VIROLOGY - CHAPTER EIGHT

VACCINES: PAST SUCCESSES AND FUTURE PROSPECTS

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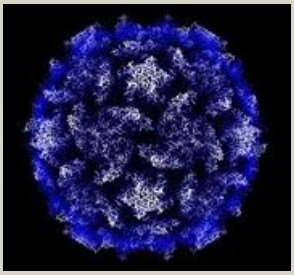


Polio Virus (From the Hogle Lab at Harvard, URL unknown)

Many of the images in the smallpox part of this file come from Fenner, Henderson, Arita et al. *Smallpox and its Eradication*. 1988 Geneva, World Health Organization and were assembled by Laura Gregorio in her essay *The Smallpox Legacy*, Pharos. Fall 1996

INTRODUCTION

What is a vaccine?

Vaccines are harmless agents, perceived as enemies. They are molecules, usually but not necessarily proteins, that elicit an immune response, thereby providing protective immunity against a potential pathogen. While the pathogen can be a bacterium or even a eukaryotic protozoan, most successful vaccines have been raised against viruses and here we shall deal with anti-viral vaccines. Vaccines may consist of a purified protein or a complex of molecules or even a whole bacterium or virus.

Immunity to a virus normally depends on the development of an immune response to antigens on the surface of a virally infected cell or on the surface of the virus particle itself. Immune responses to internal antigens often play little role in immunity. Thus, in influenza pandemics, a novel surface glycoprotein acquired as a result of antigenic shift characterizes the new virus strain against which the population has little or no immunity. This new strain of influenza virus may, nevertheless, contain internal proteins that have been in previous influenza strains. Surface glycoproteins are often referred to as protective antigens. To make a successful vaccine against a virus, the nature of these surface antigens must be known unless the empirical approach of yesteryear is to be followed. It should be noted, however, that a virally-infected cell displays fragments of internal virus antigens on its surface and these can elicit a cytotoxic T cell response that acts against the infected cell.

There may be more than one surface glycoprotein on a virus and one of these may be more important in the protective immune response than the others; this antigen must be identified for a logical vaccine that blocks infectivity. For example, influenza virus has a neuraminidase and a hemagglutinin on the surface of the virus particle. It is the hemagglutinin that provokes neutralizing immunity because it is the protein that attaches the virus to a cell surface receptor and the neutralizing antibody interferes with virus binding to the cell.

In addition to blocking cell to virus attachment, other factors can be important in the neutralization of viruses; for example, complement can lyze enveloped virions after opsonization by anti-viral antibodies.

In this chapter, we deal mostly with anti-viral vaccines, although there are also successful anti-bacterial vaccines (see here).

Major sites of viral infection

In order to develop a successful vaccine, certain characteristics of the viral infection must be known. One of these is the site at which the virus enters the body. Three major sites may be defined:

- Infection via mucosal surfaces of the respiratory tract and gastro-intestinal tract.
    - Virus families in this group are: rhinoviruses; myxoviruses; coronaviruses; parainfluenzaviruses; respiratory syncytial viruses; rotaviruses

WEB RESOURCES

Common Misconceptions about Vaccination and how to respond to them

- Infection via mucosal surfaces followed by spread systemically via the blood and/or neurones to target organs.

  Virus families in this group are: picornaviruses; measles virus; mumps virus; herpes simplex virus; varicella virus; hepatitis A and B viruses

- Infection via needles or insect bites, followed by spread to target organs:

  Virus families in this group are hepatitis B virus; alphaviruses; flaviviruses; bunyaviruses

IgA-mediated local immunity is very important in first two categories. There is little point in having a good neutralizing humoral antibody in the circulation when the virus replicates, for example, in the upper respiratory tract. Clearly, here secreted antibodies are important.

Thus, we need to know:

- The viral antigen(s) that elicit neutralizing antibody
- The cell surface antigen(s) that elicit neutralizing antibody
- The site of replication of the virus

Types of vaccines

There are four basic types of vaccine in use today

- Killed vaccines: These are preparations of the normal (wild type) infectious, pathogenic virus that has been rendered non-pathogenic, usually by chemical treatment such as with formalin that cross-links viral proteins.
- Attenuated vaccines: These are live virus particles that grow in the vaccine recipient but do not cause disease because the vaccine virus has been altered (mutated) to a non-pathogenic form; for example, its tropism has been altered so that it no longer grows at a site that can cause disease.
- Sub-unit vaccines: These are purified components of the virus, such as a surface antigen.
- DNA vaccines: These are usually harmless viruses into which a gene for a (supposedly) protective antigen has been spliced. The protective antigen is then made in the vaccine recipient to elicit an immune response

Problems in vaccine development

There are many problems inherent in developing a good protective anti-viral vaccine. Among these are:

- Different types of virus may cause similar diseases -- e.g. the common cold. As a result, a single vaccine will not be possible against such a disease
- Antigenic drift and shift -- This is especially true of RNA viruses and those with segmented genomes
- Large animal reservoirs. If these occur, re-infection after elimination from the human population may occur
- Integration of viral DNA. Vaccines will not work on latent virions unless they express antigens on the cell surface. In addition, if the vaccine virus integrates into host cell chromosomes, it may cause problems (This is, for example, a problem with the possible use of anti-HIV vaccines based on attenuated virus strains)
- Transmission from cell to cell via syncytia - This is a problem for potential AIDS vaccines since the virus may spread from cell to cell without the virus entering the circulation.
- Recombination and mutation of the vaccine virus in an attenuated vaccine.

