# 3

# Immunology and Early Phase Trials

The biological basis of successful vaccination is our own complex immune system and its response to pathogens. The immune response is also the source of many safety considerations of vaccination. Before a vaccine can be shown efficacious against infection or disease in large scale field study, it must be shown to elicit an immune response and to be safe in smaller studies. The design and analysis of vaccine studies requires an understanding of immunology and vaccines that goes beyond the scope of this book. Our goal in this chapter is to present sufficient biological background and terminology that the other chapters of the book can be read. The key ideas are the immunogenicity and safety of vaccines. Preclinical studies in animals and Phase I and II clinical studies in humans have the primary goals of assessing the immunogenicity and safety of vaccine candidates. Early phase studies as well as experimental challenge studies are discussed briefly in this chapter.

The book *Vaccines* by Plotkin, Orenstein, Offit (2008), now in its fifth edition, is the standard reference book on vaccines. *Janeway's Immunobiology* by Murphy, Travers, and Walport (2008), seventh edition, is the standard textbook on immunology, with many sections on infectious diseases and vaccines. Both of these books are highly readable. We recommend them to anyone with further interest on the topic.

## 3.1 Immunology and Infection

### 3.1.1 Innate and adaptive immune systems

The immune system is composed of a complex network of cells, molecules, and tissues that interact in an intricate fashion. The immune response can be divided into the innate immune response and the adaptive immune response. The elements of the innate immune system are encoded in a fixed way in our bodies. The innate immune system does not develop a specific response to an infectious agent. It relies on a limited and invariant repertoire of receptors

to recognize microorganisms. The innate immune response can discriminate between self and nonself, and thus is able to decide when to launch an attack. Often the innate immune system can deal with invaders that breach the skin, the mucosa or the airways. When it senses a foreign pathogen that it cannot contain, it mobilizes the adaptive immune system.

The adaptive immune system develops a specific response to a pathogen. B cells produce specific antibodies for antigens on the pathogens. An antibody is a protein that binds specifically to its antigen. An antigen is any substance that can be recognized and responded to by the adaptive immune system. T cells develop the ability to kill specific pathogens and to help B cells produce specific antibodies. Naive T cells move continuously around the body and through the various lymphoid tissues. Antibodies and T cells both bind antigens at receptors that are specific to the antigen. A nearly infinite range of specificities of antigen receptors of antibodies in B cells and in T cell receptors are encoded by a small set of genes by an irreversible rearrangement of segments of the genes. Each cell expresses a unique receptor specificity that stays with its progeny. Cells of at least $10^8$ different specificities are available in an individual at any one time (Murpy et al 2008) The adaptive immune system has the ability to remember its first encounter with a pathogen. When the pathogen invades the body again, the secondary response is much more rapid and much more intense. The adaptive immune response and its memory provide the rationale for immunization. The general idea is to the prime the body with immunization to be ready to meet the invader with a swift and aggressive response.

The five main types of pathogens are viruses (measles, mumps, yellow fever), bacteria (meningococcus, tuberculosis, pertussis, cholera, typhoid), uni- and multi-cellular organisms with nuclei (malaria, sleeping sickness), fungi (*Candida albicans*, *Pneumocystis carinii*), and worms (filariasis, river blindness, hookworm). All successful vaccines in humans up until now are directed against viruses and bacteria. Different effector mechanisms are used to clear primary infections with different pathogens and to protect against subsequent infections. With some infectious agents, such as measles or smallpox, the immune response to natural infection is quite protective against further disease. For such infectious agents, it has been fairly easy to produce efficacious vaccines that simply induce an immune response similar to that of natural infection. For some infections, such as malaria, HIV, and many of the parasites, the immune response to natural infection is insufficient to protect against disease. For such infections, vaccines have to be designed that actually do better than our own natural immune responses.

