VACCINE ENGINEERING

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HISTORICAL PICTURE OF VACCINATION
A vaccine is any preparation intended to produce immunity to a disease by stimulating the production of antibodies. Vaccines include, for example, suspensions of killed or attenuated microorganisms, or products or derivatives of microorganisms.

The most common method of administering vaccines is by injection, but some are given by mouth or nasal spray.
VACCINATION BENEFITS ......

- **Vaccination** intends to provide individuals with immunological protection before an infection actually takes place. However, the immune system is very complex, and immunity against different infectious agents is based on fine-tuned balances between the various types of cells, signal substances and antibodies that make up the total immune system.
MODERN molecular biology, recombinant DNA technology and genetic engineering have opened the road to a number of alternative strategies for vaccine production,
STAGES OF VACCINE DEVELOPMENT

• Vaccine development proceeds through discovery, process engineering, toxicology and animal studies to human Phase I, II, and III trials. The process can take more than 10 years, depending on the disease.
STAGES OF REVIEW AND REGULATION FOR DEVELOPING VACCINES

- **Phase 1** - Safety, immunogenicity (prelim)
- **Phase 2** – Immunogenicity, Safety, Dose Ranging
- **Phase 3** – Efficacy, Safety, Immunogenicity
- **BLA** – Pre-clinical and clinical data to support approval, inspection
- **Phase 4** – Inspection, Safety, Efficacy, Lot Release
- **BLA-Supplement** (post-approval changes)
A GENETICALLY ENGINEERED VACCINE IS ...

- A preparation of direct manipulation of genes of weakened or killed pathogen, such as a bacterium or virus that upon administration stimulates antibody production or cellular immunity against the pathogen but is incapable of causing severe infection.
DNA VACCINES:

- They employ genes encoding proteins of pathogens rather than using the proteins themselves, a live replicating vector, or an attenuated version of the pathogen itself. They consist of a bacterial plasmid with a strong viral promoter, the gene of interest, and a polyadenylation / transcriptional termination sequence. The plasmid is grown in bacteria (e.g., coli), purified, dissolved in a saline solution, and then simply injected into the host. In present versions only very small amounts of antigens are produced within the vaccinated individual.
RECOMBINANT (DNA) VACCINES

- Made by isolation of DNA fragment(s) coding for the immunogenic(s) of an infectious agent/cancer cell, followed by the insertion of the fragment(s) into vector DNA molecules (i.e. plasmids or viruses) which can replicate and conduct protein-expression within bacterial, yeast, insect or mammalian cells. The immunogen(s) may then be completely purified by modern separation techniques. The vaccines tend to give good antibody responses, but weak T-cell activation.
NAKED DNA VACCINES:

• They are engineered from general genetic shuttle vectors and constructed to break species barriers. They may persist much longer in the environment than commonly believed. Upon release or escape to the wrong place at the wrong time. Horizontal gene transfer with unpredictable long- and short-term biological and ecological effects is a real hazard with such vaccines. There may be harmful effects due to random insertions of vaccine constructs into cellular genomes in target or non-target species.
LIVE VECTOR VACCINES

- They are produced by the insertion of the DNA fragment(s) coding for an immunogen(s) intended for vaccination into the genome of a non-dangerous virus or bacterium, the vector. The insertion is performed in such a way that the vector is still infectious live.
RNA VACCINES

- This involves the use of in vitro synthesised RNA (a single-stranded relative of DNA). RNA are different from DNA vaccines in that there is no risk of chromosomal integration of foreign genetic material.
EDIBLE VACCINES:

- These are produced by making transgenic, edible crop plants as the production and delivery systems for subunit vaccines. Little is known about the consequences of releasing such plants into the environment, but there are examples of transgenic plants that seriously alter their biological environment. A number of unpredicted and unwanted incidents have already taken place with genetically engineered plants.
MAKING DNA VACCINES

