration by multiple pressure or by the scratch technique, vaccine stability, long shelf-life, and the possible use of polyvalent antigens. The known adverse reactions with vaccinia virus vaccines are well documented and their incidence and severity must be carefully weighed against the adverse reactions associated with existing vaccines which a new recombinant vaccine might replace. There are also reports of spread of current strains of
vaccinia virus to contacts and this may present difficulties. The possibility of using other recombinant viruses as vectors is under study.

CHEMICALLY SYNTHESIZED VACCINES

The development of chemically synthesized polypeptide vaccines offers many advantages in attaining the ultimate goal of producing chemically uniform, safe and cheap viral immunogens to replace many current vaccines which often contain large quantities of irrelevant microbial antigenic determinants, proteins and other material additional to the essential immunogen required for the induction of a protective antibody. The preparation of antibodies against viral proteins using fragments of chemically synthesized peptides mimicking viral amino acid sequences is now a possible and attractive alternative approach in immunoprophylaxis. The feasibility of synthetic vaccines was first demonstrated in studies with tobacco mosaic virus after the identification of an antigenic determinant and its amino acid sequences responsible for the immunogenic activity of the virus. Such amino acid moieties can be synthesized and, when coupled to a carrier protein, induced the production of neutralizing antibody in experimental animals. More recent studies include the induction of an immune response to an intact strain of human influenza A virus using a synthetic peptide analogous to a sequence of the haemagglutinin of H3N2 influenza A virus. The peptide was covalently linked to several macromolecular carriers, and the conjugate with tetanus toxoid was used for the immunization of rabbits and mice with the production of specific antibodies. These antibodies were protective against infection with a relatively low viral challenge in mice with the A/Texas/77 mouse-adapted influenza virus. The results indicate the potential of synthetic antigens for eliciting antiviral immunity against an important and common human pathogen. Another example is the use of peptides containing amino acid sequences from type 1 poliovirus VPI which were able to prime the immune system of rabbits for virus-neutralizing IgG antibody after a single inoculation of the intact virus. One of the peptides, corresponding to the region 93–103 in the sequence of VPI, directly induced the production of neutralizing antibodies. Veterinary vaccines have also been synthesized. For example, synthesized peptides corresponding to two regions of the major VPI polypeptide of foot-and-mouth disease virus coupled to a protein carrier produced high titres of specific neutralizing antibody in several animal species including cattle, and immunized guinea pigs were protected against virulent virus after receiving only a single dose of one peptide.

Similar approaches to the development of chemically synthesized hepatitis B vaccines were suggested a few years ago by several investigators (12–15). Successful mimicking of determinants of hepatitis B surface antigen using chemically synthesized peptides has been reported. In one laboratory, the sequence between residues 138 and 149 of the surface antigen which contained a putative dominant epitope in positions 141–146 was selected. The four cysteinyl residues were replaced by alpha-aminobutyric acid in order to prevent polymerization and other side-reactions common to sulphhydril-containing peptides, and glycine was added to the COOH terminus to aid radioimmunoassay directly on the solid phase used for synthesis. The prediction that the synthetic peptide contains a major epitope of hepatitis B surface antigen was confirmed, this sequence of amino acids eliciting antibodies directed to group-specific determinant a and the subdeterminant d, but not the epitope of subdeterminant y, or that of albumin added as a control. When the peptide was attached to aldehyde-stabilized human erythrocytes and injected into mice, it induced the formation of hepatitis B surface antibody with and without the use of Freund's complete adjuvant (16). It was previously reported that a synthetic oligopeptide of 13 amino acids in the sequence 136–147 represented a partial analogue of the a determinant of the surface antigen (14).
The result of a different approach using two predicted hydrophilic regions of the hepatitis B surface antigen molecule have also been described. Two cyclic peptides containing disulphide bonds in the region between the amino acid sequences 117–137 and 112–137 were incorporated into several adjuvants including Freund's complete adjuvant, alum, and multilamellar liposomes with and without muramyl dipeptide. Groups of BALB/c mice were immunized intraperitoneally with each of the preparations. Hepatitis B surface antibody was induced 7–14 days after inoculation in approximately 50% of the mice in each group, and in four or five out of six mice when the immunizing preparation of the 117–137 peptide was emulsified in Freund's complete adjuvant. On day 21, however, the peak levels of antibody decreased in most groups of mice. It should be noted that the antibody response was elicited in mice after a single injection without covalent linkage to a carrier protein, and the choice of adjuvant did not significantly affect the primary antibody response. It was also observed that the hepatitis B surface antibody response in mice inoculated with p25 solubilized with sodium dodecyl sulfate was similar to that observed with the synthetic peptides.

Other synthetic peptides which avoided the region 110 to 140 have also been examined because of published differences in nucleotide sequences in this region. The 13 peptides included sequences at the amino and carboxyl termini, peptides corresponding to hydrophilic domains of the protein, and peptides likely to contain exposed folds in the peptide chain indicated by proline-containing junctions between hydrophilic and hydrophobic regions. Seven out of the 13 free or protein carrier-linked synthetic peptides elicited an antipeptide response in rabbits. The carrier proteins which were used include 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 in size, reacted with the native antigen and also precipitated the M25000 and 30 000 polypeptides of hepatitis B surface antigen (18).

Later it was reported that a peptide with the sequence 110–137 stimulated a transitory antibody response to one of the surface antigen determinants, the y determinant, in inoculated chimpanzees. Repeated immunization of the chimpanzees failed to induce a stable surface antibody response. Nevertheless, intravenous challenge with hepatitis B virus with the ayw determinants, late in the immunization course, resulted in protection against infection in one chimpanzee, an attenuated infection without disease in another, and typical acute hepatitis in the third chimpanzee (19).