### 3.1.2 Immune response to infection

What happens when a person is infected by a pathogen for the first time? The innate immune system begins acting immediately. Immature dendritic cells distributed throughout the body serve as sentinels of infection. Dendritic cells

have long tentacles and migrate around the body and into tissues, continually ingesting large amounts of extracellular fluid. They can distinguish self from nonself in the material they ingest. When they encounter a foreign pathogen, several things happen. The dendritic cells develop into mature dendritic cells, capable of presenting the antigens of the pathogen to naive T cells. That is, the mature dendritic cell becomes an antigen-presenting cell, a link between the innate and adaptive immune system. Macrophages, literally "big eaters", and neutrophils are also cells that ingest and digest pathogens that are capable of presenting antigen to cells as part of the link between the innate and adaptive immune response. Sometime the dendritic cells, macrophages and neutrophils are able to contain small invasions in the immediate phase of the innate immune response.

Inflammation is another local response to infection of the innate immune system that occurs after a few hours. This part of the innate immune response is communicated by proteins secreted by the cells. Chemokines are proteins that are secreted by cells that attract other cells that have chemokine receptors into the infected area. Cytokines are proteins secreted by cells that affect cells close by that have the right receptors. In inflammation, the chemokines released by the macrophages recruit more cells of the innate immune system into the area. Once the antigen-specific cells of the adaptive immune system have been created, they too will follow the chemokines to the infected area to intensify the attack. Inflammation causes redness, soreness, swelling and warmth around the area of infection. Local inflammation at the injection site is a common side effect of vaccination.

If some threshold of infection is passed and the innate immune system is not able to clear the infection, the adaptive immune response is triggered. Triggering of the adaptive immune response depends on the transport of the infectious agent to the lymphoid organs, such as a lymph node, then recognition and proliferation by the naive T and naive B cells situated there. The antigen-presenting cell, such as a mature dendritic cell, grabs the antigen at the site of infection and migrates with it to the local lymphoid organ that contains naive T and naive B cells. The dendritic cell then presents the antigen to the naive T cell. The naive T-cell turns into specific effector cells and multiply. They become either antigen-specific CD8 cytotoxic T cells or antigen-specific helper CD4 T cells. Some of the armed effector T cells, particularly the cytotoxic T cells, leave the lymphoid tissue following the chemokine trail back to the site of infection to kill the pathogens. Some of the effector T cells, particularly the antigen-specific helper T cells, stay in the lymphoid tissue to help activate B cells that are presenting the specific antigen on their cell membranes. Antigen-specific B cells generally do not get to work until they encounter antigen-specific helper T cells. The B cells grow exponentially for a couple days and become the antibody-producing plasma cells. It takes about four days for the adaptive immune system to develop a specific response the first time an infectious agent invades a person.

Once an infection is cleared, most of the effector cells die, and a specific immunological memory is retained in memory T and memory B cells. Memory T cells last a very long time, virtually forever, and are responsible for the long term protection after infection or immunization. The second time the pathogen infects a person, the specific memory T and B cells produce a much more rapid and stronger response. Antigen-specific memory B cells replicate and produce antibodies with higher affinity, that is higher binding strength for its antigen, than the primary response.

In summary, the first encounter with an antigen produces a primary response. After a lag phase, antigen-specific antibody is produced. This is also true of a primary immunization. If the primary immunization is followed by a secondary or booster immunization, the secondary antibody response occurs after a much shorter lag, much more antibody is produced, and the antibody has a higher affinity, or strength of binding, to the antigen.

### 3.1.3 Antibodies and epitopes

Antibodies deal with extracellular forms of pathogens and their toxic products. Antibodies circulate in the fluid component of the blood called plasma. The term humors was used for body fluids, so that antibody mediated immunity is called humoral immunity (Murphy, et al 2008). Antibodies are Y-shaped and the ends of their two arms are highly variable, which provides the diversity needed to recognize specific antigens. The stem of the Y determines the class of the antibody. The antibodies are also called immunoglobulins, a particular family of proteins. There are five major classes. For understanding vaccine studies, the most important classes are IgG, IgM, and IgA. The IgG is the most abundant antibody in the plasma and the longest lasting of the antibodies. IgM is the first immunoglobulin to be secreted by the B cells and is a herald of early infection. IgA is the main antibody associated with mucosal immunity. Antibodies do three main things. They bind toxins, they bind pathogens in the blood, and they bind to pathogens in the extracellular space.