• The gene for an antigenic determinant of a pathogenic organism is inserted into a plasmid. This genetically engineered plasmid comprises the DNA vaccine which is then injected into the host. Within the host cells, the foreign gene can be expressed (transcribed and translated) from the plasmid DNA, and if sufficient amounts of the foreign protein are produced, they will elicit an immune response.
GENETIC ENGINEERING A GREAT TOOL IN DEVELOPING NEWER VACCINES

- It is possible, using genetic engineering, to introduce a gene coding for an immunogenic protein from one organism into the genome of another (such as vaccinia virus). The organism expressing a foreign gene is called a recombinant. Following injection into the subject, the recombinant organism will replicate and express sufficient amounts of the foreign protein to induce a specific immune response to the protein.
ADVANTAGES OF DNA VACCINES OVER OTHER TYPES OF VACCINES

• Cheaper and easier to produce
• Safer
• Can elicit antibody and cellular immune responses
• Stable at a broad range of temperature (no cold-chain requirement)
• Can be designed and produced by genetic engineering to have only the desired antigens or antigenic sequences (epitopes) in the vaccine
HEPATITIS B A LIFE THREATENING INFECTION

- Hepatitis B is one of the world's most common blood-borne viruses. It infects some 200,000 people per year in the U.S. alone. The virus is one hundred times more contagious than the HIV virus; like HIV, it is transmitted through blood and sexual contact and can be transmitted from mother to child at birth. The virus can exist in the bloodstream of a carrier for an entire lifetime. It is estimated that about 300 million people worldwide are carriers, of whom about 25% will die from cirrhosis or cancer of the liver brought on by the disease.
The world's first genetically engineered vaccine against a human disease--Hepatitis B--is considered one of biotechnology's greatest triumphs. The achievement stands on the shoulders of pioneering work by UW genetics professor Benjamin Hall and then-postdoctoral researcher Gustav Ammerer to develop genetic engineering techniques using yeast cultures to produce proteins of interest.
Many recombinant vaccines have been produced. These include live recombinant, vector, subunit, and DNA Vaccines.
Hall and Ammerer fused a segment of viral DNA specifying the surface antigen to the control elements of a yeast gene. When they transferred these hybrid genes into yeast cells, the resulting cultures produced Hepatitis B surface antigen. Serendipitously, these protein building blocks were found to clump together into the immunity-producing overcoat particles. With that observation, the key to a safe and effective vaccine was in hand.
HUMAN PAPILLOMA VIRUS INFECTION AND CONSEQUENCES

• Sexually transmitted HPV is the major cause of cervical cancer, the most common cause of cancer deaths among women in developing countries. About 5,000,000 cases occur each year, 80% of them in developing countries. Cervical cancer kills some 240,000 women annually.

• HPV types 16 and 18 cause around 70% of HPV cervical cancers globally, but the vaccines in development will not cover the 30% of cancers attributed to other HPV types. Because these other types are numerous and individually only contribute a small percentage, significantly expanding vaccine coverage against them may present technical challenges for manufacturers.
TWO TYPES OF GENETICALLY ENGINEERED VACCINES FOR HUMAN PAPILLOMA VIRUS PREVENTION

• Bivalent human papillomavirus vaccine (HPV2) licensed for use in females
• Either HPV2 or quadrivalent HPV vaccine (HPV4) used for females ages 19-26 years
• Quadrivalent human papillomavirus vaccine (HPV4) licensed for use in males
  • HPV4 may be administered to males aged 9 through 26 years to reduce their likelihood of acquiring genital warts.
GARDASIL
A GENETICALLY ENGINEERED VACCINE

- Gardasil, a genetically engineered vaccine, prevents cervical cancer by blocking infection with the two viruses that together cause about 70 percent of cervical cancers. HPV 16 and 18, both sexually transmitted viruses, are two of the 100-plus types of human papilloma virus.
Some genetically engineered viral vaccines consist of chimera viruses that combine aspects of two infective viral genomes. One example is the live *Flavivirus* chimera vaccine against West Nile virus (WNV) in horses (PreveNile), registered in the United States in 2006. The structural genes of the attenuated yellow fever YF-17D backbone virus have been replaced with structural genes of the related WNV. Chimera avian influenza virus vaccines have been produced on a backbone of an existing, attenuated Newcastle disease virus vaccine strain to protection against wild-type influenza virus as well as against Newcastle disease virus.
ILLUSTRATES PRODUCTION OF RECOMBINANT VACCINIA VIRUS AND ITS USE AS A RECOMBINANT VACCINE.

