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The Importance of Vaccination

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1. ABSTRACT

We have vaccines for nearly thirty of the more than seventy infectious diseases which are pathogenic for humans. Most of the vaccines, especially those to prevent childhood diseases, are highly effective with a high safety profile. Vaccines are being developed against many of the other bacteria and viruses, and some parasites. Occasionally, a new vaccine has to be withdrawn because of unexpected side effects. Smallpox remains the only infectious disease to have been eradicated. The Global Program to eradicate poliomyelitis initiated in 1988, has unfortunately run into difficulties. A few children immunised with the Sabin oral vaccine fail to clear the virus which can mutate over some years into a pathogenic form and spread rapidly unless large vaccination programs are re-introduced. Of major concern are emerging and re-emerging infectious diseases, especially HIV, for which there is currently no vaccine. Fortunately, new techniques are becoming available making it possible to consider developing vaccines based on inducing strong cell-mediated immune responses to control the agent's replication when antigenic variation in surface antigens (e.g. HIV, influenza) makes classical techniques based on induction of antibody responses less attractive.

2. INTRODUCTION: LIFE BEFORE VACCINES BECAME AVAILABLE

In his book, Guns, Germs and Steel (1), Jared Diamond describes the history of civilization over the last 13,000 years. One of the main features was the tremendous effect of infectious diseases on survival of individuals, groups and nations. Even when setting off to fight a battle, an army might not even make contact with the enemy as an infectious disease epidemic could seriously decrease the numbers of soldiers. As migrating groups of people in the Middle East and Europe began to settle down and keep groups of animals such as cattle, some animal infectious agents would over time adapt to humans and finally be regarded as human pathogens. The list includes smallpox, influenza, tuberculosis, malaria, plague, measles and cholera (2). Although there was often a high death rate, populations could survive and often expanded. But when a group travelled, some might be infected. Beginning with Columbus' voyage in 1492, followed shortly thereafter by Cortes' invasion of the Aztec Empire, the European invasion of North America, and beginning in 1788, the British invasion of Australia, the mortality rates of indigenous populations exposed to novel infections, could be 50% or higher. Within 100 years after invasion, the indigenous populations had decreased by about 90% in size, largely due to the introduced infectious diseases. When measles first reached some Pacific Islands and the Faroe Islands north of Scotland, the mortality rate was about 40%. When a second epidemic occurred in the Faroes 60 years later, those who had survived the first epidemic were protected from the second (3).

. One hundred or so years ago in a developed country, families usually included substantially more children than seen today, because it was expected that several children could die from a childhood infection. In many developed countries, loss of a child because of an infectious disease is now relatively rare. A parent was proud if he/she reached the biblical goal of three score years and ten. The life span now for the elderly is about 10 years longer.

INFECTIOUS AGENTS CAUSING DISEASE IN HUMANS

. Table 1 lists the common agents causing disease in humans (4). Of the four classes, viruses and bacteria are the most common and they cause a great variety of diseases. As indicated, some viral infections can cause cancer. Currently, all registered vaccines are specific for agents in these two groups. Despite great efforts. there is as yet no licensed parasite vaccine. Fungal infections are successfully treated with anti-fungal agents. Some bacteria and all viruses are obligate intracellular infectious agents. Some parasites, such as plasmodia, spend part of their life cycle inside the host cell.

Many viruses and bacteria cause an acute infection, i.e., when exposed to a sub-lethal dose of the agent, the immune response will clear the infection within a week or two. Most vaccines protect against such infections. In contrast, some viruses and bacteria and many parasites cause persisting infections, because the immune response of the host to the infection is evaded or subverted. In such cases, it is sometimes more difficult to make an effective vaccine. Because there is no repair mechanism, RNA viruses may mutate causing antigenic variation. This is discussed in more detail later.

3. TYPES OF VACCINES

3.1 . Early Vaccines

Smallpox was the first vaccine developed. The inoculation of young James Phipps with virus from infected cows by Edward Jenner in 1796 protected the boy from a later inoculation of smallpox. Cholera, Typhoid and Plague vaccines were developed in the late 19th century and Tuberculosis (BCG), Pertussis, Yellow Fever, Influenza and Typhus in the early 20th Century. All these involved the whole organism. The two toxoids, producing antibodies which neutralised the toxins of Diphtheria and Tetanus, became available in the 1920s. All other vaccines were developed after World War II (5).

3.2. Types Of Vaccines And Some Combinations Currently Used

Vaccines currently available especially in the USA are listed in Table 2 (4). They are mainly of five types and a brief description of each follows (2).

3.2.1. Live, Attenuated Microorganisms

Many regard most live vaccines as highly successful, one or two administrations conferring long-lasting protection. Four approaches have been widely used. Edward Jenner pioneered the approach of using a natural pathogen for another mammal (cowpox virus) as the basis of a human vaccine to control smallpox. Currently, fowlpox and canarypox viruses which undergo an abortive infection in humans are being tried as vectors of DNA coding for antigens of other infectious agents for which vaccines are not yet available (6).

The measles, mumps, rubella and yellow fever viral vaccines are typical of a second approach. The wild-type viruses are extensively passaged in tissue culture or other animal hosts until virulence is greatly reduced but immunogenicity is retained.

Type 2 polio virus is a naturally occurring attenuated strain which has been until recently a highly successful a wild type-like strain (7). More recently, rotavirus strains of low virulence have been recovered from children's nurseries during an epidemic (8).

Another approach has been to select mutants that will grow well at low temperatures but very poorly above 37^{o}C (9). Cold-adapted strains of influenza virus have mutations in four of the internal proteins and grow well at 25^{o}C. Such strains which were first described in the late 1960s in the USA, have been used successfully in Russia, and has recently been licensed for use in the USA except for young children and the elderly.