An antibody generally recognizes only a small part of a large antigenic molecule such as a protein, polysaccharide (complex sugar), or glycoprotein (a proteins with sugars attached to it), of a pathogen. An epitope or antigenic determinant is the small structure recognized by an antibody or an antigen receptor on a cell. A large molecule such as a protein, polysaccharide or glycoprotein can have many different epitopes. A T-cell epitope is a small part of the pathogen that is recognized by a T-cell receptor. Effector T cells only recognize epitopes of a pathogen when they are presented to them bound to a particular type of protein on the surface of an antigen-presenting cell, such as a dendritic cell, macrophage, neutrophil or B cell. These cell surface proteins that can hold the antigen while it is presented are encoded in a cluster of a couple hundred genes known as the major histocompatibility complex (MHC). In humans the genes in this cluster are also called the human leukocyte antigen (HLA) genes. There is many genetic variants (polymorphisms) in each

gene in the cluster across the human population. Thus, each person has his or her own set of cell-surface proteins that bind antigen to be recognized by the effector T cells. The MHC (HLA) provides a broad population-level genetic diversity as a defense against pathogens.

## 3.2 Vaccines

### 3.2.1 Smallpox

Edward Jenner is generally credited with having introduced, or at least made popular, at the end of the 18th century the use of cowpox inoculation as a protection against smallpox. The latin word for cow, *vacca*, and the vaccinia virus of coxpox, gave the name to vaccination. Smallpox was a widespread and serious, often lethal, disease. The pockmarks it left on the face could be severely disfiguring. Before vaccination for smallpox was introduced, smallpox virus itself was used intentionally to produce a protective immune response against smallpox, a process called variolization. Variolization generally, but not always, produced a milder case of smallpox than natural infection. The virus could be obtained either from fresh pustules or from the dried scabs from smallpox lesions. Several different routes of administration were used, including slight laceration of the surface of the skin or rubbing the scab between the thumb and forefinger. The practice was more widespread outside of Europe. In the 18th century, it was introduced into Europe, but apparently with limited uptake (Buchan 1792).

Vaccination against smallpox with eradication of the disease nearly two centuries after introduction of the first vaccination is a great public health success story (Fenner, Henderson, Arita et al 1988). Several characteristics of the disease and the vaccine, and the dedication of a generation of public health workers lead to the success. The disease is only moderately transmissible, it has no animal reservoir, it causes typical skin lesions in nearly everyone who acquires the disease, and immunity to natural infection is complete and apparently life-long. It has a relatively long generation time, about two weeks, so that for a viral disease, it is pretty slow-moving. The vaccine was independent of the cold-chain and easily administered subcutaneously with a bifurcated needle that held just the right amount of vaccine between its two prongs that were simply jabbed into the skin. To find the last cases towards the end of the international campaign, rewards were offered to people to turn in suspected cases. Then people in the surrounding area were vaccinated, a strategy that came to be called ring vaccination. Smallpox was declared eradicated by the WHO in 1980. Routine immunization against smallpox stopped by 1983.

### 3.2.2 Early development

After the introduction of the vaccine for smallpox, nearly a century passed before the next success (Table 3.1). In the early years of vaccine development,

two main approaches were pursued. One approach was based on attenuated live organisms that can stimulate protective immunity but not cause disease. The other approach was based on killed organisms or purified components of killed organisms. The latter have the advantage that they cannot cause disease or revert to wild-type, but since they cannot replicate, they do not stimulate the immune system in the same way as live attenuated organisms. Another consideration is that many live attenuated virus vaccines need to be kept either cold or frozen, making their widespread use dependent on a cold chain.

In the 19th century, scientists such as Louis Pasteur in Paris, among others, were experimenting with the idea of using an attenuated version of the pathogen that caused the disease of interest to immunize. This approach was radically different than using different less virulent pathogen, such as cowpox against smallpox. Louis Pasteur experimented with attenuated rabies virus vaccine. The idea of injecting a live virus into a human being, whether the virus was attenuated or not, shocked the public. Pasteur got into trouble for his experiments in humans with live rabies vaccine, but was later exonerated. Research in the latter half of the 19th century focused on developing vaccines using killed organisms. Several groups independently developed a typhoid vaccine, including A. E. Wright, who later had the argument with Karl Pearson (see Chapter 1.1.1) about efficacy of the typhoid vaccine. Killed cholera and killed plague vaccines were also developed near the end of the 19th century.

