molecular pharmaceutics

editorial

Methods of Delivery to Antigen-Presenting Cells: Development of New and Improved Vaccines

The Origin of Vaccination

The first attempts to prevent infectious disease were in 1000 A.D. in China, where contents of smallpox vesicles were used to inoculate individuals. Fatalities were uncommon in the individuals inoculated with the smallpox vesicles, compared with victims of natural smallpox infection. During the last decades of the 1700s, Edward Jenner noted that milkmaids, who had contracted cowpox, were protected against smallpox. In 1796, Jenner undertook protective tests in which he immunized an 8-year-old boy with cowpox and challenged him with smallpox; the boy was found to be immunized against smallpox. During the 19th Century, smallpox vaccination became increasingly popular, and eradication was accomplished in the decade of 1967−1977 (so far, this is the only communicable disease to be actively eradicated).

Attenuated Bacterial Vaccines. In the last quarter of the 19th Century, work by Louis Pasteur demonstrated that by attenuating a pathogen it was possible to administer the attenuated strain (the old cultures of bacteria which had undergone changes, without any loss of immunogenicity) as a vaccine. Pasteur applied this theory in an attempt to attenuate the anthrax-causing organism, *Bacillus anthrax*, and spectacular results were observed. The first attenuated bacterial vaccine (*Vibrio cholerae*) used in humans was administered in 1884 against cholera. Although the vaccine was given to ∼30000 individuals, most of which were partially protected against cholera, severe side effects were noted and the use of the vaccine was abandoned. Today, a vaccine for cholera is still a challenge. Another attenuated bacterial vaccine is the Bacille-Calmette-Guerin (BCG) vaccine against tuberculosis. In 1921, the first administration of BCG vaccine was given to a human, when a newborn infant's mother had died of tuberculosis and the infant was given BCG orally. No side effects were noted, and in the late 1920s, ∼50000 French infants had received the BCG vaccine. Despite the efficacy of the BCG vaccine, there is still no cure for tuberculosis.

Live Virus Vaccines. Pasteur and his colleagues noted that they were unable to culture the virus of rabies as they could a bacterium. Nevertheless, they found that the infectious agent resided in the spinal cord and brain of infected animals. Pasteur removed the spinal cord of infected rabbits and dried it for 2 weeks. The virus lost its virility yet retained its immunogenicity. An immunization schedule was set up with 42 dogs, and although the results were extraordinary, the vaccination procedure was quite controversial, in that deaths occurred that could not be explained, and Pasteur and his colleagues received much criticism.

Dead Organisms as Vaccines. The simplest way to destroy the ability of microbes to cause disease yet maintain their antigenicity is to prevent replication by killing the microbe in the appropriate manner. An example of a killed vaccine is against poliomyelitis (Salk), which involved treating the virus with formalin. This vaccine had an enormous impact on the incidence of the vaccine before being replaced by the live attenuated vaccine developed by Sabin. The Sabin vaccine was inexpensive and reliable, whereas the Salk (dead) vaccine was difficult to produce with inadequate quality. Inactivated (dead) vaccines which are easy to produce with higher potencies are now available but are impractical for a global scale even though they may be more effective than live attenuated vaccines. Measles and poliomyelitis vaccines have now been administered to infants and children as live attenuated.

Disasters. In spite of the progress in developing vaccines for a majority of diseases, there were a number of disasters in humans, in particular, in 1932 the Lubeck disaster for BCG vaccine, the Bundaberg tragedy in 1928 for the diphtheria vaccine, and the Cutter disaster in 1955 for the Salk-type vaccine. All these disasters were due to improper lab manufacturing and handling, and consequently, these incidents led to improved procedures and the safety of vaccines and led to regulatory measures that ensured proper laboratory conditions, training of personnel, and improved procedures in laboratories where vaccines were manufactured. With attempts to control more complex diseases and the need to improve vaccine safety, stability, efficacy, and cost, there is pressure for more precisely defined vaccines.

Development of Safer and Effective Vaccines

Public awareness of health and safety issues is now far greater than it was 60 or more years ago. Vaccines must now meet higher standards of safety and biochemical characterization than they did in the past. Some of the vaccines developed in the past would not even meet the minimum standards required today. Hence, we need to use new molecular and biological technologies that have become available in the past 20−25 years; these technologies are useful in the generation of new and improved vaccines. Advances in the fields of genetics, chemistry, and immunology are now included in the development of new and

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improved vaccines, in an attempt to move from traditional live virus vaccines to the theoretically safer but less immunogenic vaccines. Results of investigations into these areas, as they relate to the production of highly purified vaccines, are the identification and the isolation of the antigens responsible for protection. Ideally, the protective antigen is highly purified and highly immunogenic.