Thus, chemical synthesis of small peptides representing specific regions of the hepatitis B surface antigen is now feasible. Antisera to these peptides cross-react with the native surface antigen particles. Antibodies to a chemically synthesized nonapeptide, representing amino acids 139 to 147 of the 226 amino acids in the molecule, were shown by a different approach to bind the antibodies present in the hepatitis B immunoglobulin issued in the United Kingdom and in the sera of patients recovered from acute hepatitis B, monoclonal anti-a and antibodies in the serum of healthy laboratory personnel immunized with the plasma-derived hepatitis B vaccine. The affinity and level of hepatitis B surface antibody were determined using three different antigens, synthetic linear and cyclical amino acid sequences 139 to 147 and the p25–gp30 complex of the native surface antigen prepared by solubilization. Antibody levels, expressed as total antibody combining sites in fixed volumes of immune sera, increased throughout the course of immunization and correlated with the development of surface antibody as measured by radioimmunoassay (20, 21). The values of the total antibody combining sites were similar for both forms of the synthetic peptide, although higher affinity values were found with the cyclical structure.

The antibody response to hepatitis B surface antigen (anti-HBS), induced in 25 recipients of a recombinant yeast-derived hepatitis B vaccine, was compared with that induced in 25

* Vaccine from Merck, Sharp & Dohme, West Point, PA, USA.
recipients of a vaccine prepared from plasma-derived HBsAg. Anti-HBs antibody affinity and specificity were examined using antibody affinity assays with two different antigens, a complex of the major M, 25,000 polypeptide of HBsAg (p25), covalently linked to its glyco- sylated form (gp30) prepared from native purified HBsAg, and a cyclic synthetic peptide representing amino acid residues 139–147 of the major polypeptide of HBsAg and known to represent a major part of an α determinant. There was no difference in anti-HBs antibody affinity or Abt values (molar antigen binding sites of the antibody) measured with either antigen between the two vaccine groups. All subjects in both groups produced antibody which bound to the gp30–p25 complex antigen, whereas only 88% of recipients of the plasma-derived vaccine (compared with 96% of those receiving the yeast-derived vaccine) produced antibodies which bound to the cyclical synthetic peptide 139–147. The results were in agreement with the finding of similar levels of anti-HBs, as measured by commercial solid-phase radioimmunoassay, between the two vaccine groups after 3 doses of vaccine. Thus, there was no statistical difference in quantity, quality, or specificity of the anti-HBs response induced by the recombinant yeast hepatitis B vaccine and the vaccine prepared from plasma (27).

Enhancement of the immunogenicity of the pre-S region of hepatitis B surface antigen has been demonstrated in mice (9), using chemically synthetic amino acid residues. The immune response to the pre-S2 region was shown to be regulated by H-2 linked genes which are distinct from those which regulate the response to the S region. It was also demonstrated that immunization of a "non-responder" murine strain with particles which contain both S and pre-S2 can circumvent non-responsiveness. More recently, a protein sequence which mediates the attachment of hepatitis B virus to human hepatoma cells was identified. A synthetic peptide analogue, which is recognized by both cell receptors and viral antibodies, elicited antibodies reacting with the virus (22). Such a preparation may elicit protective antibodies by blocking the attachment of virus to the cells. Whether these results can be translated to man remains to be established.

However, designing proteins with the correct tertiary structure and with functional activities is exceedingly difficult, since it is not possible to predict the tertiary structure of a protein from its amino acid sequence alone. X-ray crystallography and interactive computer graphics are essential and available tools. The first step is to obtain a highly purified protein which can be crystallized to diffraction quality. The electron density of the crystal can then be calculated and since crystallography provides information on the non-hydrogen atoms in proteins, it is possible to build a scaffold model for fitting the known amino acid sequence into this structure. The model can then be refined by using sets of test coordinates to improve the density map. More recent techniques using synchrotron X-ray sources may allow the collection of structural information from protein in solution.

Two-dimensional proton nuclear magnetic resonance techniques, which assign peaks to specific protons in the protein are now available and the results can be converted to a set of coordinates for the molecule. An alternative approach is to develop comprehensive algorithms to simulate the mechanisms which determine protein structures coupled with establishing libraries of the protein data base. Another approach is to design synthetic proteins based on the natural folding patterns of the alpha helix configuration and the beta pleated sheet. However, as van Brunt (22) points out, there are no proven principles yet for de novo protein design, although it is equally clear that significant advances are being made in the construction of secondary structure patterns of proteins.

Nevertheless, the studies referred to above are in agreement with several reports which show that the modification of peptides based on secondary structure predictions and model building is now feasible (24). Peptides have been synthesized which retain biological function and appropriate secondary structure, even though they have a limited sequence homology with the natural peptide or are much smaller. For example, studies with hormones have shown that it is possible to stabilize a beta-turn by cyclization of the
molecule either by introducing a disulfide bond, or by designing a cyclic peptide.

Synthetic peptides may therefore be employed in due course as vaccines, although mixtures of more than one of the peptides may be required. Of the many questions that remain to be answered, the critical issues are whether antibodies induced by synthetic immunogens will be protective and whether the protective immunity will persist. Some of the carrier proteins and some of the adjuvants which had been linked to the synthetic molecules cannot be used in man, and it is therefore essential to find acceptable and safe material for covalent linkage, or, alternatively, to synthesize sequences that do not require linkage. The prospect of multivalent synthetic vaccines against a variety of microbial agents is now within reach.

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REFERENCES