The serious diseases associated with tetanus bacteria and diphtheria bacteria are caused by specific protein toxins that they release. So it is sufficient for an immunization to induce antibodies against the toxins. The vaccines against tetanus and diphtheria, chemically weakened toxins, called toxoids, were available in the 1920s.

The tuberculosis vaccine Bacille Calmette-Guérin was developed by Albert Calmette and Camille Guérin by severe attenuation over 13 years of a bovine tubercle bacterium and introduced in 1927. BCG vaccine is a live attenuated bacterial vaccine. Today it is the most widely used vaccine in the world, though its efficacy is variable, partly due to variability of the BCG strains around the world. The live virus yellow fever vaccine was available for human use in 1935. Whole cell killed pertussis vaccine became available in 1926. Safety concerns about the whole cell pertussis vaccine lead to a search for an alternative. Natural immunity to pertussis induces antibodies to pertussis toxin, filamentous hemagglutinin, pertactin and fimbrial antigens (Storsaeter et al 1992). Acellular pertussis vaccines containing pertussis toxoid and possibly one or more of the three other antigens became available in the 1990's.

The development of a safe and easy cell culture method to grow viruses, by John Enders, Thomas Weller, and Fred Robbins, started the golden age of vaccine development in 1949 (Plotkin and Plotkin 2008). The live oral polio vaccine (OPV) of Albert Sabin and the injected inactivated polio vaccine (IPV) of Jonas Salk were both developed in the early 1950's. The live virus measles, mumps, rubella and varicella vaccines followed in succession between

the 1960s and the 1990s. Various killed influenza virus vaccines were available since the 1930's, and the live cold-adapted influenza virus (CAIV) vaccine was licensed finally in 2003 in the US.

### 3.2.3 Recent developments and beyond

**Table 3.1.** History of Human Vaccine Development (from Plotkin and Plotkin 2008) permission from publisher has been requested.

| Live, Attenuated | Killed Whole Organism | Protein or Polysaccharide | Genetically Engineered |
|---|---|---|---|
| | | 18th Century | |
| Smallpox (1798) | | | |
| | | 19th Century | |
| Rabies (1885) | Typhoid (1896) | | |
| | Cholera (1896) | | |
| | Plague (1897) | | |
| | | First Half 20th Century | |
| Tuberculosis (1927) | Pertussis (1926) | Diphtheria toxoid (1923) | |
| (Bacille Calmette-Guérin) | | Tetanus toxoid (1926) | |
| Yellow Fever (1935) | Influenza (1936) | | |
| | Typhus (1938) | | |
| | | Second Half 20th Century | |
| Polio (oral) | Polio (injected) | Pneumococcus polysaccharide | Hepatitis B surface antigen |
| Measles | Rabies (cell culture) | Meningococcus polysaccharide | recombinant |
| Mumps | Japanese encephalitis | *Hemophilus influenzae* type b | Cholera (recombinant |
| Rubella | Tick-borne encephalitis | polysaccharide | Toxin B) |
| Adenovirus | Hepatitis A | Meningococcal conjugate | |
| Typhoid | | *H. influenza* conjugate | |
| (*salmonella* Ty21a) | Hepatitis B (plasma derived) | | |
| Varicella | | Typhoid (Vi) polysaccharide | |
| Rotavirus reassortants | | Acellular pertussis | |
| Cholera | | Anthrax secreted proteins | |
| | | 21st Century | |
| Cold-adapted influenza | | Pneumococcal conjugates | Human papillomavirus recombinant |
| (CAIV) (2003) | | (2000) | (2006) |
| Rotavirus (attenuated | | Meningococcal quadrivalent | |
| and new reassortants) | | conjugates (2005) | |
| Zoster (2006) | | | |

Many bacteria including meningococcus, pneumococcus, and *Hemophilus influenzae* have an outer capsule composed of polysaccharides (complex sugars). The capsules are species- and type specific. There are more than 90 types of pneumococcal bacteria, a subset of which cause most of the disease. Important meningococcal bacteria types are A, B, and C. Vaccines are generally effective against only the types that they contain, though some cross-protection can occur. The best defense against bacteria with polysaccharide capsules is to coat them with antibody (opsonization). A bacterium, or other antigen, coated with antibodies is recognized as foreign by certain cells (phagocytes) that eat it and destroy it. Vaccination aims to elicit antibodies against the polysaccharide capsules. The first vaccines for these bacteria were made from the purified polysaccharide capsule. However, complex sugars are not as immunogenic as proteins, especially in very young children. The newer conjugate vaccines for such bacteria link the bacterial polysaccharide to a protein carrier to be able to elicit the innate immune response and the T-cell dependent antibody response and be more strongly immunogenic.