Although BCG (Bacillus-Calmette-Guerin) is still used by WHO to vaccinate young children, variable results

Table1. Common Infectious Agents Causing Disease in Humans

Infectious agent	Diseases
Bacteria	
Bacillus anthracis	Anthrax
Bordetella pertussis	Whooping cough
Borellia burgdorferi	Lyme disease
Chlamydia trachomatis	Pelvic inflammatory disease in women (STD)
	Blindness (trachoma)
Clostridium botulinum	Botulism
Clostridium tetani	Tetanus
Corynebacterium diphtheriae.	Diphtheria
Coxiella burnetii	Severe fever (Q fever) in abattoirs
Eschericia coli	Diarrhoea
Haemophilus influenzae	Meningitis , epiglotitus, pneumonia type
b (Hib)	
Helicobacter pylori	Gastritis, duodenal ulcer, stomach cancer
Legionella pneumophila	Legionnair's disease
Listeria monocytogenes	Meningitis, septicaemia
Mycobacterium leprae	Leprosy
Mycobacterium tuberculosis	Tuberculosis
Neisseria gonorrhoere	Gonorrhoea (STD)
Neisseria meningiitidis	Meningitis, septicaemia
Pseudomonas aeruginosa.	Nosocomial infections
Tick-born typhus fever Rickettsia	Typhus A fever
Salmonella	Typhoid fever
Shigella	Dysentery
Staphylococcus aureus	Impetigo,toxic shock syndrome in women
Streptococcus pneumoniae	Pneumonia, otitis media, meningitis
Streptococcus pyogenes	Tonsilitis, scarlet fever, rheumatic fever
Treponema pallidum	Syphilis (STD)
Vibrio cholerae	Cholera
Yersinia pestis	Bubonic plague
Viruses	
Adenovirus	Respiratory disease
Corona virus	Respiratory and gastric disease
Cytomegalovirus	Mononucleosis (cancer)
Dengue virus	Dengue fever, dengue shock syndrome
Ebola virus	Haemorrhagic fever
Epstein-Barr virus	Glandular fever (infectious mono-nucleosis), Burkitt's lymphoma (cancer)
Hantaan virus.	Acute lung injury
Hepatitis A, B,C, D, E, viruses	Liver disease (hepatitis) (cancer)
Herpes simplex virus, type 1.	Brain infection, mouth lesions
Herpes simplex virus, type 2.	Genital lesions (STD)
Human herpes virus,. type 6.	Kaposi's sarcoma (cancer)
Human immunodeficiency viruses, Types 1 and 2	Acquired immunodeficiency syndrome (AIDS) (STD)
Human T cell lymphotrophic virus, type 1	Cancer of some blood cells
Influenza virus, A B and C	Respiratory disease, Influenza
Japanese encephalitis virus	Brain infection
Lassa fever	Fever, haemorrhage
Measles virus	Respiratory infection, SSPE
Mumps virus	Mumps, meningitis, orchitis (sterility)
Papilloma virus	Warts, cervical carcinoma (cancer, STD)
Parvovirus	Respiratory disease, anaemia
Polio virus	Poliomyelitis, paralysis
Rabies virus	Rabies
Respiratory Syncytial virus	Respiratory disease in infants
Rhino virus	Common cold
Rotavirus	Diarrhoea in infants
Rubella virus	German measles, foetal malformations
Smallpox (vaccinia) virus	Generalised infection (smallpox)
Yellow fever virus	Jaundice, kidney and liver failure
Varicella zoster virus	Chickenpox, shingles
Parasites	
African trypanosomes	Trypanosomiasis, sleeping sickness
Cryptosporidium spp	Diarrhoea
Entamoeba histolytica.	Dysentery
Giardia lambiia	Diarrhoea
Filaria	Elephantiasis
Dracunculasis	Guinea worm
Leishmana	Kala azar, tropical sores
Plasmodium	Malaria
Schistosomes	Schistosomiasis
Toxoplasma gondii	Mononucleosis
Trichomonas vaginalis	Vaginal infection (vaginitis)
	(trichomoniasis)
Fungi	
Aspergiillis fumigates	Pneumonia
Candida albicans	Thrush
Histoplasma	Pneumonia
Pneumocystis carinii	Pneumonia in AIDS patients

SSPE = subacute sclerosing panencephalitis, STD = sexually transmitted disease

Table 2. Currently Registered Viral and Bacterial Vaccines

Viral.	Bacterial
Live, attenuated	
Vaccinia (smallpox)	BCG
Polio (OPV)	Salmonella typhi
Yellow fever	Vibrio cholerae
Measles	
Mumps	
Rotavirus	
Rubella	
Adeno	
Varicella-Zoster	
CA influenza	
Inactivated, whole organism	
Influenza	Bacillus anthracis
Polio (IPV)	Bordetella pertussis
Rabies	Coxiella burnetii
Japanese encephalitis	
Hepatitis A	
Subunit	
Influenza	Salmonella typhi Vi
Hepatitis B (Hep B)	Bordetella pertussis (acellular)
Polysaccharide	Neisseria meningiditis (A.C.Y. W135)
	Streptococcus pneumoniae, 23 Valent
Conjugate	Haemophilus influenzae, type b (Hib)
	Streptococcus pneumoniae, heptavalent
	Neisseria meningiditis
Toxoids	
	Corynebacterium diphtheriae
	Clostridium tetanus
Combinations	
Measles, Mumps, Rubella (MMR)	Diphtheria,tetanus, pertussis (whole organism) (DTPw), (DTPa) acellular DTPa, Hib, HepB, IPV

are obtained with adults. A fifth and more general approach has been to selectively delete or inactivate one or more genes in bacteria (10). Salmonella strain Ty21a has a faulty galactose metabolism and strains with other deletions are being made. A similar approach is being used with complex viruses. Thus, 18 open reading frames have been selectively deleted from the Copenhagen strain of vaccinia virus. The product, NYVAC, has low virulence but has retained immunogenicity (11). Attempts to use this approach with simian immunodeficiency virus, SIV, have been less successful.