Despite these problems, anti-viral vaccines have, in some cases, been spectacularly successful (see addendum) leading in one case (smallpox) to the elimination of the disease from the human population. The smallpox vaccine is an example of an attenuated vaccine, although not of the original pathogenic smallpox virus. Another successful vaccine is the polio vaccine which may lead to the elimination of this disease from the human population soon. This vaccine comes in two forms. The Salk vaccine is a killed vaccine, while that

developed by Albert Sabin is a live attenuated vaccine. Polio is presently restricted to parts of Africa (Nigeria) and south Asia (Pakistan and Afghanistan).

Although smallpox is the only human disease that has been eradicated using vaccination, it is likely that one animal disease has also been eradicated. Rinderpest (cattle plague or steppe murrain) is a viral disease with high mortality that infects cattle and other ruminants and causes fever, diarrhea and lymphoid necrosis. It is a member of the measles family (Family: Paramyxoviridae; Genus: Morbillivirus) and was eradicated using a live attenuated vaccine. In 2010, the Food and Agriculture Organization reported that no case of rinderpest had been diagnosed for nine years. It is thus the only disease of agricultural livestock that has been successfully eradicated.

PAST SUCCESSES

Smallpox (Variola)

Smallpox is a devastating and disfiguring disease that is highly infectious. It is caused by variola virus (also known as smallpox virus), a member of the *orthopoxvirdae* (figure 2A). The disease of smallpox has been known for thousands of years and probably originated in Asia. It spread westwards into the middle east and among its victims was Pharaoh Rameses V (figure 2B). The disease may have reached Europe with the crusaders. Smallpox was introduced to the New World by European colonists and caused devastating epidemics in the indigenous population who had no natural immunity. Indeed, some early colonists used smallpox as a biological weapon again the original inhabitants of North and South America.

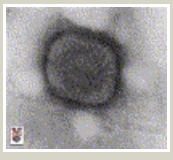
Smallpox is characterized by numerous pustules containing infectious virus all over the body (figure 2 C and D). The fatality rate is more than one quarter of infected patients infected by the most serious form caused by *Variola major*. Another form of smallpox caused by *Variola minor* has a much lower fatality rate (up to 5%).

The first attempts to control smallpox occurred in the 10th century and used variolation (so called because small pox virus is *Variola*). In variolation (figure 2E), material (scabs) was obtained from the pustules of an infected person who did not die of the disease. This person, therefore, had a milder form of smallpox as a result of a naturally occurring variant. This material was used to infect another person who usually also got a milder disease. If the person did not die, there was lifelong immunity. Another reason for the success of variolation was that virus in the scabs was less virulent because it had been partially desiccated and was complexed with and inactivated by antibodies from the donor.

The fatality rate of variolation was about 1 - 2% and so it was still a dangerous procedure. This technique was used in Pakistan, Ethiopia and Afghanistan until 1970. Variolation was widespread in England in 1700s where it was introduced by the wife of the British Ambassador to Turkey, Mary Wortley Montague (figure 1b).

Figure 1b
Mary Wortley Montague

Figure 2

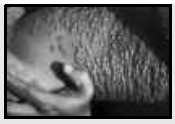


A. Smallpox virus
Copyright
1994 Veterinary Sciences
Division Queen's University Belfast

B. The mummified head of Ramses V (died 1157 BCE) with rash that is probably the result of smallpox

C. Infant with smallpox

E. Powdered smallpox scabs were inhaled to protect against smallpox in Chinese medicine



D. Smallpox lesions on skin of trunk. Photo taken in Bangladesh. CDC/James Hicks

Figure 3

A. Edward Jenner

B. Dr Jenner about to vaccinate a child

C. Blossom the cow

D. The last known person in the world to have a natural case of smallpox. Variola minor in 23-year-old Ali Maow Maalin, Merka, Somalia
CDC

In 1796, Edward Jenner (figure 3A), who was at the time experimenting with variolation, discovered vaccination using vaccinia virus, the agent of cowpox (*vacca* is the Latin for cow).

Jenner was a physician living in rural Gloucestershire in the west of England and it was widely known at that time that people who contracted cowpox (such as dairy maids) appeared to gain protective immunity against the much more virulent smallpox. Jenner vaccinated a Mr Phipps (who worked for him) and own son (figure 3B) with cowpox from a cow called Blossom (figure 3C), and then challenged them with virulent smallpox. Both vaccinees were, fortunately, protected. Jenner's original virus is not the vaccinia that was used in smallpox vaccinations until recently. The vaccine virus may have arisen as recombinant from cowpox or horse pox. For a long time the vaccine virus was maintained in horses or buffalo.

The last case of natural smallpox in the U.K. occurred in the 1930s; the last in the U.S.A. was in the 1940s. The last natural case in the world was in Somalia and occurred in October 1977 (figure 3D). Although the virus had been eliminated in the wild, smallpox was retained in the laboratory and as a result of a laboratory accident there was subsequently a fatal case of smallpox in England. Worldwide stocks were reduced to laboratories in the United States and the Soviet Union. It is not known whether infectious virus from the Russian laboratory was distributed after the dissolution of the Soviet Union.

