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The Newer Vaccines

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The newer vaccines

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Vaccination is a powerful weapon in the control of animal diseases and many highly successful vaccines have been developed, particularly for virus diseases such as rinderpest, foot-and-mouth disease and Newcastle disease. Despite their extraordinary success, however, there are sufficient problems associated with their production and quality control to warrant a re-examination of the methods in current use. Dissection of virus particles into biologically active fragments has shown that their immunizing activity is usually carried on a single protein. With the identification of the genes coding for these individual proteins it is now possible to express these immunogens in both prokaryotic and eukaryotic cells. Moreover, the immunogenic sites of some of these proteins have been identified, synthesized in *E. coli* cells or by chemical methods and shown to possess immunizing activity.

It is too early to put a time-scale on the commercial availability of the new vaccines. However, the potential advantages of such products over conventional vaccines has led to considerable effort in this field of research and progress has been so rapid that new vaccines could be available within the next few years.

Introduction

Vaccination against viral and bacterial diseases has been one of the great success stories in human and veterinary medicine. In the field of human medicine, for example, the eradication of smallpox and the control of diseases such as yellow fever, poliomyelitis, diphtheria and tetanus by vaccination are well recognized. There are equally successful experiences in veterinary medicine with vaccines that control leptospirosis, rinderpest and foot-and-mouth disease in cattle, clostridial diseases in sheep, diarrhoea caused by enterotoxigenic *E. coli* in pigs and Newcastle and Marek's disease in poultry. To take one example, Western Europe is now essentially free from foot-and-mouth disease because of the highly successful vaccination policy that was started in the mid-1960s. This policy has also been of considerable benefit to neighbouring countries such as the United Kingdom where vaccination is not used. Thus there has been only one outbreak in the U.K., limited to one farm on the Isle of Wight, in the last 16 years.

Because the greatest advances with the newer vaccines have been made with viral diseases, this paper considers the newer viral vaccines and the principles underlying their development.

Virus vaccines in current use

The vaccines in use at present are prepared in one of two ways. First, in the preparation of inactivated vaccines, the virus is grown in large amounts in tissue culture cells and then inactivated with an agent such as β-propiolactone or an imine, with the use of conditions that retain the immunogenic activity of the protective antigen. Second, with attenuated vaccines the virulent virus obtained from the infected animal host is weakened by growing it in an

'unnatural host' or tissue culture cell so that it will grow in the natural host without causing disease. The immune mechanisms of the host are triggered in the same way as they would be by a natural infection. All the attenuated vaccines in use at present have been obtained by this empirical approach.

In view of their success, one might ask why there is any need to seek improved vaccines. Apart from the natural desire to improve current products there are, however, other and possibly more pressing reasons to seek better methods for preparing vaccines. For example, there are certain disadvantages in the preparation and use of the current vaccines. Thus with attenuated vaccines the possibility exists for contamination with other viruses as a result of their adventitious presence in the cells or medium used for production; this must be carefully monitored. Storage of the vaccines also presents a problem because it is essential that the infectivity is maintained. This is usually achieved by storage at refrigeration temperatures but such storage may be difficult to maintain, particularly in tropical countries. Not least, the occurrences of post-vaccination incidents caused by a reversion to virulence is not an insignificant feature of attenuated vaccines. Indeed, the occurrence of post-vaccinal encephalitis after vaccination against smallpox is the reason for the current debate on the possible use of *vaccinia* virus as a carrier for heterologous genes in vaccination programmes.

The manufacture and control of inactivated vaccines also present problems. There is the constant problem of ensuring that the product is innocuous. Moreover, the hazard to personnel and the immediate environment when large amounts of a virulent virus are being handled is clear. Storage of inactivated vaccines can also present problems similar to those encountered with attenuated vaccines if the immunogen is not a stable structural unit.