Live, attenuated viral and bacterial vaccines can stimulate strong antibody and cell-mediated immune responses. Potentially, this is important with viruses which can show antigenic variation in surface antigens but less variable internal antigens , e.g., influenza viruses. Thus, using an approach to inactivate this virus but retain component of the polio vaccine. With the current prolonged attempt to eradicate this virus, there have been a few cases of the virus in some recipients persisting for several years and mutating back to the ability of the product to generate strong CMI responses has given interesting results and will be described later.

3.2.2. Inactivated Whole Microorganisms

Viruses and bacteria can be inactivated (loss of infectivity) and the product used with varying efficacy as a vaccine though larger doses and sometimes more frequent administration is required (Table 2). Inactivated viral vaccines are generally effective in preventing disease. The continuing antigenic drift which is characteristic of influenza viruses (12) makes it difficult to exactly match the specificity of the circulating strains when the vaccine finally becomes available. Of the three bacterial vaccines in this category, pertussis is the only one widely used. It is effective but has been replaced by a subunit preparation (acellular) which is less reactogenic (13). Inactivated whole agent vaccines are effective at inducing infectivity-neutralising antibodies but usually not class I MHC-restricted responses which are necessary for clearing intracellular infections.

3.2.3. Subunit And Conjugate Vaccines

Antibodies that block the infectivity of viruses or bacteria generally recognize peptide or carbohydrate epitopes of proteins expressed on the organism's surface. Examples are the haemagglutinin and neuraminidase (principally the former) of influenza virus and the surface antigen (HBsAg) of hepatitis B virus.

Blood from HBV-infected people was found to contain HBsAg and was the first source for a vaccine. Production of the antigen in DNA-transfected yeast cells initiated the era of genetically engineered vaccines (14,15). The epitope recognised by an antibody may consist of a single linear peptide but sometimes an epitope is formed by discrete peptides juxtaposed as a consequence of protein folding and/or oligomerisation.

The activity of toxins secreted by tetanus and diphtheria bacteria were counteracted by modification of their properties to form toxoids which induced neutralizing antibody. Such modification is now being achieved by genetic manipulation.

Encapsulated bacteria especially have a coating of polysaccharide that is very poorly recognized by the immune system of < 2 year old children and relatively poorly by that of older people (mainly an IgM response). As a result, infections by these bacteria were often fatal in young children while elderly people gained some protection using a 23 valent pneumococcal vaccine. It was found in 1929 (16) that immunizing with a polysaccharide/protein conjugate gave a much stronger anti-polysaccharide antibody response, mainly composed of IgG due to T cells being involved. It took about 50 years for this finding to be fully utilized by vaccine manufacturers but there are now three highly immunogenic and effective conjugate vaccines (Table 2) and their use is saving many young lives. For example, within one year after the introduction in 1999 of the *Neissseria meningitides*, serogroup C conjugate vaccine in the United Kingdom, the incidence of meningitis was reduced by 92% among young children and by 95% among teenagers (17)

4. VACCINE SAFETY

All available data are reviewed by regulatory authorities before registration of a vaccine (18). Potential safety hazards which occurred at a frequency of about 1/5,000 doses or more often should have been detected (19). Within the first 24 hours, reactions can include fever,

Table 3. Vaccine efficacy (per cent decrease in the incidence of different infectious diseases) in the USA, as assessed by comparing maximum morbidity levels (before vaccine availability) and the levels some years after compulsory vaccination was introduced

Before vaccination			After vaccination		
	No. of cases	Vaccine available	No of cases		% decrease in disease incidence
			1996	2003 [1]	
Diphtheria	206,919	1921 (1942)	1	1	>99.9
Measles	894,134	1941 (1963)	500	56	>99.9
Mumps	152,200	1968 (1971)	600	231	>99.6
Rubella	57,686	1969 (1971)	210	7	>99.6
Pertussis	265,269	1934 (1945)	6400	11647	>94
Poliomyelitis	21,269	1952 (1952)	0	0	100
Paralytic					
Haemophilus	20,000	1984 (1987)	1065	259	>99

[1] Calculations based on 2002 figures, Dates in parenthesis indicate the year the vaccine was widely introduced. MMR vaccine was introduced in 1971. IPV (Salk) vaccine was introduced in 1952 and OPV (Sabin) in 1963.

prolonged crying, syncope, seizures and rarely anaphylaxis. Effects which usually occur at low frequencies (detected by immunosurveillance after registration) include the Guillain-Barre syndrome after influenza virus vaccination, usually about 1 case per million doses but in 1976-77, the incidence was 1 case per 60,000 doses (20) and encephalopathy - one case per million doses after measles vaccination compared with one case per thousand doses after infection (21). Sometimes, a vaccine may be withdrawn after registration due to an unexpected effect. A rotavirus vaccine released in the United States was associated with an unacceptably high incidence (1/10,000) of intussusception (22). In several countries, including the United States in 1999, the oral polio vaccine was replaced by the inactivated polio vaccine because use of the former resulted in a low number of cases of paralysis (23).

There is no firm scientific or clinical evidence that the administration of any vaccine causes a specific allergy, asthma, autism, multiple sclerosis, or the sudden infant death syndrome. A widely cited report claimed an association between the measles component of the measles, mumps and rubella {MMR} vaccine and the subsequent occurrence of inflammatory bowel disease or autism (24). At least 10 studies (25) found no such association. This claim was apparently made by the anti-vaccination lobby simply because the increase in cases of autism in the United States late last century coincided with the introduction of a second MMR vaccination.