Reassortant vacines are produced by coinfection of cell culture with wild-type and attenuated virus strains so their genomes can mix. This approach can be used with viruses with segmented genomes, such as influenza and rotavirus (Plotkin and Plotkin 2008). A modern approach to live attenuated vaccines is to use recombinant DNA technology to put mutations into the genes responsible for virulence in a way that makes reversion to wild-type nearly impossible.

Several new approaches to vaccines are being tried. DNA vaccination injects small bits of the DNA encoding an immunogenic part of virus directly into the muscle. Surprisingly, the elicited immune response is able to protect against infection with the whole virus. Subunit vaccines contain only parts of the antigenic material of the pathogen. They induce a response against only some proteins in the pathogen. Vector-based vaccines integrate genes of the pathogen of interest into the DNA of another pathogen that serves as the vector. When the vector pathogen replicates in the host it expresses the genes of the pathogen of interest, inducing an immune response to that pathogen. Many more vaccines are in the pipeline, including vaccines against malaria, HIV, dengue fever, new vaccines against tuberculosis, and new generations of vaccines against numerous infectious agents for which vaccines already exist.

### 3.2.4 Adjuvants

Adjuvants are substances that enhance the ability of an antigen to induce an immune response. Many of the antigens used in vaccines by themselves do not produce a strong immune response, partly because they do not themselves induce the innate immune response needed to activate the naive T cells. Adjuvants are included in many vaccines to enhance the immunogenicity. Different adjuvants promote different types of immune response. Adjuvants are often made of bits of cell walls of bacteria, but may be too strong to be used in

human vaccines. The pertussis toxin protein has adjuvant properties. In the combination vaccine diphtheria, pertussis, tetanus, the components of pertussis serve as an adjuvant.

## 3.3 Safety

Prophylactic vaccines are generally given to healthy people, so that safety is a primary consideration at all phases of clinical testing and after licensure. Safety concerns of vaccination result partly from the immune response to foreign material in the body, either from the pathogen antigen of interest or the adjuvant. Unwanted postvaccination reactions are called side effects or adverse events or adverse experiences (AEs). Some adverse events could immediately follow vaccination, and others could appear over the next few days. Typical adverse events local at the injection site include inflammation with swelling, redness, soreness, and/or warmth. Systemic adverse events include fever, malaise, chills, or muscle aches. Serious adverse events (SAEs) include anaphylactic shock immediately following vaccination, serious ulceration or abscesses at the vaccination site, or death, among others.

Other safety issues arise with vaccines that contain whole attenuated or killed pathogens. Attenuated pathogens in vaccines can be shed. Shedding is not synonymous with transmission, but occasional transmission might occur. One transmission event of the cold-adapted influenza virus vaccine was documented, but without causing disease (Vesikari et al 2006). However, in some cases transmission of the vaccine virus to contacts can result in disease, such as with the live oral polio vaccine. Some attenuated pathogens can revert to wild-type and cause disease. In immunocompromised people, that is, people with weakened immune systems, such as people with HIV, on cancer chemotherapy, or for other reasons, live attenuated vaccine viruses can cause severe disease. For this reason, live attenuated vaccines are not supposed to be given to most immunocompromised people or close contacts of immunocompromised people.

If whole pathogens are not completely killed before being put into the vaccine, they could also cause disease. Shortly after the killed (Salk) polio vaccine trials in the US, when manufacturing of the vaccine ramped up, vaccine from Cutter Laboratories contained virus that was not sufficiently inactivated. Over 200 paralytic polio cases were traced to vaccine from Cutter (Oshinsky 2005). The incident resulted in much stricter manufacturing requirements, but also damaged the public trust in being vaccinated against polio. Widespread immunization against Swine influenza in the US in 1976 caused several hundred cases of Guillain-Barré syndrome, resulting in several deaths from pulmonary complications (Neustadt and Fineberg 2005). A rotavirus vaccine was withdrawn shortly after introduction when a few cases of a rare type of intestinal obstruction occurred that might have been attributable to the vaccine (Murphy et al 2001). Perception of the safety of vaccination is also important for

people to agree to be vaccinated or to have their children vaccinated. Safety of vaccines has become increasingly important as the threat of disease has been reduced.