An additional problem is the inability in some instances either to grow the organism in sufficient amounts to enable an inactivated vaccine to be produced or in an attenuated form so that it is safe to use. It is in this area that recombinant DNA technology is likely to have the greatest impact, at least in the first phase of the development of new vaccines.

Structural basis for vaccination

Electron microscopy has revealed the great variety of virus structures. By application of the techniques designed for the purification and analysis of macromolecules it has been possible to purify most viruses and determine their chemical composition in fine detail. Moreover, most viruses can be dissected into subunits which possess biological activity. The most important fact to emerge from these studies with regard to vaccination is that the immunogenic activity is usually carried by a single protein. With the viruses causing, for example, influenza, rabies, or vesicular stomatitis it is the surface projection that carries the immunizing activity. This observation has been critical to the development of the new vaccines because it means that the coding information is carried by a single gene. This has allowed their ready manipulation in a variety of organisms.

Expression of protective antigens

There are two main methods for the expression of protective antigens. In the first, the DNA coding for the protein is inserted into prokaryotic or eukaryotic host cells and expressed as a fusion protein. The antigen is then extracted from the cells and purified, usually by the use of affinity columns charged with the appropriate antibody.

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Yields of foreign protein in amounts as high as 20% of *E. coli* cell protein have been obtained with the immunizing protein of foot-and-mouth disease virus (Kleid *et al.* 1981). Other proteins have also been expressed in acceptable yields. However, the products have been uniformly disappointing in the level of protective antibody response they elicit and it is apparent that much remains to be learned about ways of presenting the antigen to the host's immune system in a form that evokes a better response.

In the second method, the gene coding for the immunogenic protein is inserted in a heterologous virus that is attenuated so that it does not cause disease in the host. The new virus then expresses the immunogenic protein directly in the host. This approach to immunoprophylaxis has been investigated most extensively by using *vaccinia* virus as the attenuated vector, and very encouraging results have been obtained with several different heterologous genes (Mackett *et al.* 1982; Panicali & Paoletti 1982). While emphasis has been placed on its possible application to the vaccination of man, there seems to be no reason why *vaccinia* virus should not be used in the same way in cattle. Indeed this species could serve as an admirable testing ground for this new approach to vaccination.

Peptides as antigens

Fragments of proteins have also been found to elicit protective antibody. The major problem in the use of peptides is the identification of the sequence or sequences of amino acids constituting the antigenic site. The direct approach of isolating and testing fragments derived by cleavage of the protein with enzymes or chemical agents has yielded valuable results (Strohmaier *et al.* 1982). Predictions of potential antigenic sites have also been made from the three-dimensional structure of the protein, sequence analysis of viral antigens that have undergone antigenic change in nature (Bittle *et al.* 1982) and sequence analysis of viral mutants selected in the laboratory for their ability to grow in the presence of monoclonal neutralizing antibody (Minor *et al.* 1983). From a comparison of the sequences of the parent and mutant viruses, it has been possible in some instances to identify potential immunogenic sites.

Peptides can be synthesized by enzymic methods but the most frequently used is the solid-phase method devised by Merrifield (1965). In addition peptides can be expressed in *E. coli* cells as part of a fusion protein by coupling the DNA coding for the peptide with the gene coding, for example for *E. coli* β-galactosidase. The product can then be extracted from the mixture by use of an affinity column charged with anti-β-galactosidase antibody. Peptides corresponding to the antigenic site of foot-and-mouth disease virus which have been synthesized either by the solid-phase method (Bittle *et al.* 1982) or as part of a fusion protein (Yansura *et al.* 1983) have been shown to elicit neutralizing antibody and protect cattle against challenge infection.

Anti-idiotypes as antigens

Study of the structure of antibody molecules has revealed that the idiotype of an antibody molecule is located at or close to its antigenic binding site. It would be expected that an antibody against this site would mimic the configuration of the antigen that induced the original antibody response. Several examples have now been described that demonstrate the validity of this concept (Kennedy *et al.* 1984). Although we are far from realizing the potential of this approach at the practical level, the concept is valuable because it would be possible to immunize with a product free of infectivity, viral proteins and viral nucleic acids.