The eradication of smallpox has been one of the great triumphs of public health. There are several reasons for this:

- There is no animal reservoir for variola, only humans are infected by this virus
- Once a person has been infected by the virus, there is lifelong immunity, although this may not be the case with people immunized using the vaccine strain
- Subclinical cases are rare and so an infected person can be identified and isolated
- Infectivity does not precede overt symptoms, that is there is no prodromal phase
- There is only one Variola serotype and so the vaccine is effective against all virus strains

- The vaccine is very effective
- There has been a major commitment by the World Health Organization and governments to smallpox erradication.


Figure 4. Louis Pasteur

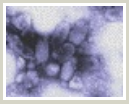


Figure 5. Rabies virus

Rabies

Almost a century after Jenner's pioneering work on smallpox, in 1885 Louis Pasteur (figure 4) and Emile Roux developed the first vaccine against rabies (figure 5) (*rabhas*, Sanskrit: to do violence). Pasteur discovered that if he took spinal cord material from a dead rabid rabbit and kept it for a period of 15 days in a dry atmosphere (a flask containing potassium hydroxide) and then injected it into a dog, the latter did not get rabies. He developed a protocol in which he carried out the same procedure with spinal cord tissue that had been kept in a dry atmosphere for less and less time (each was separated by an interval of two days), until he finally injected spinal tissue containing virulent virus (only a day or two in the flask). He found that the dog was then immune to rabies. Pasteur successfully treated a boy (Joseph Meister) bitten by a rabid dog sixty hours earlier with this protocol in which he used successively more virulent virus. In fact, according to Pasteur, the rabies in the final inoculation was more virulent than that of ordinary canine rabies. Fortunately, Mr Meister survived both the initial bites and the virulent virus!

Current anti-rabies vaccines are not prepared in the way that Pasteur used. Human Diploid Cell Vaccine (HDCV) is made in tissue culture using normal human WI-38 fibroblasts. The rabies virus is purified by passage through a filter and inactivated by beta-propriolactone. This inactivated virus vaccine is used almost exclusively in the developed world for pre- and post-vaccination of rabies. Purified Chick Embryo Vaccine (PCEC) is also purified virulent virus. It is made by ultracentrifugation and also inactivated by beta-propriolactone. HDCV gives a high titer of neutralizing antibody after 10 days. When used properly, it can confer 100% protection.

There is also a live attenuated vaccine (Flury strain) that is grown in chick embryos and is for use in animals only.

A recombinant anti-rabies vaccine (VRG, Raboral) has been made by inserting the gene for the surface glycoprotein of rabies into vaccinia virus, the virus used in smallpox vaccinations. The recombined virus appears safe for humans but is used for treating wild animals since (because it is a live virus) it can give herd immunity. The vaccine virus is stable to elevated temperatures and can be delivered orally. It is therefore fed to animals in food baits. Raboral V-RGŪ is approved for immunization of raccoons and coyotes, two of the most significant wildlife carriers of rabies in North America.

CASE REPORT

Poliovirus Infections in Four Unvaccinated Children --- Minnesota, August--October 2005

Poliomyelitis

In western countries, wild type polio is no longer a problem but it is still endemic in some less developed countries such as Nigeria, Pakistan and Afghanistan (figures 6). However, wild poliovirus has been imported into some countries that have stopped transmission of indigenous virus and outbreaks can result from these importations. A number of countries continue to be affected by such outbreaks. Most of these are in the “wild poliovirus importation belt” – a number of countries stretching from west Africa to central Africa and the Horn of Africa (figure 6).

Until the 1950s, when anti-polio vaccination became routine, summer outbreaks of polio were common in western countries, often spread via the oral-fecal route while using swimming pools. These outbreaks led to widespread paralytic polio that necessitated help in breathing and the use of "iron lungs" (figure 7).

Anti-polio vaccines

There are two types of polio vaccine, both of which were developed in the 1950s. The first, developed by Jonas Salk, is a formalin-killed preparation of normal wild type polio virus. This is grown in monkey kidney cells and the vaccine is given by injection. It elicits good humoral (IgG) immunity and prevents transport of the virus to the neurons where it would otherwise cause paralytic polio. This vaccine is the only one used in some Scandinavian countries where it completely wiped out the disease.

A second vaccine was developed by Albert Sabin. This is a live attenuated vaccine that was produced empirically by serial passage of the virus in cell culture. This resulted in the selection of a mutated virus that grew well in culture and, indeed, in the human gut where the wild type virus grows. It cannot, however, migrate to the neurones. It replicates a normal infection since the virus actually grows in the vaccinee and it elicits both humoral and cell-mediated immunity. It is given orally, a route that is taken by the virus in a normal infection since the virus is passed from human to human by the oral-fecal route. This became the preferred vaccine in the United States, United Kingdom and many other countries because of it ease of administration (often on a sugar lump), the fact that the vaccine virus replicates in the gut, and only one administration is needed to get good immunity (though repeated administration was usually used). In addition, the immunity that results from the Sabin vaccine lasts much longer that that by the Salk vaccine, making fewer boosters necessary. Since it elicits mucosal immunity (IgA) in the gut (figure 10), the Sabin vaccine has the potential to wipe out wild type virus whereas the Salk vaccine only stops the wild type virus getting to the neurons.