## 3.4 Immune Assays

### 3.4.1 Antibody assays

Measuring the immune response to vaccination is important to understand how immunogenic the vaccine is. For a vaccine to be licensed, evidence of its potency must be demonstrated. Potency is the specific ability or capacity of the vaccine as measured by a laboratory test. Increasingly, immune measure are being used as outcomes in definitive studies leading to licensure of vaccine candidates. The most important assays measure the antibodies circulating in the plasma, the fluid part of the blood. Once blood is collected, it is allowed to clot. Serum is the fluid component of clotted blood, and when the antibodies in it are of interest, it is called antiserum. Assays make use of the high specificity of the antibody is for its antigen. Assays for antibodies are also called serological assays and the use of antibodies called serology. Serial dilutions of the antiserum are performed, usually diluting at each step by half, a process called titration. The titer of an antiserum is the dilution at which binding of the antibody to its antigen falls to 50% of the maximum.

The enzyme-linked immunosorbent assay (ELISA) is one of the most common assays. It can be used to detect antibody or to detect antigens in viral infections. The assay relies on direct measurement of antibody binding to its antigen.

The hemagglutination assay is based on the ability of some viral surface or envelope proteins to agglutinate, or stick to human or animal red blood cells and cause them to clump. Hemagglutinin is the main surface protein of the influenza virus. Protective immunity against influenza is generally attributed to neutralizing antibodies directed against the hemagglutinin. The antibodies against hemagglutinin are measured by the ability to inhibit the hemagglutination assay. The titer, or dilution, in a person's antiserum at which this is measured is called the hemagglutination assay inihibition (HI or HAI) titer.

Immunoblots can be used to test sera for the presence of antibodies to specific proteins. Immunoblots, also known as Western blots, are used to separate proteins (antigens) of different sizes. Antibodies are then exposed to the size-separated proteins on the blots to allow them to bind to their specific antigens. The bound antibodies are then labeled so they can be seen. If a vaccine is composed of just parts, or subunits, of a pathogen, then the antibody response to the vaccine will look different from the antibody response to the whole pathogen. Thus, the response to natural infection can be differentiated from the response to a subunit vaccine because there will be fewer bands on the immunoblot in a person who did not have a natural infection.

Several statistical issues related to analyzing and interpreting assays are not discussed in this book. These include interval censoring of the titer measurements and interpretation of null results when the result may be positive but simply below the limits of detection of the assay.

### 3.4.2 T cell assays

A number of assays can be used to characterize T cells. T cells are more difficult to characterize than B cells or their antibodies because there are different types of T cells with different functions. Also measurement of the T cell receptors in the cell membrane is more difficult than measuring antibodies. Cytotoxic T cells can be measured by seeing if they kill specific target cells. CD4 help T-cells can be measured by the amount of cytokines they release when exposed to the specific antigen. The ELISPOT assay is a modification of the ELISA assay that allows the measurement of the frequency of T cells in a population of T cells that respond to a specific antigen. The ELISPOT can also be used to detect specific antibody secretion of B cells. T cell assays are available that allow identification of functional subsets of T cells, T-cell receptor specificity, frequency of certain subsets of lymphocytes, assessment of the diversity of the T-cell repertoire, among others. As development of vaccines based on stimulation of cellular immunity becomes more important, it would be important to include more discussion of the assays.

## 3.5 Herd immunity

*Herd immunity* describes the collective immunological status of a population of hosts, as opposed to an individual host, with respect to a given pathogen (Fox and Elveback, 1975; Anderson and May, 1982). Herd immunity can be thought of as a collective biological state of a population of hosts. Herd immunity of a population can be high if many people have been immunized or have recovered from infection with immunity or be low if most people are susceptible. The level of herd immunity can decrease if the proportion of susceptibles increases or vaccinated protection wanes in individuals. The term "herd immunity" is sometimes somewhat incorrectly used to refer to the threshold at which circulation of an infectious agent is essentially eliminated. We prefer the definition of herd immunity that considers it a continuum rather than a threshold. If herd immunity is high enough, then a threshold may be reached at which infectious hosts no longer contact enough susceptible hosts to maintain transmission.