5. VACCINE EFFICACY

In the USA, The Centers for Disease Control and Prevention (CDC) have recorded each year the number of cases of different childhood infections occurring in the country from as far back as 1912 and until the present day. To indicate the efficacy of vaccines, the levels of infections during a major epidemic before vaccines became available and those in recent years after vaccines were available, in most cases many years after the introduction of the specific vaccine, have been compared.

In Table 3, such data are compared for seven different infections (4, 2). In most cases, the last two columns show data usually many years after the vaccines first became available. The findings vary from very encouraging to extraordinarily good. Elimination/eradication will be discussed later, but to have decreased the incidence of a disease by more than 99% which was achieved with several vaccines is most impressive. The result with pertussis is not quite so encouraging but 94% protection is still good. An acellular vaccine has now been introduced.

Measles is a great example to consider in more detail. It is highly infectious so that vaccination levels must reach about 95% to prevent transmission. In the USA since records were kept from 1912, case numbers were never less than 100,000 each year before vaccination. There was an epidemic every 2 or 3 years, reaching as high as nearly 900,000 cases on one occasion. After vaccination was introduced in 1963, levels fell to about several thousand– see Figure 1. A 3 year epidemic then occurred reaching about 28,000 cases in 1990. This led to the introduction of a two dose immunization schedule which resulted in the prevention of transmission of the disease in the USA. This success in turn led to the decision of the Ministers of Health in other American countries to attempt to eliminate measles from the Western Hemisphere which has had some success (4).

6. TOWARDS THE ERADICATION OF SOME INFECTIOUS DISEASES

The eradication of one or more infectious diseases represents the crowning achievement of any vaccination program. Jenner (4) was the first to propose that his vaccination technique could be used to achieve such an aim with smallpox.

6.1. Smallpox

Based on their experience with the control of smallpox (a DNA virus) with a vaccination level of 80% in their country, the Russians proposed to the World Health Assembly (WHA) in 1954 that eradication of the disease could be achieved globally. A 10 year voluntary program (unfunded) was initiated in 1954 but by 1964, despite great progress in developed countries, no progress was made in developing countries, with up to 2 million deaths and 1.5 million disease cases still occurring per annum. Two years later, a 10 year funded ($US300 million) WHO program was begun initially under D. A Henderson and later Isao

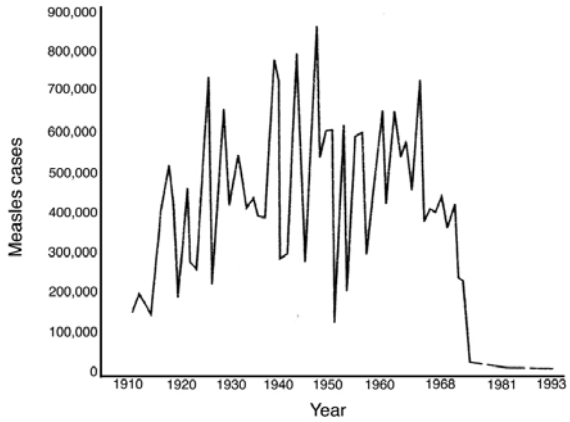

Figure 1. Reported cases of measles each year in the USA from 1912 to 1966, and at three times after 1966, Mass immunization against measles was initiated in 1963. Note that the highest recorded number of measles cases was 894,134 in 1941, but following the introduction of mass immunization , the number of cases reported decreased rapidly to 22,231 in 1968, to 3124 in 1981 and to 312 in 1993.

Arita. Despite many difficulties including wars and vaccine shortages, the goal was achieved. Three years after the last case of smallpox was treated in 1977, F. Fenner, Head of the Committee which certified when each country was free of the disease, announced to the WHA that smallpox had been eradicated (4) He became the chief author of the 1400 page book, Smallpox and its Eradication (26) which took 8 years to write. Fenner, Henderson and Arita shared the 1988 Japan Prize for this wonderful achievement

Based on the success of the smallpox Program, it became clear that to achieve eradication, three properties of the agent were necessary and several others desirable. Necessary properties included a safe and effective vaccine was available, the infection was specific to humans and there was only one or a few strains of virus. Desirable characteristics included the absence of sub-clinical / carrier cases, a simple marker of successful vaccination, the vaccine was heat stable and the agent was only moderately infectious. The first of the desirable characteristics has turned out to be quite important in the case of poliomyelitis.

Of two other candidates which fulfilled the necessary requirements, but only one or two of the desirable properties, poliovirus and measles, the former was chosen for the next Eradication Program. Factors in its favour included that some countries had controlled the infection using this vaccine, OPV was relatively inexpensive and that oral administration of OPV was much simpler than a vaccine requiring injection.