The attenuated Sabin vaccine, however, came with a problem: back mutation. This may result from recombination between wild type virus and the vaccine strain. Virulent virus is frequently isolated from recipients of the Sabin vaccine. The residual cases in countries that use the attenuated live virus vaccine (about 8 per year in the US until recently; figure 9a, b and c) resulted from mutation of the vaccine strain to virulence. About half of these cases were in vaccinees and half in contacts of vaccinees. Paralytic polio arises in 1 in 100 cases of infection by wild type virus and 1 in 2.4 million initial vaccinations as a result of back reversion of the vaccine to virulence. This was deemed acceptable as the use of the attenuated virus means that the vaccine strain of the virus still replicates in the body and gives gut immunity via IgA.

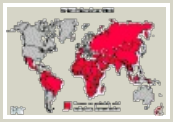
The vaccinee who has received killed Salk vaccine still allows wild type virus to replicate in his/her gastro-intestinal tract, since the major immune response to the injected killed vaccine is circulatory IgG (figure 10). As noted above, this vaccine is protective against paralytic polio since, although the wild type virus can still replicate in the vaccinee's gut, it cannot move to the nervous system where the symptoms of polio are manifested. Thus, wild type virus is unlikely to die out in populations who have received only the killed vaccine since it would be shed in the feces. It should be noted, however, that studies in The Netherlands during a polio outbreak in 1992 (among people who had refused the vaccine) showed that immunity produced by the Salk vaccine did prevent circulation of wild type virus in the general population.

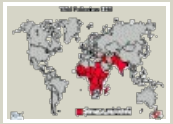
An additional problem of using a live attenuated vaccine is that preparations may contain other pathogens from the cells on which the virus was grown. This was certainly a problem initially because the monkey cells used to produce the polio vaccine were infected with simian virus 40 (SV40) and this was also in the vaccine. SV40 is a polyoma virus and has the potential to cause cancer. It appears, however, not to have caused problems in vaccinees who inadvertently received it. There have been some allegations that the original attenuated polio vaccine used in Africa may have been contaminated with human immunodeficiency virus (HIV). This has been found not to be the case. Of course, there can also be similar problems with the killed vaccine if it is improperly inactivated. This has also occurred.

Current recommendations concerning polio vaccines

Once the only polio cases in the US were vaccine-associated, the

Figure 6 - Polio Statistics Comparison of worldwide incidence in 1988, 1998, 2004 and 2013


Polio - 1988 WHO


Polio - 1998 WHO


Polio - 2004 WHO


Polio - 2013 polioeradication.org

previous policy of solely using the Sabin vaccine was reevaluated. At first, both vaccines were recommended with the killed vaccine first and then the attenuated vaccine. The killed vaccine would stop the revertants of the live vaccine giving trouble by moving to the nervous system. Thus, in 1997 the following protocol was recommended:

> *To reduce the vaccine associated cases (8 to 10 per year), the CDC Advisory Committee on Immunization Practices (ACIP) has recommended (January 1997) a regimen of two doses of the injectable killed (inactivated: Salk) vaccine followed by two doses of the oral attenuated vaccine on a schedule of 2 months of age (inactivated), 4 months (inactivated), 12-18 months (oral) and 4-6 years (oral). Currently four doses of the oral vaccine are typically administered in the first two years of life. It is thought that the new schedule will eliminate most of the cases of vaccine-associated disease. This regimen has already been adopted by several European countries and some of Canada.*

The regimen of polio vaccination was subsequently amended again in 2000:

> *To eliminate the risk for Vaccine-Associated Paralytic Poliomyelitis, the ACIP recommended an all-inactivated poliovirus vaccine (IPV) schedule for routine childhood polio vaccination in the United States. As of January 1, 2000, all children should receive four doses of IPV at ages 2 months, 4 months, 6-18 months, and 4-6 years.*

Figure 7 (right) - Poliomyelitis


A. Child in iron lung WHO


B. Iron lung ward


C. Child with polio sequelae WHO


D. Child with polio sequelae WHO


E. Victims of polio WHO

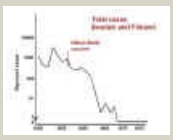


Figure 8
Total reported cases in Sweden and Finland (1950-76) which use the killed vaccine only developed by Jonas Salk. The Salk

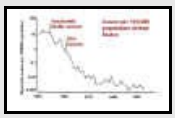


Figure 9a
Reported (cases per 100,000 population) cases of paralytic poliomyelitis in the United States, 1951-1992, which initially used the killed Salk vaccine. This was subsequently replaced by the live attenuated oral vaccine developed by Albert Sabin. The Sabin vaccine is swallowed. It is often given on a sugar lump

vaccine is injected

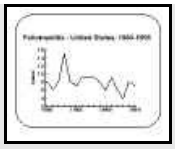


Figure 9b
Poliomyelitis in the US 1980-1995 CDC

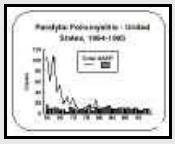


Figure 9c
Vaccine-associated paralytic polio - VAPP in US 1964-1995 CDC

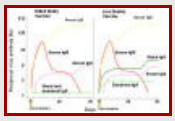


Figure 10
Secretory antibody (nasal and gut IgA) and serum antibody (serum IgG, IgM and IgA) in response to killed polio vaccine (left) administered by intramuscular injection and to live attenuated polio vaccine (right) administered orally

Rotavirus disease: An initial problem but later success

Rotaviruses are found worldwide, causing major gastroenteritis and diarrhea-associated hospitalization and over half a million deaths per year in children under five years of age. According to WHO, five countries (India, Nigeria, the Democratic Republic of the Congo, Ethiopia and Pakistan) accounted for more than half of all rotavirus disease deaths under age five in 2008. Symptoms include: fever, vomiting, diarrhea and abdominal pain. Seroprevalence studies show that antibody is present in most infants by age 3 years.