Herd immunity can be measured in several different ways. Seroprevalence is the proportion of a population that has antibodies to a particular antigen. Seroprevalence of protective antibodies against an infectious agent is a measure of herd immunity. In Figure 3.1, the age-specific seroprevalences,

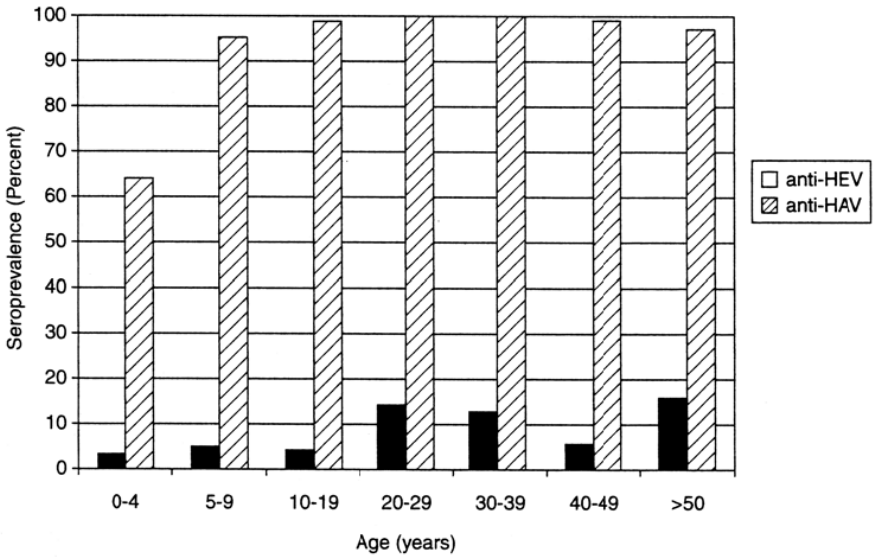


**Fig. 3.1.** Age-specific prevalences of anti-HEV and anti-HAV immunoglobulin G in Vietnam. Data from Hau et al 1999.

that is proportions of people with anti-hepatitis A virus (HAV) IgG and anti-hepatitis E virus (HEV) IgG in a collection of communities in Vietnam (Hau, et al, 1999) are plotted. Seroprevalence of anti-HAV IgG rises very quickly with age essentially reaching 1.00. Seroprevalence of anti-HEV IgG, on the other hand, is very low. The area under the bar graphs, adjusted for the varying sizes of the age groups, can be regarded as the level of herd immunity. The herd immunity for HAV is high and that for HEV is low. On average, 97% versus 16% of the people have antibodies against the two diseases. There is concern that the population is susceptible to an outbreak of HEV. Fine (1993) reviews herd immunity. Fine and Mulholland (2008) use the term community immunity, which is a useful alternative to herd immunity.

The indirect effects of vaccination are primarily due to herd immunity resulting from increased levels of protection in individuals. Recently impressive indirect and overall effects have been observed with the conjugate pneumococcal vaccines, meningococcal, and Hib vaccines, indicating important herd immunity (Chapter 13).

## 3.6 Early Phase Vaccine Studies

The early phase of vaccine development involves searching for candidate vaccine antigens. These include *in vitro* studies as well as testing in animals. Once a candidate antigen is found, then a vaccine is formulated. The decision to

move from preclinical testing to Phase I, Phase II and finally Phase III in humans is a complex process involving the immunogenicity and safety of the vaccine candidate, the cost and potential market for the vaccine, and many other factors.

If appropriate animals are available for that particular infectious agent, then the vaccine candidate will be tested in preclinical studies in animals. The vaccine candidate is evaluated for safety, immunogenicity, and possibly efficacy against experimental challenge with the infectious agent. In early preclinical studies, knowledge about the immune response may affect decisions about choice of antigen, broadness of coverage, and delivery systems. The immune response to antigens is often quite specific to the animal host, so that using animal immune responses to make conclusions about human responses is uncertain. However, immunogenicity in animals can give some help in making the decision to move a vaccine forward to clinical testing in humans (Sadoff and Wittes 2007).