6.2. Poliomyelitis

In 1985, the Director of the Pan American Health Organization (PAHO) proposed the Initiative to eliminate the transmission of indigenous wild-type polio virus from the whole of the Americas. In 1988, WHO extended this to achieve global eradication by 2,000. The campaign was led by WHO, UNICEF, Rotary International and the CDC. In view of the perceived difficulties, four strategies were used.1. Achieving and maintaining high routine vaccination (OPV) coverage; 2. Administering more doses to children on National Immunization Days; 3. Establishing sensitive methods for detecting vaccine virus-induced paralysis; and 4, Conducting mopping-up and catch-up campaigns. There were some remarkable achievements. In one day in December, 1997 in India, 123 million children were vaccinated. In other countries, war-like hostilities could be stopped temporarily to allow vaccination to proceed. One major difficulty was that the virus could persist in some individuals, especially immunocompromised children, for months allowing mutant forms to be expressed. One by one different regions claimed to be free of poliomyelitis but the deadline had to be extended finally to 2005. By the end of 2004, the virus was still endemic in India and Pakistan and in 6 African countries but especially in Nigeria. In one province, a religious group refused to be vaccinated, claiming that the vaccine was contaminated with HIV and contained substances which could affect the fertility of their children. By the time the vaccine was thoroughly tested in a Moslem country, infectious cases had occurred in 18 previously virus -free countries. However, the incidence of mutated forms of OPV, which can cause paralysis occurring some years after vaccination, is increasing – in 2005, there were 22 cases in Hispaniola, 4 cases in Madagaska, 3 in the Philippines and 2 in China (27) so that some experts are now warning that the eradication campaign will fail (28). To minimize mutant forms of virus appearing some years after vaccination, there is discussion on whether the program should switch to IPV, which would not cause this difficulty but this would not give protection in the intestines or throat (28). At least a very high number of children would need to be revaccinated.

6.3. Measles

Measles like polio is an RNA virus. Although measles vaccine world wide has proved to be very stable, laboratory experiments show that mutations can occur. Indigenous measles transmission has been interrupted in Cuba since 1988, in England and Wales in 1995, and in the USA in 2000. However, some countries like Japan, Italy, Germany and France do not consider a measles eradication program a top priority (29). Ciro de Quadros, head of the PAHO Vaccination Program has discussed the feasibility of measles eradication in view of the American experience, and what a better place for young children the world would be if this could be achieved, but acknowledges that a decision would be subject to the outcome of the poliomyelitis eradication Program (30).

7. A NEED FOR NEW AND IMPROVED VACCINES

7.1. Established Diseases

Vaccines are not yet available for most of the agents mentioned in Table 1. Generally, the vaccines against infectious diseases to date have been so successful that major efforts are being made to develop vaccines against most of the remaining agents in that Table. Considerable progress is being made and most of this advanced group are mentioned in Table 4. In addition,

Table 4. A need for improved or new vaccines

Improved	New
Viral	
Japanese encephalitis	Corona
Measles [1]	Cytomegalo
Rabies	Dengue [1]
Smallpox	Epstein-Barr
Hepatitis A [1]	Hepatitis C [1]
Varicella [1]	HIV [1]
	Papilloma [1]
	Respiratory syncytial [1]
	Rota [1]
	West Nile
Bacterial	
Mycobacterium	Chlamydia trachomatis
Tuberculosis [1]	Haemophilus ducreyi (common STD)
	Helicobacter pylori [1]
	Mycobacterium leprae
	Neisseria gonorrhea
	Shigella spp [1]
Others	
	Malaria [1]
	Filariasis
	Giiardi (diarrhea)
	Schistosomiasis
	Treponema pallidum (syphilis)

[1] Recent advances in the development of vaccines against these agents are described in the book described in references 28,29,31.

some of the existing vaccines could be improved. But why improve the measles vaccine when it has been so effective? Currently it is not administered in developing countries before the recipient is 9 months of age. Many lives in those countries would be saved if a new vaccine could be given several months earlier, in the presence of maternal anti-measles antibody.

HIV, *M. tuberculosis* and malaria would be at the head of many lists and each remains a major challenge. Slowly, advances are being made. But in late January, 2006, the Bill and Melinda Gates Foundation announced funding (over 1 billion dollars) to carry out a program to eradicate tuberculosis.

Very encouraging and inspiring news is the progress being made with a prophylactic vaccine designed by Ian Frazer and based on virus-like particles composed of the L1 protein of the human papillomavirus (HPV). In clinical trials so far, 100% efficacy has been obtained in preventing cervical cancer to one or a few selected types of the approximately 15 types of HPV which cause virtually all cases of this cancer world-wide. A recent perspective article is entitled - The Promise of Global Cervical-Cancer Prevention (31). Progress is also being made, based on inducing a strong cell-mediated immune response, towards the development of a therapeutic vaccine to cure existing cases of this cancer (32).

7.2. Emerging And Re-emerging Diseases

A recent article (33) describes the worldwide distribution of selected emerging and re-emerging diseases. Nineteen are due to viral infections and 5 to bacterial infections. Many induce a high mortality in humans. HIV-1 which causes AIDS is very likely the most serious hazard as there are now 50 - 60 million cases worldwide. About 70 years ago, it jumped from non-human primates because of the consumption by humans of infected 'bushmeat'. In developed countries, the disease is controlled by a complex drug mixture. In adults in the absence of drugs, the average time to death is about 10 years. There is a small group of long-term 'non-progressors' who live longer than 20 years because viral levels are kept low due mainly to strong cytotoxic T lymphocyte (CTL) responses. In Africa, some prostitutes have strong CTL responses and continue to resist HIV infection provided they continue prostitution. In some developing countries, especially in Africa, there is about a 30% incidence of untreated infections so that the average lifespan has dropped from about 50 to 30 years. Development of an effective vaccine is still some years away.

Dengue, Yellow Fever, Cholera, and West Nile are re-emerging viruses. For example West Nile virus, previously confined to Africa, appeared in New York in 1999 and had spread over nearly all of the USA by 2003 (33,34). Multidrug resistant tuberculosis and vancomycin-resistant *Staphylococcus aureus* represent re-emerging bacterial infections (34).

SARS (severe acute respiratory syndrome), a previously unidentified corona virus, was identified in China in early 2003 and within a few months had spread through Asia, Europe and had reached the Americas. There were over 8,000 cases and nearly 800 deaths.

