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Regular Review

Prospects for vaccines against HIV

A vaccine for routine use is at least five to 10 years away

The best and cheapest method of preventing illness is immunisation, and we urgently need effective vaccines against the human immunodeficiency virus (HIV). This enormous undertaking will be facilitated by the rapid advances in molecular biology and recombinant DNA technology, the understanding of immunological mechanisms, and the production and application of monoclonal antibodies so that the structure and location of important antigenic epitopes may be determined. Chemical synthesis of oligopeptides has been simplified, and computer programs, x ray crystallography, and other methods provide the tools for determining the three dimensional structure of proteins so that the structure and location of antigens or epitopes may be predicted. These techniques have already led to novel vaccines against hepatitis B virus and the virus causing foot and mouth disease.¹⁻⁵

HIV is placed taxonomically in the lentivirus group, a subfamily of the retroviruses. This virus encodes DNA synthesis directed by ribonucleic acid (RNA) (reverse transcription). The enzyme reverse transcriptase catalyses synthesis from the single stranded RNA of the virus of a double stranded DNA intermediate, the provirus, which becomes integrated into the host DNA and serves as a template for viral genomic and messenger RNA transcription by the host cell.

The development of vaccines against HIV is beset with difficulties because of the nature of the virus and because antibodies to the structural components of the virus (figure) are present in infected people, often at a high titre, although such humoral antibodies may be protective before the onset of infection; in addition, the surface lipoprotein envelope of the virus is subject to variation; and there is a lack of suitable small laboratory animals that are susceptible to this infection.

Inactivated virus vaccines

Traditionally vaccines against virus infections are based on inactivated or killed whole virus preparations—for example, the influenza vaccine and the Salk (killed) poliovirus vaccine—or its surface antigen component—for example, the plasma derived hepatitis B surface antigen vaccine. This approach, however, presents several problems. HIV will have to be grown in large amounts under conditions of high and secure containment. Complete inactivation of the virus without loss of antigenicity and immunogenicity must be assured—a difficult task without small animal models. The use of cell culture systems for this purpose raises the difficulty of

inserting viral nucleic acids into the host cell chromosome and encouraging the production of new and infectious viruses.⁶

Immunisation and vaccination after infection against two animal lentiviruses have led to severe clinical syndromes on subsequent exposure to live virus—for example, goats

Anatomy of HIV (after Dr H Gelderblom). The virus particle consists of a lipid bilayer in which the envelope protein is incorporated with the transmembrane pedicle (gp41) and the protruding knoblike structures (gp120). The core, which contains the RNA genome and reverse transcriptase, is surrounded by two membranes—an outer layer (p18) and an inner membrane (p24)

inoculated with inactivated preparations of the virus causing carpine arthritis and encephalitis develop a more severe disease when challenged with live virus. Caution will therefore be required in developing and testing such vaccines against HIV.⁷ An additional problem is that inactivated HIV vaccines may induce immunosuppression if by analogy with animal retroviruses this syndrome is induced by a viral component.

Attenuated live virus vaccines

Using live virus vaccines against HIV is precluded by many of the considerations that make inactivated virus vaccines difficult. The construction in vitro of a non-pathogenic virus by gene deletion seems an attractive option, but this approach has several disadvantages. Firstly, reverse transcription of the viral genome into copies of DNA and integration into the cellular genome may occur. Secondly, recombination with endogenous retroviral sequences may restore pathogenicity.

Thirdly, recombination with cellular proto-oncogenes may generate acute tumour virus. Fourthly, promotion of adjacent cellular oncogenes or their activation near the integration site may occur through viral enhancers.

Production of vaccines by recombinant DNA techniques

Techniques for identifying and analysing genes coding for biologically active substances and for transferring genetic material within and between organisms with expression of proteins under controlled conditions have been applied successfully to the development of vaccines—for example, against hepatitis B. In particular, recombinant DNA techniques have been used for expressing the *gag* and *env* genes of HIV in prokaryotic and eukaryotic cells. This approach has a considerable safety advantage as such preparations do not contain viral nucleic acid.

But will such subunit protein preparations elicit neutralising or protective antibodies? The extensive variation and genomic diversity among isolates of HIV from different geographical regions and different patients pose difficulties, although there are conserved as well as variable epitopes for neutralising antibodies. This genomic variability may enable the virus to escape the immune system of the host, infect different cell types, and adapt to a different environment.