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New approaches to attenuated vaccines

A sufficient number of cases of disease occur after administration of even the most successful attenuated vaccines (such as those against poliomyelitis and smallpox) to warrant investigation of the chemical basis for the difference between virulent and attenuated strains. Advances in our knowledge of the structure of viruses have provided a basis for the construction of attenuated vaccines in a more rational manner.

With viruses that have a segmented genome, co-infection of cells with two strains allows reassortment of gene segments. The objective of this approach is to reassort one virus which has low virulence for the host (the donor strain) with a second which contains the gene coding for the desired protective antigen so that a new virus is produced which contains all the genes from the donor strain except that coding for the protective antigen, which is derived from the second virus. This approach is theoretically admirable but in general has proved disappointing in practice.

For viruses with an unsegmented genome a new approach to attenuation has recently emerged. Sequencing of the RNAs of the virulent and attenuated strains of poliovirus has shown that, in serotype 1 where there are many base differences between the two strains, there is no evidence of reversion to virulence of the attenuated vaccine. However with the corresponding viruses of serotype 3 where there are few differences in base sequence, many instances of reversion to virulence of the attenuated strain have been and are still being reported.

The new approach has been made possible by the demonstration by Racaniello & Baltimore (1981) that the DNA complementary to the single-stranded RNA of poliovirus is infectious. By ligating the appropriate fragments of the (complementary) cDNAs from different viruses it should be possible to produce new viruses that will not revert to virulence.

Prospects for the future

The rapid advances made during the last few years in the manipulation of nucleic acid molecules have led many to believe that products of practical and commercial value would emerge just as quickly. These hopes have not been realized because some crucial problems remain to be solved. It is clear that proteins identical in primary structure with the 'natural proteins' can be produced in large amounts in a variety of cells. However, presentation to the host of these proteins in the configuration they assume on the infecting virus still requires study. It has been known for several years that the activity of subunits derived from viruses is several orders of magnitude lower than that of the intact virus. Similar considerations apply to the genetically engineered products. Several attempts over many years to present subunits of viruses in the native configuration have met with limited success. However, recent work by Morein *et al.* (1984) with immunostimulating complexes (ISCOMS) has shown that the antibody response to the subunit of a virus can be enhanced so that it approaches that elicited by the intact virus particle. This work represents a major advance in the presentation of antigens and will be of considerable importance in the use of genetically engineered products.

The application of *vaccinia* virus as a carrier for heterologous genes coding for protein antigens will depend very much on 'socio-political' rather than scientific considerations. The debate is based on whether, in a world now free from smallpox, it is justifiable to use a virus that is

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known to cause side effects – some serious – in a small proportion of the recipients. The risk–benefit ratio varies from country to country and it seems logical that the medical authorities in each particular country should be allowed to reach their own conclusions. However, the application of this particular technique to animals would seem to be more acceptable. The advantages of using *vaccinia* virus recombinants in cattle, for example, are too obvious to require further comment.

The use of fragments of proteins, i.e. peptides, provides a more intellectual challenge (Lerner 1982). The extremely promising results obtained with short linear sequences of the immunizing protein of foot-and-mouth disease virus indicate that it may be possible to define immunogenic sites precisely and consequently to reach an understanding of their structural features at the atomic level. It has been found, for example, that the alteration of a single amino acid in the immunogenic fragment of the immunizing protein of foot-and-mouth disease virus is sufficient to alter its antigenic spectrum (Rowlands *et al.* 1983). This observation suggests that peptides could be designed which would overcome the problems encountered in vaccinating against viruses that exhibit antigenic variation. Clearly peptide molecules, with their smaller molecular weight, are more amenable to studies of this kind than the higher molecular mass proteins. The same considerations apply, in a much more general way, to the study of the structural conformations that are necessary to evoke a good immune response which is the basis of vaccination.