Last century, there were three influenza pandemics but the 1918 'Spanish flu' was by far the most lethal, causing probably 50 million deaths. Due apparently to the properties of the surface haemagglutinin protein of the virus, the structure in the lungs which facilitated the transfer of oxygen to the blood was rapidly destroyed probably due to the activation of different cytokines, so the blood turned blue and death often occurred well within 24 hours (35). It takes several days for the adaptive immune response to begin to become effective. Bird flu (H5N1) has been around now for possibly 6 years but a pandemic has yet to occur. The death rate is about 60% but so far passage from human to human has not occurred. The maximum opportunity for a pandemic to occur is if the bird virus infects a person who is currently infected with a human flu strain. If the two viruses infect a cell at about the same time, there is a major opportunity for the exchange of genetic material while RNA replication occurs so that many different combinations of RNA can occur. If this takes place in the upper respiratory tract, one or more of the progeny virus could spread into the environment and be capable of inducing a human pandemic.

Table 5 lists agents which are likely to be of animal origin, indicating they are zoonoses and the disease is transferred by vectors. Infections in the Table have mortality rates varying from about 9 % (SARS) to about 90 % (Ebola and Marburg viruses). A variety of small animals has been implicated to different extents as vectors of emerging infectious agents, but there is now increasing evidence, indicated in Table 5, that bats are major contributors in this area (36). During seasonal fruiting, fruit

Table 5. Newly emerging infectious agents

Agent	Vector
SARS	Bats
H5N1 influenza	Wild and domestic birds
Ebola haemorrhagic fever	Probably bats
Marburg haemorrhagic fever	Probably bats
Human immunodeficiency virus	Chimpanzees, other non-human primates
Lassa fever	Wild rodents?
Lyme disease	Deer mice and ticks
Hendra virus	Bats
Nipah virus	Bats
Variant Creutzfeldt-Jakob disease	

Bovine spongiform encephalopathy, SARS: Define Severe Acute Respiratory Syndrome

Table 6. Properties and roles of components of the adaptive immune system during an intracellular infection

Response	Cytokines expressed	Role		
		Prevent	Control	Clear
Antibody		+++	+	+/-
CD4 Th2	IL-3,4,5,6,10,13			
CD4 TH1	IL-2, TNFα, IFNγ		++	+?
CD8+CTLs	TNFα, TNFβ, IFNγ		+++	+++

IL interleukin, IFN interferon, TNF tumor necrosis factor (64, 2).

bats with virus-infected saliva feed in or below fruit-bearing trees, and regurgitate half digested fruit (now infected) which is often collected with intact fresh fruit for human consumption or eaten by other small animals. And so transmission of the infection occurs. In contrast, anal swabs of the horseshoe bats in China showed the presence of SARS-Corona Virus (37).

8. A BRIEF OVERVIEW OF THE ADAPTIVE IMMUNE SYSTEM

There are two immune systems; most organisms possess innate immunity whereas vertebrates also have the adaptive system which is necessary for developing vaccines. The former involves cells such as dendritic cells and macrophages which also have important roles in the adaptive system. The latter is characterised by lymphocytes which possess specific receptors which recognize foreign antigens.

8.1. Lymphocytes

There are two lymphocyte classes, B lymphocytes which are formed in the bone marrow and migrate to the spleen or lymph nodes, and T lymphocytes, which develop in the thymus from bone marrow derived progenitors. T cell receptors (TCR) are expressed in the thymus following gene rearrangement events. Many T-cells are destroyed in the thymus because they recognize self antigens. Those cells with TCR recognising non-self antigens mature in the thymus and then often migrate to lymphoid tissues. B lymphocytes express mainly IgM receptors but after activation, other immunoglobulins, IgG, IgE and IgA are made, all of which recognise antigenic epitopes. One type of T lymphocyte expresses the CD4 marker and exists in one of two forms, Th1 and Th2. By producing and secreting different cytokines (Table 6), Th2 cells help B cells to differentiate and replicate to become plasma cells producing and secreting different antibodies. CD4 Th1 cells help B cells to a lesser extent but because of the secretion of different cytokines, they also mediate delayed-type hypersensitivity (DTH) responses and the activation of other cells such as macrophages. If infected, the latter are more readily recognised by effector T cells. A second important role for CD4 Th1 cells is helping the activation of the second class of T cells, those with the CD8 marker. These cells are as crucial as CD4 T cells in the response to infections. They are called cytotoxic T cells (CTLs) or killer T cells because not only can they recognize and kill an infected cell but this process can occur within an hour or so after infection and many hours before that cell produces infectious progeny (38). In the body, this gives a 'window' of some hours for the effector T cell to find and kill the infected cell. It is important to realize that the T lymphocyte response by the body generally occurs before antibody is produced.

The receptors on T lymphocytes recognize particular patterns on the infected cell surface, a complex between the major histocompatibility (MHC) antigens and peptides from the infectious agent. In the case of CD4 T cells, the average length of peptide binding to a cleft at the protruding front of the class II MHC antigen is 15 amino acids (derived from foreign antigen being degraded in lysosomes) whereas the average length of peptide binding to a class I MHC molecule, and recognized by CD8 T cells is 9 amino acids. The class II MHC antigen:peptide complex is mainly expressed on antigen-presenting cells (APCs), especially dendritic cells. In contrast, nearly all cells in the body express class I MHC molecules. Thus, CTLs have been viewed as performing a continuous molecular audit of the body (39).