Figure 31-12 Brock Biology of Microorganisms 11/e © 2006 Pearson Prentice Hall, Inc.
NEW AND FUTURE VACCINES – POSSIBLE NEW DEVELOPMENTS

• New prophylactic and therapeutic vaccines will prevent and potentially cure a wide range of diseases by stimulating immune mechanisms. Advances in vaccinology will provide an efficient way to produce long-lasting protective immunity. Vaccines against non-infectious diseases are being trialled and will provide alternative treatments for conditions such as allergies, cancer, Alzheimer’s, diabetes, other autoimmune conditions and addictions. Advances in DNA Vaccines will allow rapid development of vaccines against potential agents for biological warfare. New delivery technology will provide easier routes of delivery, such as nasal, transcutaneous and oral, without compromising efficacy.
REVERSE GENETICS
(REVERSE VACCINOLOGY)

By knowing the genetic code of an organism it is possible to extrapolate the codes for all genes and therefore the proteins that they code for. Some of these will include peptides with antigenic properties. For example, this technique is being applied in the development of a meningococcal vaccine that would be protective against all strains. The search for a vaccine against all strains of this disease has been going for 40 years with little success. The main reason for failure has been the sequence and antigenic variability of the antigens which have been identified during this period using conventional methods. Recently the genome for Neisseria meningitides was sequenced.
In reverse vaccinology the genome sequence can be fed into a computer, which can predict the proteins located in the bacterium’s surface. Using this method for meningococcal there were 600 predicted proteins identified in silico (computational biology). Of these, 350 were successfully expressed in E.coli as fusion products, purified and used to immunise mice. There were 91 novel surface proteins identified, 29 of these induced bactericidal antibodies – a prediction of a good vaccine candidate. Prior to this, only 12-15 had been identified and only 4 –5 of these had bactericidal properties. Vaccine candidates are in clinical trials.
Reverse genetics is an important tool used to study the interaction of the virus with the host cell and the host immune response. Currently, reverse genetics is used to generate vaccines against the influenza virus.
ADVANTAGES IN REVERSE VACCINOLOGY

• Fast access to virtually every antigen Non-cultivable organisms can be approached – the whole organism does not need to be grown to produce antigens Non-abundant antigens can be identified – less common antigens which may be immunogenic can be identified. Antigens not expressed in vitro can be identified. On-structural proteins can be used

• This technology is currently also being applied to vaccines for pandemic influenza
DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. Nucleic acid vaccines are still experimental, and have been applied to a number of viral, bacterial and parasitic models of disease, as well as to several tumour models. DNA vaccines have a number of advantages over conventional vaccines, including the ability to induce a wider range of immune response types.
Gene vaccines may be relatively new, but they're the logical outgrowth of two familiar strands of medical science. First is the 200-year-old practice of vaccination, in which the body is infected with a weakened form of a disease that prepares the immune system for a future encounter with the real thing.
Bananas have potential to become the world's first edible vaccine due to Agrobacterium. An edible vaccine doesn't need sterile syringes, costly refrigeration, or multiple injections. According to the World Health Organization (WHO), more than 2 million children die worldwide each year from diarrhea that can be prevented easily with vaccines.
There are vaccines in the pipeline for bacterial diseases like anthrax, viral pathogens like Ebola, and inheritable diseases, including several forms of cancer and Alzheimer's. An Alzheimer's vaccine, for example, would stimulate the immune system to attack the protein deposits in the brain that are caused by the degenerative disorder.
The most promising areas of Alzheimer’s disease research involves vaccine-based therapies which stimulate the body to produce antibodies to amyloid-beta protein and remove it from the brain.
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