Prior to the introduction in the United States of widespread vaccination in 2006, there were up to three million cases of rotavirus infection per year. In about 1 to 2.5% of cases, there was severe dehydration. This resulted in 20 to 60 deaths of children under five each year. In addition, there were 50,000 to 70,000 hospitalizations and over 500,000 visits to doctors' offices per year.

Since the introduction of vaccination there has been a drop in rotavirus-related hospitalizations by up to 86 percent. It is likely that vaccination has also protected non-vaccinated infants by limiting circulating infection. Deaths have also been markedly reduced. In 2008, there were an estimated 14 deaths from rotavirus disease in the United States and fewer than 10 in the United Kingdom compared to 98,621 in India.

Rotashield and intussusception

Reassortant vaccines are created by genetic reassortment in which non-human rotavirus strains express the antigens of human rotaviruses on their surface. The non-human strains replicate but do not cause disease and are of low pathogenicity in humans. A live, tetravalent rhesus-human reassortant vaccine (Rotashield - Wyeth Laboratories) was first licensed for use in infants in August 1998. It contained human G types 1, 2, 4, and simian G type 3. However, post-licensure surveillance indicated a possible relationship between the occurrence of intussusception 3 to 20 days after the vaccine was administered, especially the first dose (15 cases/1.5 million doses were reported). Intussusception is a rare intestinal obstruction that occurs when the intestine folds on itself or telescopes into itself resulting in reduced blood supply. It is most common among young children. The most common place in the intestine for intussusception to occur is where the small intestine joins the colon. However, it can occur in many parts of the intestine. With prompt treatment, almost all patients fully recover. It is more common in boys than in girls.

Use of the Rotashield vaccine was suspended and it was eventually removed from the market in October 1999, when studies confirmed the link between vaccination and intussusception.

RotaTeq

RotaTeq (Merck) is a live oral vaccine licensed in the United States in 2006. It contains five reassortants (WC3 bovine rotavirus strain with surface proteins of the G1-4 and P1A human serotypes). It does not contain preservatives or thimerosal. Three doses are given at 2, 4 and 6 months of age with the minimum age for the first dose of 6 weeks. The series should not be initiated after 12 weeks. The efficacy of the RotaTeq vaccine is high with 98% reduction in severe rotavirus gastroenteritis within the first year of vaccination and a 96% reduction in hospitalization rate. There is also a 74 and 71% reduction of rotavirus gastroenteritis within the first and second years after vaccination.

Rotarix

Rotarix (Glaxo Smith Klein) is a human, live attenuated rotavirus vaccine which contains a rotavirus strain of G1P[8] specificity. It is used for the prevention of rotavirus gastroenteritis caused by G1 and non-G1 types (G3, G4, and G9) when administered as a 2-dose series in infants and children.

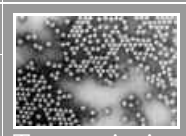
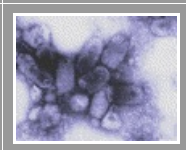
Both of these rotavirus vaccines are very effective (85% to 98%) at preventing infection-associated gastroenteritis and diarrhea. CDC recommends routine vaccination of infants with either of the two available vaccines. Both are administered orally.

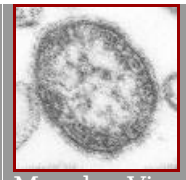
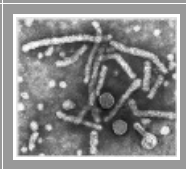
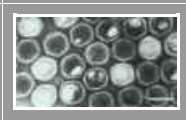
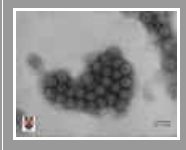
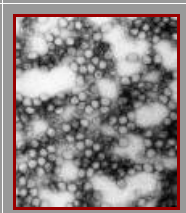
- RotaTeqŪ (RV5). This is given in 3 doses at ages 2 months, 4 months, and 6 months
- RotarixŪ (RV1). This is given in 2 doses at ages 2 months and 4 months.