8.2. Specific Roles For Different Components

As anticipated, specific roles can be attributed to different components of the adaptive immune response during an intracellular infection (Table 6) and during an extracellular infection. These are

- Specific antibody is the major adaptive immune response for preventing or substantially limiting any infection. Antibody should also clear an extracellular infection, as the final antigen/antibody complex should bind to Fc or complement receptors on cells such as macrophages. These cells can engulf and often destroy such antibody-coated complexes.
- CTLs are the major mechanism for controlling and finally clearing most acute intracellular infections. Ectromelia, Theiler's virus and lymphocytic choriomeningitis virus are natural pathogens of mice. There is substantially impaired clearance of these viruses in CTL-deficient mice, and HIV levels in HIV-infected chimpanzees treated with anti-CD8 antiserum are not reduced. In contrast, CTL-deficient mice have survived infection with influenza or vaccinia viruses which are not natural mouse pathogens. Extracellular replicating agents may stimulate CD4+ Th1 responses but do not induce CTL

formation. Generally, inactivated infectious agents do not induce the formation of CTLs, but a later report shows this is not always the case – section 10.7.

Sometimes, "dominant' cytokines such as IFN-gamma can clear an infection like vaccinia virus in nude mice (40).

It is important to realize that the immune response is genetically controlled. Members of outbred populations such as humans show tremendous variation in their antibody and T cell-mediated immune response to antigens. By making inbred stains of mice by brother/sister mating for many generations, it was found that all mice in a given strain made very similar amounts of antibody to a given antigen.

In 1975, and based on their experimental observations, Doherty and Zinkernagel (41) predicted that CTLs could distinguish normal and infected cells because the latter expressed at the cell surface a complex between a MHC molecule and a protein (later shown to be a peptide) from the infecting virus. X-ray crystallography later showed this interpretation to be correct (42).

At birth, the human baby contains 18 genes coding for MHC molecules (nine from each parent). But on a population basis, there are sometimes > 100 DNA molecules coding for different MHC antigen specificities, anyone of which can occupy the same loci on chromosome 6 in different individuals (43). Thus, one person has only a small sample of the total number of MHC specificities available so that responses to infectious diseases will vary greatly. No one would have the best genes to combat all infections. A similar variation is found between different stains of inbred mice. For example, Balb C mice are highly susceptible to ectromelia virus whereas C57Bl mice are relatively resistant.

A more detailed account of the role of different immune responses during an infection or following vaccination is given elsewhere (44).

9. SOME RECENT DEVELOPMENTS FAVORING IMPROVED VACCINE DESIGN OR DELIVERY

With the great success of many vaccines especially those for children on the one hand, and the challenges posed by many other diseases yet to be conquered, including the threats posed by emerging diseases, the interest in vaccine development is probably at an all time high. The worldwide effort to make effective vaccines to control HIV-1, malaria and tuberculosis has been especially impressive. With the last two there is some progress but HIV seems to be the ultimate challenge. This section examines some recent developments.

9.1. Enlarging Combination Vaccines

Making combination vaccines has two advantages. One is the cost of delivery. If several vaccines can be administered as mixtures or combinations, instead of each vaccine given separately, this is a big saving. Secondly, their use involves far fewer injections. Of course, there must be compatibility and no interference by one or more components on the others especially at the T cell level. Some current combinations are shown in Table2. Avoiding interference should be easier if with mixtures of conjugate vaccines, the same protein carrier is used. Similarly if live vectors begin to be widely used, choosing the same vector would be advantageous.

9.2. Vaccine Availability

It dawned on some scientists that some immune products, such as subunit vaccines and antibodies might be made in plants – hence the term, plantibodies. The original concept was that by simply eating the plant (edible vaccines), one might be immunized - an idea very attractive for developing countries (45). But it has since been ruled that such products must be isolated under strict conditions and subject to the same safeguards as conventional products (46).

9.3. Mixed Vaccine Formulations; The Prime/Boost Approach

Particularly in the case of non-infectious preparations, several doses of the preparation were often required to obtain high antibody titers, and the concept arose of priming with one formulation of an antigen and later boosting with a different formulation. Thus, immunization of naïve volunteers with a HIVgp160 vaccinia virus construct and then boosting with a gp160 preparation gave higher anti-gp160 antibody titers compared to using either preparation for both priming and boosting (47). It was then shown that mice immunized with a chimeric DNA preparation and later boosted with chimeric fowlpox virus and both expressing influenza haemagglutinin (HA) gave anti-HA tiers up to 50 fold higher than those found after two injections of the same preparation (48). This approach has also been used to induce high and persistent CTL responses to HIV-1, SIV, Ebola virus, *M. tuberculosis* and plasmodia antigens in mice and/or monkeys (6). Most unfortunately, in some clinical trials in humans, the results have been disappointing (49). The reasons are not clear but one factor may be that the dose of DNA used in the trial may be insufficient. One of the major advantages of using DNA for priming is that the response can occur in the presence of specific antibody to the protein form. The earliest time that measles vaccine can be applied is 9 months when the amount of maternal antibody has waned sufficiently. An alternative approach using different chimeric live vectors for priming and boosting is progressing (see 10.5).

9.4. Analysis Of The Genome Of Complex infectious Agents

A recent development, the whole genome sequencing of complex microorganisms, bacteria and parasites, has the potential to revolutionize the way different components are chosen to form the basis of a vaccine (50). For example, potential candidate proteins that might be recognized by infectivity-neutralizing antibodies might have transmembrane sequences near one end of the

molecule. Other sequences could represent important T cell determinants. In one case, mice immunized with 6 out of 108 proteins from *Streptococcus pneumoniae* that had been identified from the DNA sequence as having appropriate structural characteristics, were protected from disease when later challenged with this organism (51)

9.5. The Use Of Live Vectors

Viruses and bacteria have mainly been used for this purpose (2, Table 5). The greatest experience has been with vaccinia virus and its derivatives such as the highly attenuated Ankara virus and the New York preparation, NYVAC. They have a wide host range, have many different promoters , and DNA coding for up to 10 average-sized proteins can be accommodated. Canary and fowlpox (avipox) viruses undergo abortive infection in mammals, making them very safe as vectors though the duration of the response may be shorter (52). Adenovirus (53), polioviruses (54) and Salmonella (55) are often used to obtain a mucosal response, although BCG and Vaccinia have been administered both intranasally and orally.