Genetic and Recombinant DNA Approaches. The application of genetic and recombinant DNA approaches to vaccine development has led to new possibilities of safer and more efficient vaccines. Recombinant DNA technology can be applied to antigen identification and isolation, and because we are able to clone and express all the antigens of an organism individually, this technology overcomes two major hurdles associated with traditional vaccines. First, before the recombinant DNA era it was difficult to obtain sufficient quantities of particular antigens, in a sufficiently pure form, to allow the appropriate testing. Recombinant DNA technology overcame this problem, and second, recombinant DNA technology has made the study of pathogenic organisms safer because single genes and their translation products are examined rather than the whole organism.

Chemistry/Protein Chemistry Approaches. Synthetic peptide chemistry has contributed significantly to vaccine development, where peptides could be made easily from 2 to >100 amino acids, and if the primary sequence of an antigen is known, then it is possible to identify T and B cell epitopes of an antigen to be used for immunization. In addition, the identification of antigens from cancer and infectious diseases has allowed their isolation and production by recombinant means, in high yields and highly purified. Numerous vaccine approaches use purified proteins for immunization.

Approaches to Enhancing Immunogenicity

Recombinant DNA and peptide/protein approaches for immunization have been designed to be safer and more efficient than traditional vaccines. Unfortunately, there are still many obstacles for their clinical use. The limited immunogenicity of many of these candidates has hindered their development as potential vaccines. Strategies to enhance the immunogenicity of candidate vaccines are therefore required.

Conventional Adjuvants. The most commonly used adjuvant in experimental animals is complete Freund's adjuvant. Although effective in inducing effective and long-lasting immune responses, complete Freund's adjuvant is not suitable for human use. The first registered human adjuvant (aluminum hydroxide or aluminum phosphate) is used in diphtheria, tetanus, and hepatitis B vaccines. Aluminum salt adjuvants are limited in their use, in that they preclude lyophilization or freezing, they are not effective with all antigens, and they do not stimulate cell-mediated immunity. Candidates for alternative adjuvants for vaccine development include the Syntex formulation, SAF-1 [containing squalene oil, an amino acid derivative of muramyl dipeptide (threonyl-MDP), and nonionic block copolymers]; Ribi formulation (containing mycobacterial cell walls and monophosphoryl lipid A); and the Cambridge Biotech saponin derivative, QS21 (also called Quil A). The development of new adjuvants, however, has been dominated by concerns about safety, since most of the adjuvants developed to date are too toxic for use in humans. They can cause tissue damage at the site of injection, granulomatous reactions, arthritis, and pyrogenicity.

Particulate Antigens. Liposomes (phospholipid-based vesicles) have been used extensively since 1970 as a system for the delivery of a drug to specific sites. In addition, liposomes have been shown to induce humoral and/or cell-mediated immune responses for liposome-entrapped bacterial, viral, and peptide antigens. Another approach has been to incorporate antigens into solid particles called ISCOMs (immunostimulatory complexes). ISCOMs have been demonstrated to induce both antibody- and cell-mediated immune responses to a number of antigens.

Cytokines. Recombinant cytokines or cytokines fused to peptides and/or proteins, such as IL-2, GM-CSF, IL-12, IFNγ, and IL-4, have been used to increase the immunogenicity of synthetic peptides and proteins.

Other Approaches. Several viral vectors have been tested in the development of vaccines, such as vaccinia virus, adenovirus, and avipox virus. Humoral and cellular immune responses have been shown to be generated. Microbial toxins (e.g., diphtheria toxin, *Bordetalla* pertussis toxin, and anthrax toxin), membrane-fusing agents (HIV Tat protein, measles virus fusion peptide, and *Drosophila* transcription factor *Antennapedia*), lipopeptides, heat shock proteins, and modifying MHC anchor amino acids of a peptide are among other approaches being used to enhance the immunogenicity of peptides and/or proteins.

Delivery of Proteins and Genes to Antigen-Presenting Cells

There has been much emphasis recently on the use of ex vivo-generated autologous dendritic cells loaded with antigen prior to re-administration into patients. Although this approach has resulted in strong immune responses and in some patients clinical responses, it is not a realistic method for worldwide use. There is the risk of contamination; it is a long process, and it is very expensive to undertake clinical trials. A more direct and less laborious strategy is to target proteins and DNA to antigen-presenting cells, in particular, dendritic cells. In this issue, recent methods used to deliver proteins and genes to antigen-presenting cells are discussed. Dr. Mark Saltzman and his colleagues describe delivery methods using synthetic materials, and Dr. David Kaplan and his colleagues describe the use of natural materials for antigen delivery. Dr. Blaine Pfeifer, the guest editor for this special issue, and his colleagues introduce us to numerous delivery methods utilizing bacterial vectors, and Dr. Margaret Liu and her colleagues detail approaches using viral vectors.

Finally, Dr. Magdalena Plebanski and her colleagues describe applications used for infectious diseases, in particular, the use of delivery of nanobeads into antigen-presenting cells, and I and my colleagues describe applications used for cancer in particular approaches to receptor-mediated targeting of antigens to antigen-presenting cells.

The future holds promise for new vaccines to prevent, control, and possibly eradicate diseases, including cancer and infectious diseases. The delivery methods described in this issue and our growing knowledge of the immune system should lead to the production of new and effective vaccines.

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