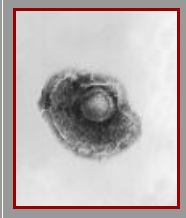
OTHER ANTI-VIRAL VACCINES

There are a number of other commonly used anti-viral vaccines and these are listed below

TABLE 1
Common currently used anti-viral vaccines

Virus	Vaccine Type	Micrograph	CDC Links
Polio (Salk)	Inactivated	 Transmission electron micrograph of poliovirus type 1. **CDC/Dr. Joseph J. Esposito jje1@cdc.gov**	Updated Recommendations of the Advisory Committee on Immunization Practices (ACIP) Regarding Routine Poliovirus Vaccination (2009)
Polio (Sabin)	Attenuated		
Rabies	Current human vaccine is inactivated. There is an attenuated vaccine for animal use.	 Rabies Virus New York State Department of Health	ACIP Recommendations Use of a Reduced (4-Dose) Vaccine Schedule for Postexposure Prophylaxis to Prevent Human Rabies (2008)
Mumps	Attenuated	 Mumps Virus CDC PHIL	Use of Combination Measles, Mumps, Rubella, and Varicella Vaccine Recommendations of

Measles	Attenuated	 Measles Virus CDC PHIL	the Advisory Committee on Immunization Practices (ACIP)
Rubella	Attenuated	 Rubella virus CDC PHIL	
Influenza	Inactivated	 Influenza virus Copyright 1994 Veterinary Sciences Division Queen's University Belfast	CDC Vaccine Information Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011
Hepatitis A	Inactivated		Prevention of Hepatitis A Through Active or Passive Immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2006
Hepatitis B	Subunit	 Hepatitis B Virus Copyright Dr Linda M Stannard, University of Cape Town, South Africa, 1995.	Hepatitis B Vaccine Recommendations (2005, 2006, and 2011) Part 1 - Infants, Children, & Adolescents Part 2 - Adults
Varicella	Attenuated	 Varicella Virus John Curtin School of Medical Research Australian National University Canberra, Australia. Micrograph: Dr Frank Fenner	Prevention of Varicella Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2007
Rotavirus	Attenuated	 Rotavirus Copyright 1994 Veterinary Sciences Division , Queen's University, Belfast	Prevention of Rotavirus Gastroenteritis Among Infants and Children Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009 Rotavirus and Intussusception
Yellow Fever	Attenuated	 Yellow fever virus CDC PHIL	Yellow Fever Vaccine Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010
Human Papilloma	Subunit		Quadrivalent Human Papillomavirus Vaccine Recommendations of the Advisory Committee on

			Immunization Practices (ACIP), 2007
Japanese Encephalitis	Inactivated		Japanese Encephalitis Vaccines Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010
Varicella	Attenuated	 Varicella (Chickenpox) Virus CDC PHIL	Prevention of Varicella. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2007
Herpes Zoster	Attenuated		Prevention of Herpes Zoster. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008
For more information on anti-HIV (AIDS) vaccines go here			

KILLED VERSUS ATTENUATED VACCINES

Attenuated Vaccines

Attenuation is usually achieved by passage of the virus in foreign host such as embryonated eggs or tissue culture cells. From among the many mutant viruses that exist in a population (especially so in RNA viruses), some will be selected that have a better ability to grow in the foreign host (higher virulence). These tend to be less virulent for the original host. To produce the Sabin polio vaccine, attenuation was only achieved with high inocula and rapid passage in primary monkey kidney cells. The virus population became overgrown with a less virulent strain (for humans) that could grow well in non-nervous (kidney) tissue but not in the central nervous system. Non-virulent strains of all three polio types have been produced for the vaccine.

Molecular basis of attenuation

We do not know the basis of attenuation in most cases since attenuation was achieved empirically. The empirical foreign-cell passage method causes many mutations in a virus and it is difficult to determine which are the important mutations. Many attenuated viruses are temperature-sensitive (that is, they grow better at 32 - 35 degrees than 37 degrees) or cold adapted (they may grow at temperatures as low as 25 degrees). In the type 1 polio virus attenuated vaccine strain, there are 57 nucleotide changes in the genome, resulting in 21 amino acid changes . One third of the mutations are in the VP1 gene (this gene is only 12% of genome). This suggests that attenuation results from changes in surface proteins of the virus

An attenuated nasal vaccine for influenza (FluMistŪ) has been developed (see below). This contains cold-adapted vaccine strains of the influenza virus that have been grown in tissue culture at progressively lower temperatures. After a dozen or more of these passages, the virus grows well only at around 25° and *in vivo* growth is restricted to the upper respiratory tract. Studies showed that influenza illness occurred in only 7 percent of volunteers who received the intra-nasal

influenza vaccine, versus 13 percent injected with trivalent inactivated influenza vaccine and 45 percent of volunteers who were given placebo. Both vaccine comparisons with placebo were statistically significant.

Advantages of attenuated vaccines

- They activate all phases of immune system. They elicit humoral IgG and local IgA (figure 8)
- They raise an immune response to all protective antigens. Inactivation, such as by formaldehyde in the case of the Salk vaccine, may alter antigenicity
- They offer more durable immunity and are more cross-reactive. Thus, they stimulate antibodies against multiple epitopes which are similar to those elicited by the wild type virus
- They cost less to produce
- They give quick immunity in majority of vaccinees
- In many cases (e.g. polio and adenovirus vaccines), administration is easy
- These vaccines are easily transported in the field
- They can lead to elimination of wild type virus from the community

Disadvantages of Attenuated vaccine

- Mutation. This may lead to reversion to virulence (this is a major disadvantage)
- Spread to contacts of the vaccinee who have not consented to be vaccinated (This could also be an advantage in communities where vaccination is not 100%)
- Spread of the vaccine virus that is not standardized and may be mutated
- Sometimes there is poor "take" in tropics
- Live viruses are a problem in immunodeficiency disease patients

Advantages of inactivated vaccine

- They give sufficient humoral immunity if boosters given
- There is no mutation or reversion (This is a big advantage)
- They can be used with immuno-deficient patients
- Sometimes they perform better in tropical areas

Disadvantages of inactivated vaccines

- Some vaccinees do not raise immunity
- Boosters tend to be needed
- There us little mucosal / local immunity (IgA). This is important (figure 8)
- Higher cost
- In the case of polio, there is a shortage of monkeys
- In the case of smallpox, there have been failures in inactivation leading to immunization with virulent virus.