It has also been very convenient to use such chimeric vectors to evaluate the potential of different cytokines to modify immune responses. Inserting DNA coding for both the foreign antigen and the cytokine allows the effect of the latter to be assessed under optimum conditions as its maximum effect should occur. Thus, interleukin IL-12 and IL-4 are major means for enhancing a CMI or a humoral response respectively. In contrast, incorporation of DNA coding for IL-4 into the DNA of ectromelia greatly increased the virulence of this virus in otherwise resistant mice, and even if the latter had been immunized to increase resistance before challenge (56).

9.6. Oligo, Poly And Lipopeptides

The Ig receptors on B lymphocytes may recognize a pattern on an antigen often formed by a linear peptide sequence. Sequences may contain epitopes recognised by B cells alone or T cell determinants, or both. Some of the obvious advantages of this approach are that the final product contains the critical components of an antigen and avoids other sequences which, for example, may mimic host antigen sequences and thus potentially induce an autoimmune response. When used for the production of antibodies, one restriction is that the isolated or synthetic peptide may not reflect the conformation adopted when it is part of a protein. Sometimes, this can be overcome. Group A streptococci cause rheumatic fever and heart disease, especially in indigenous populations (57). A minimum 'helical' non-host cross-reactive peptide from the conserved C terminal (cryptic sequence) of the M protein was displayed so that the helical folding was maintained. This led to seven serotypic peptides being attached to an alkane backbone so that each was displayed. Outbred mice were immunized several times, the first time with complete Freund's adjuvant. This construct was highly immunogenic and protected the mice against a bacterial challenge, an encouraging result. Maintaining conformation could also be less of a problem if the protein involved had a low molecular weight.

No such restriction would apply when the peptide was used to generate a cell-mediated immune response. Nevertheless, several administrations might be necessary together with a suitable adjuvant. The attachment of appropriate lipids to the peptide began to be practiced in the late 1980s and overcame most of these difficulties when it was found that the lipid/peptide conjugate was self adjuvanting. The following presentation is largely taken from two recent reviews (58,59).

. Linking a synthetic self hormone, luteinizing hormone-releasing hormone (LHRH) which is only 10 amino acids in length, to a strong epitope for CD4+ T cell induction as well as the incorporation of Pam2 Cys greatly increased the immunogenicity of the hormone (60). Coupling the lipid to an internal lysine to form a branched structure further enhanced the antibody response. This approach has been further modified to use a short polylysine sequence to which is attached 3 lipoamino acids.

The induction of CD8 T cell responses using this approach has now been achieved against a variety of viruses, bacteria, the malaria parasite as well as tumors, by using a preparation containing the lipid and epitopes inducing CD4 and CD8 responses. The ability of such preparations to clear infections varied, but some were successful. Not surprisingly, it has been more difficult for the lipopeptides to control persisting infections such as HIV.

Lipopeptides could readily induce cytokine production in antigen-presenting cells (APCs) of which dendritic cells (DCs) are the most important for priming naïve T cells. DCs express a range of Toll-like receptors (TLRs) which recognize conserved molecular patterns produced by microorganisms. TLR 2 is important for the recognition of lipopeptides and lipoproteins , but TLR1 and TLR6 may also be involved. The interaction with TLR 2 initiates a process which results in the maturation of the DCs so that T lymphocytes can be readily activated (thus explaining the self adjuvant effect of the lipopeptides).

A number of clinical trials has shown that the vaccine candidates are safe and immunogenic although protection or clearance have yet to be demonstrated.

9.7. Generation Of CTLs

One of the advantages of using infectious agents for vaccination is that as well as inducing good antibody responses, strong CTL responses are also generated. Furthermore, while the useful antibody response is directed towards surface antigens, most antigens in the agent may contribute epitopes for a CTL response. It may not always be safe to use an infectious form of an agent,eg., a vaccine containing live, modified HIV. Many methods of inactivation such as the use of most chemicals result in preparations which induce good antibody responses but very poor if any CTL responses (61). As described above, the use of lipopeptides and lipoproteins should help to overcome this. But a colleague, Arno Mullbacher, compared the use of UV versus gamma irradiation on different viruses and found that only the latter product,

while clearly non-infectious, induces strong CTL responses in mice. This is very clearly shown with influenza viruses. The two surface antigens, the haemagglutinin (HA) and neuraminidase (NA) can vary by up to 10 % by mutation in some sequences, whereas the membrane and internal antigens are much less variable. Of the three subtypes naturally infecting humans, H1,H2 and H3, antibody to the HA of one subtype does not protect against infection by the other two subtypes. In contrast, gamma-inactivated but not UV inactivated A/Jap (H2N2) immunized mice survived a lethal challenge with heterologous A/PC (H3N2) or A/WSN (H1N1) virus as effectively as mice primed with infectious virus (62). Though it has not yet been conclusively shown that this cross protection is due to CTL activity, this is most likely. Memory CTL immunity is clearly important in reducing disease severity in birds challenged with H5N1 virus (63).

10. LIFE IN THE 21ST CENTURY: THE CONTINUING CHALLENGES

The world population continues to expand and there is increasing interaction between mankind and other mammals especially those who live in isolated areas and who have had only occasional contact with mankind before. HIV is a good example. It was first transferred from apes to mankind probably in the 1930s but such transfers have occurred six more times since then. Hopefully, future disease agent transfers will not prove to be as difficult to control as HIV. But the present list is sufficiently large to keep researchers and vaccine manufactures busy for quite a few years.

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