Cold Spring Harbor meeting

Building new vaccines

from Nigel Williams

HEPATITIS B virus may be one of the first viruses to come under large-scale attack from a synthetic subunit vaccine and, if an acceptable way of boosting the immune response can be found, many more viruses face a similar challenge. One unorthodox way of tackling this problem may be to use recombinant vaccinia virus, a descendant of Jenner's original protocol against smallpox, as a vector. With the focus on viruses, these were some of the points arising from the first of what promises to be an annual series of meetings to consider the modern approach of vaccines.*

The modern approach is dominated by efforts to produce synthetic subunit vaccines which, in the long term, may be able to overcome problems of contamination and storage often found with conventional vaccines. Also, viruses for which there are no conventional vaccines can be tackled. Because it appears that only a restricted number of sites on the viral surface are important targets for neutralizing antibodies, the aim is to produce synthetic analogues of these regions by expression of viral proteins in recombinant heterologous cells or by chemical synthesis of short peptides. If such subunits retain the configuration found normally on the viral surface and can induce a specific immune response, the hope is that they can form the basis of safe and effective vaccines.

The appeal of this approach was reflected by the number of groups reporting work on a wide range of animal and human viruses. While many have obtained antibodies to synthetic products that cross-react with the intact virus, this is only the first step — a potential vaccine must stimulate an antibody response strong enough to protect the animal from subsequent infection. It is clear that this is a major challenge.

Hepatitis B virus is of particular interest because no conventional mass vaccine exists against this virus — the cause of widespread and serious human disease — but a natural, highly immunogenic 'subunit' can be found in the serum of chronically infected individuals. This consists of particles containing aggregated viral surface antigen (HBsAg) molecules in both glycosylated and non-glycosylated forms. Purification of these particles from serum has formed the basis of a small-scale but successful vaccine by Merck, Sharp and Dohme. It is the proven potency of these particles (and their very limited supply and high cost) that has been the impetus for obtaining expression of

recombinant HBsAg DNA in cell vectors.

Valenzuela *et al.*¹ and subsequently others identified the part of the hepatitis B genome encoding the 226 amino acids of the HBsAg by cloning viral DNA in *Escherichia coli*. The use of *E. coli* for expression however, has been difficult; but using yeast cells as vectors, W.N. Burnette (Amgen) and D.E. Wampler (Merck, Sharp and Dohme) were among those reporting successful expression. The molecule is not glycosylated or secreted but forms aggregated particles in yeast cells which appear similar to those recovered from serum. The yeast derived particles seem as potent as the plasma derived particles in stimulating antibodies; indeed, Wampler reported that the Merck, Sharp and Dohme product, purified by immune affinity, is about to undergo clinical trials.

Since publication of the first nucleotide sequence of HBsAg, several groups have also produced synthetic peptides corresponding to specific regions of the molecule. So far the ability of such peptides to induce antibodies that cross-react with native HBsAg has been surprisingly good but the strength of the response has been variable and difficult to predict. However R.A. Lerner (Scripps Clinic) has encouraging results with a peptide of 38 amino acids in length spanning a relatively hydrophilic region of HBsAg. In a trial on three chimpanzees one was protected, one developed infection without disease and one developed acute infection when subsequently challenged with HBsAg. But a problem with this and many other peptides is that a carrier protein and adjuvant are needed to boost the immune response; the majority of these agents are unacceptable for use in man.

Encouraging for a different reason is the production (E. Wimmer, SUNY Stony Brook) of several peptides representing regions of the poliovirus (type 1) capsid protein, VP1³. Although only one peptide was able to induce neutralizing antibodies in rabbits, when attached to a carrier protein and injected with adjuvant, all the peptides were able to prime the immune response so that the animals responded vigorously to a subsequent challenge with whole virus vaccine. It may be that priming an immune response is sufficient to protect an individual against viruses with a long incubation time but again, the carrier/adjuvant system used puts human use some way off.

G.R. Dreesman (Baylor College, Houston) described one way that may overcome the need for a carrier protein; he has introduced a cross-linking disulphide bond into a peptide, 16 amino acids in length, corresponding to a region of

HBsAg². This cyclic form of the peptide gave a much better antibody response than the linear form.

A quite novel way of overcoming the need for carrier proteins and adjuvants may be the use of recombinant vaccinia virus as a live vector to present heterologous virus subunits. M. Mackett (NIH) described the production of vaccinia recombinants expressing HBsAg⁴. Vaccination of three chimpanzees with these recombinants provided total protection when the animals were subsequently challenged with HBV (G.L. Smith, NIH). E. Paoletti (New York State Department of Health) reported on other vaccinia recombinants expressing the coat glycoprotein (gD) of herpes simplex virus, that were able to protect mice from subsequent, normally lethal, challenge with live virus.

These are impressive results but the use of a live recombinant vector for subunit vaccination in man is clearly controversial. Among the many potential problems are the possibly different and more dangerous tropisms of recombinant viruses and reversion of recombinants to a more virulent form. However, the enormous worldwide experience (and undoubted success) in using vaccinia virus for smallpox vaccination and the impressive immunogenicity of the recombinants suggest that it is well worth tackling these potential problems.

Although the problem of boosting the response to subunits may be overcome, the problem of antigenic lability of certain viruses remains. Foot and mouth disease virus (FMDV) appears to have a dominant neutralizing site on the capsid protein, VP1. Synthetic peptides spanning the region have been produced and are able to induce protective antibody in rabbits against the corresponding FMDV strain⁵. But FMDV is extremely labile and exists in many variant forms. D.J. Rowlands (Animal Virus Research Institute, Pirbright) described three antigenic variants within a single isolate which differ at only two amino acids within the immunogenic site. Peptides representing the immunogenic site of the different variants induce good neutralizing antibody only against the corresponding variant. The antigenic lability of FMDV suggests that a synthetic vaccine may have to comprise several different peptides tailored each time to cope with local FMDV variants.

The power of the subunit approach which allows the viral surface to be explored and the nature of specific antibody-antigen interactions examined, justifies the optimism and the growing interest in these techniques. □

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*Modern Approaches to Vaccines: A meeting held at the Cold Spring Harbor Laboratory, New York, 31 August–4 September 1983.

1. Valenzuela, P. *et al.* *Nature* **280**, 815 (1979).
2. Dreesman, G.R. *et al.* *Nature* **295**, 158 (1982).
3. Emini, E.A. *et al.* *Nature* **304**, 669 (1983).
4. Smith, G.L. *et al.* *Nature* **302**, 490 (1983).
5. Bitte, J.L. *et al.* *Nature* **298**, 30 (1982).