NEW METHODS OF VACCINE PRODUCTION

Selection for mis-sense


Figure 11 Attenuated influenza vaccine strain using a cold-sensitive mutant that can be reassorted with new virulent strains

Conditional lethal mutants. Temperature-sensitive mutants in influenza A and RSV have been made by mutation with 5-fluorouracil and then selected for temperature sensitivity. In the case of influenza, the temperature-sensitive gene can be reassorted in the laboratory to yield a virus strain with the coat of the strains circulating in the population and the inner proteins of the attenuated strain. Cold adapted mutants can also be produced in this way. It has been possible to obtain mis-sense mutations in all six genes for non-surface proteins.

The attenuated influenza vaccine, called FluMist, uses a cold-sensitive mutant that can be reassorted with any new virulent influenza strain that appears (figure 11). The reassorted virus will have the genes for the internal proteins from the attenuated virus (and hence will be attenuated) but will display the surface proteins of the new virulent antigenic variant. Because this is based on a live, attenuated virus, the customization of the vaccine to each year's new flu variants is much more rapid than the process of predicting what influenza strains will be important for the coming flu season and combining these in a killed vaccine.

Synthetic peptides

Injected peptides which are much smaller than the original virus protein raise an IgG response but there is a problem with poor antigenicity. This is because the epitope may depend on the conformation of the virus as a whole. A non-viral example that has achieved some limited success is a prototype anti-malarial vaccine.


Figure 12 Anti-idiotype antibodies

Anti-idiotype vaccines

An antigen binding site in an antibody is a reflection of the three-dimensional structure of part of the antigen, that is of a particular epitope. This unique amino acid structure in the antibody is known as the idiotype which can be thought of as a mirror of the epitope in the antigen. Antibodies (anti-ids) can be raised against the idiotype by injecting the antibody into another animal. This gives us an anti-idiotype antibody and this, therefore, mimics part of the three dimensional structure of the antigen, that is, the epitope (figure 12). This can be used as a vaccine. When the anti-idiotype antibody is injected into a vaccinee, antibodies (anti-anti-idiotype antibodies) are formed that recognize a structure similar to part of the virus and might potentially neutralize the virus. This happens: Anti-ids raised against antibodies to hepatitis B S antigen elicit anti-viral antibodies.

Recombinant DNA techniques

Attenuation of virus

Deletion mutations can be made that are large enough that they are unlikely to revert (though suppression of the mutation remains a problem. This has been seen in some of the Nef deletion mutants developed as potential anti-HIV vaccines). Another problem with this approach in some vaccines is that the virus could still retain other unwanted characteristics such as oncogenicity (e.g. with adenovirus, herpes virus, HIV).

Single gene approach (usually a surface glycoprotein of the virus)
A single gene (for a protective antigen) can be expressed in a foreign host. Expression vectors are used to make large amounts of antigen to be used as a vaccine. The gene could be expressed in and the protein purified from bacteria using a fermentation process, although lack of post-translational processing by the bacteria is a problem. Yeast are better for making large amounts of antigen for vaccines since they process glycoproteins in their Golgi bodies in a manner more similar to mammals. An example of a vaccine in which a viral protein is expressed in and purified from yeast is Gardasil, an anti-human papilloma virus vaccine that is very effective in preventing cervical cancer. The current hepatitis B vaccine is also this type. A similar anti-human papilloma vaccine, Cervarix, is made by expressing viral genes recombined into a bacculovirus and expressed in insect cells.

These vaccines have many of the disadvantages of a killed vaccine. This approach has been used to make several potential HIV vaccines but they provoke little cell-mediated immunity.

Cloning of a gene into another virus

By cloning the gene for a protective antigen into another harmless virus, we present the antigen just as the original virus does. In addition,

cells become infected, leading to cell-mediated immunity. Vaccinia (the smallpox vaccine virus) is a good candidate since it has been widely used in the human population with no ill effects. We can make a multivalent vaccine virus strain in this way as vaccinia will accept several foreign genes. A candidate HIV vaccine has undergone clinical trials. However, the use of vaccinia against smallpox has shown rare but serious complications in immuno-compromised patients and alternatives have been sought. One is a recombinant canary pox virus that does not replicate in humans but does infect cells. Immunization with live recombinant canary pox vector expressing the HIV envelope gene has induced an HIV-1 envelope specific CTL response. Similar constructs with gag, protease, nef and parts of pol genes are in clinical trials but all have, so far, shown no clinical efficacy.