With the increasing interest in reaching a basic understanding of the structural features required for successful vaccination, progress in this field should be rapid. It is the author's view that the vaccines of the 1990s will be defined in strict chemical terms and may even be synthesized by chemical methods.

References

Bittle, J. L., Houghten, R. A., Alexander, H., Shinnick, T. M., Sutcliffe, J. G., Lerner, R. A., Rowlands, D. J. & Brown, F. 1982 Protection against foot-and-mouth disease by immunization with a chemically synthesised peptide predicted from the viral nucleotide sequence. *Nature, Lond.* **298**, 30–33.

Kennedy, R. C., Sparrow, J. T., Sanchez, Y., Melnick, J. L. & Dreesman, G. R. 1984 Enhancement of the immune response to a cyclic synthetic HBsAg peptide by prior injection of anti-idiotype antibodies. In *Modern approaches to vaccines. Cold Spring Harbor Symposium* (ed. R. M. Chanock & R. A. Lerner), pp. 427–430.

Kleid, D. G., Yansura, D., Small, B., Dowbenko, D., Moore, D. M., Grubman, M. J., McKercher, P. D., Morgan, D. O., Robertson, B. H. & Bachrach, H. L. 1981 Cloned viral protein vaccine for foot-and-mouth disease; Responses in cattle and swine. *Science, Wash.* **214**, 1125–1129.

Lerner, R. A. 1982 Tapping the immunological repertoire to produce antibodies of predetermined specificity. *Nature, Lond.* **299**, 592–596.

Mackett, M., Smith, G. L. & Moss, B. 1982 Vaccinia virus: a selectable eukaryotic cloning and expression vector. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7415–7419.

Merrifield, R. B. 1965 Automated synthesis of peptides. *Science, Wash.* **150**, 178–185.

Minor, P. D., Schild, G. C., Bootman, J., Evans, D. M. A., Ferguson, M., Reeve, P., Spitz, M., Stanway, G., Cann, A. J., Hauptmann, R., Clarke, L. D., Mountford, R. C. & Almond, J. W. 1983 Location and primary structure of a major antigenic site for poliovirus neutralisation. *Nature, Lond.* **301**, 674–679.

Morein, B., Sundquist, B., Hoglund, S., Dalsgaard, K. & Osterhaus, A. 1984 ISCOM, a cagelike immunostimulating complex of membrane proteins. In *Modern approaches to vaccines. Cold Spring Harbor Symposium* (ed. R. M. Chanock & R. A. Lerner), pp. 363–367.

Panicali, D. & Paoletti, E. 1982 Construction of poxviruses cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4927–4931.

Racaniello, V. R. & Baltimore, D. 1981 Cloned poliovirus complementary DNA is infectious in mammalian cells. *Science, Wash.* **21**, 916–919.

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Rowlands, D. J., Clarke, B. E., Carroll, A. R., Brown, F., Nicholson, B. H., Bittle, J. L., Houghton, R. A. & Lerner, R. A. 1983 Chemical basis of antigenic variation in foot-and-mouth disease virus. *Nature, Lond.* **306**, 694–697.

Strohmaier, K., Franze, R. & Adam, K-H. 1982 Localization and characterization of the antigenic portion of the foot-and-mouth disease virus immunizing protein. *J. gen. Virol.* **59**, 295–306.

Yansura, D. G., Dowbenko, D., Weddell, G. N., Hoatlin, M. E., Shire, S. J., Bock, L. A., Patzer, E. J., Kleid, D. G., Moore, D. M., Robertson, B. H., Grubman, M. J. & McKercher, P. D. 1983 Biosynthetic vaccine for foot-and-mouth disease. In *Advances in gene technology. Molecular genetics of plants and animals. Proc. 15th Miami Winter Symposium 20*, pp. 479–489. New York: Academic Press.

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