If the vaccine candidate looks safe with possibly good immunogenicity, then a Phase I clinical trial in humans is conducted. In Phase I clinical trials, safety is the primary outcome of interest, but immunogenicity is also assessed. Phase I trials are usually small and conducted in healthy adults generally not at risk to be naturally exposed to infection. Phase I trials may involve different vaccine candidates, doses, or schedules of administration (number and timing of doses).

Phase II studies are further safety and immunogenicity testing in humans. Decisions to move forward to the larger Phase II trials are based on the results of the safety and immunogenicity data in the Phase I studies. Phase II studies are often conducted in populations more similar to the target population for the final vaccine than Phase I studies. When an immune marker is or immune markers are considered to be a reliable measure of protection against disease, Phase II studies can be the definitive study for licensure with immune markers as outcomes. Examples include the meningoccal C vaccine in Great Britain (REF) and the current study of meningococcal A vaccine in Africa and India (REF). The immune response is also used for licensing vaccines when the incidence of disease is very low, making vaccine field studies unfeasible or for vaccines against biological threat agents. Concomitant use trials are designed to show that administration of two or more vaccines at the same time do not interfere with the immunogenicity of the antigens. For example, when varicella (V) was added to the measles, mumps, rubella vaccine (MMR) to make MMRV, it had to be shown that varicella would not interfere with the immunogenicity of the other three.

During and after licensure, immune responses allow generalization to populations that were themselves not tested for efficacy (Sadoff and Wittes 2007). We return to the topic of using immunological surrogates of protection as outcomes in vaccine studies in Chapter 15. Phase IIb studies are intermediate sizes trials, still Phase II studies, that are large enough that some information on vaccine efficacy may be available (Rida et al 1997). The preliminary

efficacy results can also be used to expand enrollment to a full-scale Phase III field study.

During a clinical study, all adverse events and serious adverse events are recorded for study participants. A decision must be made whether the adverse event is due to the vaccine. For example, a person might have died in a car accident. Likely, the conclusion would be made that this SAE (death) was not due to the vaccine. Phase I and II trials can detect common adverse or serious adverse events. Some Phase III trials can detect relatively infrequent serious adverse events. Sometimes serious adverse events do not become associated with a vaccine until millions of people have been vaccinated. These events are followed in postlicensure, or Phase IV, observational studies. Central registries have been set up in many countries to record adverse events and serious adverse events following vaccination. The problem with observational studies is to decide whether there is an increased rate of adverse events in people receiving the vaccine that is caused by the vaccine. Methods have been developed to analyze such observational safety studies, which we consider in Chapter 16.5.

## 3.7 Human Challenge Studies

Some pathogens have characteristics that make ethical and feasible studies to measure vaccine efficacy using experimental infection in humans, called challenge studies. The pathogen should either not generally cause lethal infection or a very effective treatment must be available, or both. Human challenge studies have been conducted with malaria (Patarroyo et al 1987), influenza (Clements et al 1984, 1986, 1990), and other vaccines. Occasionally, such as in influenza, the challenge is with the attenuated vaccine virus (Belshe et al 2000).

## Problems

**3.1. Testing for vaccine efficacy in small sample preclinical studies**
(a) In preclinical vaccine studies in nonhuman primates, one wants to minimize the number of animals used at the same time getting appropriate information. Sample sizes are small and exact inference is used. Albert (1996) considered three approaches to computing the sample size in preclinical studies of an AIDS vaccine. (b) How would you decide which approach to inference to choose on which to base your inference? [1]

**3.2. Phase II trials**
(a) What would be some of the difficulties to have a blinded Phase II trial?

[1] This could have consequences for going forward to clinical studies.

(b) Assume that you have done a Phase II trial of a vaccine candidate and there is evidence of good immunogenicity and safety. How would you decide to take a particular candidate forward to an expensive, large-scale Phase III vaccine field trial?
(c) How would you decide if you have similar evidence on three vaccine candidates?

**3.3. Rotavirus safety trial**
(a) Merck Rotavirus vaccine safety trial