Hybrid virus vaccines

Live vaccines using recombinant viruses have been constructed against hepatitis B virus and other viruses. Foreign viral DNA is introduced—for example, into vaccinia DNA—by constructing chimaeric genes. This is accomplished by homologous recombination in cells as the large size of the genome of vaccinia virus (198 000 base pairs) precludes the insertion of genes *in vitro*.⁸ The hepatitis B surface antigen made by vaccinia virus recombinants is similar or identical in its polypeptide composition, buoyant density, sedimentation rate, and antigenicity to the surface antigen obtained from the plasma of carriers of hepatitis B. The recombined vaccinia virus containing hepatitis B surface antigen coding sequences has been used to vaccinate rabbits and produces typical vaccinia lesions in the skin and high titres of hepatitis B surface antibody in the circulation.

Preliminary studies in the United States in chimpanzees showed the feasibility of using a recombinant vaccinia virus. The vaccinated chimpanzees produced antibodies when challenged intravenously with live hepatitis B virus of a heterologous subtype with only mild inapparent infection. 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 because of newly synthesised surface antigen after challenge with live hepatitis B virus.³ Recombinant vaccinia HIV *env* gene vectors have been constructed and are under evaluation in the United States and Zaire.

There are, however, no laboratory markers of attenuation or of virulence of vaccinia virus in man. Alterations in the genome of vaccinia virus and possible changes in the viral envelope that are concomitant with selecting recombinants may alter the virulence and indeed the tropism of the virus. Furthermore, the vaccinia vector may generally be used only once as antibody is induced to the vaccinia vector. Little is also known of how orthopox viruses are maintained in nature, and recombinant strains may become established in nature.

The advantages of recombinant vaccinia virus as a vaccine include low cost, easy administration by multiple pressure or

by the scratch technique, stability, long shelf life, and the possibility of using polyvalent antigens. The known adverse reactions with vaccinia virus vaccines are well described⁹; there are also reports of current strains of vaccinia virus spreading to contacts, which may present difficulties—particularly in populations where infection with HIV is common.¹ This problem may be overcome by including the gene for interleukin-2 in a live vaccinia virus vaccine. Other recombinant viruses have also been used as vectors.

Novel vaccines using hybrid virus particles

Using the envelope proteins of hepatitis B virus (hepatitis B surface antigen) in a particulate form by expressing the proteins in mammalian cells is an attractive option for producing vaccines against HIV. Variable lengths and sequences of another virus (poliomyelitis virus type I) are inserted in different regions of the S gene of hepatitis B virus. The envelope proteins carrying the surface antigen and the insert are assembled with cellular lipids in the cultured mammalian cells after transfection. The inserted polio neutralisation peptide was found to be exposed on the surface of the hybrid envelope particles and induced neutralising antibodies against poliovirus in mice immunised experimentally.⁴ Several other immunogenic peptides including HIV peptides could be incorporated into the surface of an organised multimolecular complex in secreted hybrid envelope particles by established cell lines licensed for vaccine production.

Other potentially attractive carrier vehicles for vaccines are the core particles of hepatitis B virus. The advantage of the core particles include their ability to induce antibody with about 100 times greater efficiency than surface antigen particles and an ability to augment the function of T helper cells. The feasibility of this approach was recently shown with synthetic and biosynthetic peptides of the virus causing foot and mouth disease after fusion to hepatitis B core antigen.⁵

Chemically synthesised vaccines

The development of chemically synthesised polypeptide vaccines offers many advantages in attaining the ultimate goal of producing chemically uniform, safe, and cheap viral immunogens. These might then replace many current vaccines, which often contain large quantities of irrelevant and sometimes undesirable microbial antigenic determinants, proteins, and other material. The preparation of antibodies against viral proteins by using fragments of chemically synthesised peptides mimicking viral amino acid sequences is now a possible alternative approach for immunoprophylaxis. Several amino acid moieties mimicking determinants of viral antigen have been synthesised and when coupled to a carrier protein have induced neutralising antibody in experimental animals.² Whether these results can be translated to man remains to be seen.

Designing proteins with the correct tertiary structure and functional activities is, however, difficult as it is not possible to predict the tertiary structure of a protein simply from its amino acid sequence. X-ray crystallography and interactive computer graphics are essential. The first step is to obtain a highly purified protein that can be crystallised to a quality capable of causing diffraction. The electron density of the crystal may then be calculated, and as 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 may then be refined by various techniques, and techniques using synchrotron X-ray sources may allow scientists to collect structural information from proteins in solution.

Other techniques are also used, and several reports have shown the feasibility of modifying peptides through secondary structure predictions and model building. Peptides have been synthesised that retain biological function and appropriate secondary structure even though they have different sequences from the natural peptides or are much smaller.