DNA VACCINES

The Third Vaccine Revolution

These vaccines are based on the deliberate introduction of a DNA plasmid into the vaccinee. The plasmid carries a protein-coding gene that transfects cells *in vivo* at very low efficiency and expresses an antigen that causes an immune response. These are often called DNA vaccines but would better be called DNA-mediated or DNA-based immunization since it is not the purpose to raise antibodies against the DNA molecules themselves but to get the protein expressed by cells of the vaccinee. Usually, muscle cells do this since the plasmid is given intramuscularly. It should be noted that the plasmid does not replicate in the cells of the vaccinee, only protein is produced.

WEB RESOURCES
DNA vaccine web

It has also been shown that DNA can be introduced into tissues by bombarding the skin with DNA-coated gold particles. It is possible to introduce DNA into nasal tissue in nose drops. In the case of the gold bombardment method, one nanogram of DNA coated on gold produced an immune response. One microgram of DNA could potentially introduce a thousand different genes into the vaccinee.

Advantages of DNA vaccines

- Plasmids are easily manufactured in large amounts
- DNA is very stable
- DNA resists temperature extremes and so storage and transport are straight forward
- A DNA sequence can be changed easily in the laboratory. This means that we can respond to changes in the infectious agent
- By using the plasmid in the vaccinee to code for antigen synthesis, the antigenic protein(s) that are produced are processed (post-translationally modified) in the same way as the proteins of the virus against which protection is to be produced. This makes a far better antigen than, for example, using a recombinant plasmid to produce an antigen in yeast (e.g. the HBV vaccine), purifying that protein and using it as an immunogen.
- Mixtures of plasmids could be used that encode many protein fragments from a virus or viruses so that a broad spectrum vaccine could be produced
- The plasmid does not replicate and encodes only the proteins of interest
- There is no protein component and so there will be no immune response against the vector itself
- Because of the way the antigen is presented, there is a cell-mediated response that may be directed against any antigen in the pathogen. This also offers protection against diseases caused by certain obligate intracellular pathogens (e.g. Mycobacterium tuberculosis)

All of the above means that DNA vaccines are cheap and therefore likely to be developed against pathogens of lesser economic importance (at least to drug companies)

Possible Problems

- Potential integration of plasmid into host genome leading to insertional mutagenesis
- Induction of autoimmune responses (e.g. pathogenic anti-DNA antibodies)
- Induction of immunologic tolerance (e.g. where the expression of the antigen in the host may lead to specific non-responsiveness to that antigen)

Initial studies

Most work has been done on DNA vaccines against viruses since DNA-based plasmid immunization actually resembles virus infection. When they have been well-characterized, the immune responses are broad-based and mimic the situation seen in a normal infection by the homologous virus. The immune response can be remarkably long-lasting and even more so after one booster injection of plasmid. Cytotoxic T lymphocyte (CTL) responses are also well produced as might be expected since the immune system is seeing what is a model of an infected cell.

One important demonstration using a DNA vaccine has been the induction of cytotoxic cellular immunity to a conserved internal protein of influenza A to determine if it might be possible to overcome the annual variation (antigenic drift and shift) of the virus. CTLs were derived in mice against the conserved flu nucleoprotein and this was effective at protecting the mice against disease, even when they were challenged with a lethal dose of a virulent heterologous virus with a different surface hemagglutinin. Because transfer of anti-nucleoprotein antibodies to untreated mice does not protect them from disease, the protective effect of the vaccine must have been cell-mediated.

The current influenza vaccine is an inactivated preparation containing antigens from the flu strains that are predicted to infect during the next flu season. If such a prediction goes awry, the vaccine is of little use. It is the surface antigens that change as a result of reassortment of the virus in the animal (duck) reservoir (see influenza). The vaccine is injected intramuscularly and elicits an IgG response (humoral antibody in the circulation). The vaccine is protective because enough of the IgG gets across the mucosa of the lungs where it can bind and neutralize incoming virus by binding to surface antigens. If a plasmid-based DNA vaccine is used, both humoral and cytotoxic T lymphocytes are produced, which recognize antigens presented by plasmid-infected cells. The CTLs are produced because the infected muscle cells present flu antigens in association with MHC class I molecules. If the antigen presented is the nucleocapsid protein (which is a conserved protein), this overcomes the problem of antigenic variation. Such an approach could revolutionize the influenza vaccine.

Other studies have used a mix of plasmids encoding both nucleoprotein and surface antigens. Protection by DNA vaccines has also been demonstrated with rabies, mycoplasma and *Plasmodium yoelii*. Anti-HIV vaccines are also being tested. In the HIV chapters, it was noted that progress on AIDS vaccines has been stymied by the fact that many vaccines only elicit humoral antibodies while the use of whole virus vaccines (which might elicit CTL responses) has been rejected because of other potential problems. Plasmid-based vaccines may overcome these problems
.


Figure 13 Vaccine-preventable diseases,

by year of vaccine development or licensure - United States, 1798-1998 (MMWR/CDC)

WEB RESOURCES

Baseline 20th century annual morbidity and 1998 provisional morbidity from nine diseases with vaccines recommended before 1990 for universal use in children in the United States
MMWR/CDC

Are your child's vaccines up to date?
CDC

Today, many anti-viral vaccines are available and more are being developed. These vaccines have made a considerable impact on public health around the world (figure 13 and and see here).

Return to the Virology section of Microbiology and Immunology On-line

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