Synthetic HIV peptides may therefore eventually be used as vaccines, although mixtures of more than one of the peptides may be required. But will antibodies induced by synthetic immunogens be protective and will protective immunity persist? Some of the carrier proteins and some of the adjuvants that have 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 synthesise sequences that do not require linkage; using a non-toxic threonyl analogue of muramyl dipeptide in a pluronic polymer formulation seems promising.¹⁰ Another approach is synthesising sets of overlapping linear peptides extending the entire range of the *gag* and *env* gene coding sequences. Alternatively, the sequences for preparing HIV synthetic peptides may be based on the predictions of computer programs. Promising results have been reported, including the induction of neutralising antibodies.

Novel antigen presentation

Protein micelles are aggregates of polypeptides arranged so that the hydrophobic regions are sequestered inside the particles with the hydrophilic residues on the surface so that the resulting particulate forms are water soluble. Antigenic hepatitis B polypeptides have been extracted with the non-ionic detergent Triton X-100, the detergent is removed, and the membrane polypeptides reassociated into water soluble protein micelles.¹¹ The immunogenicity for each unit of weight of the hepatitis B viral surface polypeptide is greatly increased by breaking down the cross linked lipopolypeptide complex and converting these to monomeric polypeptide units that are then aggregated into the micellar form. This presentation of the antigen has been very effective in eliciting neutralising antibodies. Safety tests and protective efficacy studies of the micelle vaccine have been completed in non-human primates, and limited clinical trials have been done. More recently polypeptide micelles have also been prepared from hepatitis B surface antigen protein expressed by recombinant DNA technology in yeast and mammalian cells.

Immune stimulating complexes based on the use of Quil A and consisting of several glycosides have been used successfully for preparing several experimental animal vaccines (including a vaccine against the virus causing feline leukaemia). The viral antigens interact with the complexer to form artificial rosettes that are like cages and composed of both proteins and lipids. These structures seem to present the viral proteins to the immune system as a repetitive array and may therefore circumvent the poor immunogenicity of the antigens. It is not known yet whether protective immunity is induced by such preparations, but the use of proteins expressed by recombinant DNA techniques may overcome the problems of preparing and purifying subunit antigens from whole virus from cultured cells.

Cell fusion and syncytia

Cells infected with viruses may serve as an important means of infection given the propensity of HIV to induce cell fusion and the formation of syncytia so that little free virus remains in the tissue fluids. Activating T and B cells and producing antibody would not be enough to provide complete immunity

or to eliminate the virus. Secondly, the importance of macrophages and phagocytosis in establishing immunity have received little attention. Indeed, the transient neutralisation by antibody may be of little value if the virus-antibody complexes are simply phagocytosed by macrophages, particularly as macrophages seem susceptible to infection by HIV and may well be a primary target cell for the virus. Thus if most macrophages can be infected by virus-antibody complexes the prospects for a conventional vaccine are poor.¹²

Anti-idiotypic antibodies and cell mediated responses

In simple terms, an anti-idiotypic is an antibody to an antibody. A region of the antibody recognises the shape of a particular antigen, and this variable part of the antibody is the idiotypic. The idiotypic binds to the part of the antigen referred to as the epitope.

Antibodies may act as antigens; on injection into a second host the antibodies are treated as foreign, and the antibodies produced against them are referred to as anti-idiotypic antibodies as they recognise the idiotypic of the first antibody. The result is therefore an antibody cast in the shape of the original antigen.

Anti-idiotypic antibodies may neutralise HIV and closely related strains. One experimental approach is to produce antibodies against the CD4 receptor so that the binding site on the envelope glycoprotein of the virus, which is believed to be highly conserved, is blocked. The task is to produce human monoclonal antibodies directed against identified epitopes on HIV that are essential for infectivity. These antibodies could then be used as antigens to produce anti-idiotypes. This is technically an attainable goal and offers an important approach to immunisation against HIV.

Finally, little attention has been directed to cell mediated responses against HIV, but such studies are now underway.

Conclusion

Any candidate vaccine must be subjected to scrupulous evaluation. Premature unsubstantiated claims on efficacy may result in loss of confidence by the public and may have a serious impact on other immunisation programmes. Because of the lengthy incubation of five to eight years it is unlikely that an HIV vaccine will be generally available for at least another five to 10 years.

ARIE J ZUCKERMAN

Professor of Microbiology,
Department of Medical Microbiology and
World Health Organisation Collaborating
Centre for Reference and Research on Viral Hepatitis,
London School of Hygiene and Tropical Medicine,
London WC1E 7HT

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