Chapter 19

Viral Vaccines

Fighting Viruses with Vaccines

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Chapter Outline

1. INTRODUCTION

Vaccines are biological preparations that stimulate protective immune responses against pathogens. They have had a profound impact on human health: decreasing illness, extending life spans, and improving quality of life. Edward Jenner's introduction of vaccinia immunization to ameliorate smallpox is an eighteenth-century landmark in public health and one of the earliest demonstrations of the basic principles of immunology. However, immunization may have been practiced as early as 1000 CE in Asia and Africa; there is evidence that the Chinese inoculated people against smallpox by variolation, a procedure in which a small amount of material from smallpox pustules was inoculated into the skin or nostrils of naïve individuals. Vaccinia also provided a precedent for the use of live attenuated viruses to induce effective long-lasting protection, an example that even today inspires vaccinologists. During the last half of the twentieth century, a large number of safe and effective viral vaccines were developed for use in humans and animals. Vaccine development has been largely an empirical science, but systems biology approaches are helping to unravel mechanisms of protection and to predict vaccine efficacy.

This chapter is based on the premise that vaccine-induced protection can best be understood in the context of viral pathogenesis, which identifies potential steps in the infectious process where immunity might intervene to prevent disease. Importantly, the pathogenesis of a specific viral disease helps to determine the immunobiological requirements for a vaccine to protect against that particular infection. We first describe the major vaccine modalities, with their strengths and limitations, followed by an analysis of the mechanisms of vaccine-induced protection as exemplified by a few of the best-studied vaccines. Finally, we explore the utility of systems approaches to vaccine characterization and development.

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2. VACCINE MODALITIES

There are certain immunological principles that govern the induction of protective responses by any vaccine modality (Sidebar 1). Delivery of an immunogen to professional antigen-presenting cells (APCs) is the most effective way to initiate immune induction, which can be modulated to emphasize either cellular or humoral responses. There is a physiological limit to the expansion of naïve T lymphocytes during the primary response but, once rested, committed memory lymphocytes can be restimulated to undergo further expansion (often called an anamnestic or booster response). Adjuvants can bring professional APCs into contact with antigens through their proinflammatory action or exploit cytokines to increase proliferation of antigen-responsive lymphocytes. Newer vaccine modalities attempt to exploit these immunological principles to both enhance and focus the immune response to maximize protective efficacy.

Most vaccines licensed today protect through the induction of functional antibodies. The reason for this is that the diseases for which we have vaccines are largely those in which the agent replicates on the mucosa where antibodies can prevent implantation, or in which the agent disseminates from the mucosa through the bloodstream. Antibodies in the blood can neutralize those viruses that spread via a cell-free viremia and prevent invasion of organs. However, if vaccination does not entirely prevent infection and spread, cellular immune responses may then kill infected cells and thus reduce viral replication. Most effective viral vaccines in use are directed against acute infections, and they do not give 100% protection. Vaccinated individuals—when exposed to a wild virus—may undergo a modest infection that is subclinical and is evidenced only by an anamnestic jump in antibody titer.

Vaccine modalities fall into three broad categories: attenuated live viruses, nonreplicating inactivated viruses or purified antigens, and vectors with limited replicative capacity. Each of these modalities has its advantages and disadvantages, and it is unpredictable which one will produce the most successful vaccine for a given viral disease (Table 1). For instance, in the case of poliomyelitis, there are two vaccines: both the inactivated poliovirus vaccine (IPV) and the oral poliovirus vaccine (OPV) have their complementary advantages.

The earliest vaccines were attenuated viruses derived using chemicals or oxygen to weaken them, often leading to the development of attenuated strains that were relatively safe. Later vaccines were derived by serial passage of viruses in animals or cell cultures to select for attenuated mutants. In some instances, molecular sequencing and virus cloning have been used to produce improved versions. With the beginnings of experimental virology, technology was developed that led to the earliest nonreplicating viral vaccines, formulated by chemical or physical inactivation of virulent viruses. Further advances permitted the production of recombinant viral proteins that could be used as immunogens. Most recently, a variety of vector systems have been introduced to express viral proteins, and these are currently under active development as potential vaccine modalities.

2.1 Attenuated Viruses

Attenuated viruses produce infections that are milder than the illnesses produced by the virulent wild-type counterparts from which they are derived. Attenuated variants may differ in several ways from wild-type isolates. They are often host range mutants so that their replicative capacity—relative to their wild-type counterparts—is high in selected cell culture systems but much lower in vivo. Also, attenuated vaccine viruses are selected for differential tropism in vivo compared to their virulent parents. For instance, the cold-adapted viruses that constitute the live attenuated influenza vaccine (LAIV) will replicate quite well at 33 °C but poorly at 37 °C. In vivo, the cold-adapted virus replicates in the upper respiratory tract (nasal epithelium) but very little in the lower respiratory tract (alveolar epithelium), whereas the virulent

Sidebar 1 Principles of immune induction relevant for vaccine efficacy

- Immune induction is more efficient if an immunogen is presented by professional APCs, such as macrophages and dendritic cells.
- There is a relationship between the amount of antigen presented and the number of naïve lymphocytes that are induced to respond. The number of T lymphocytes induced during the active response determines the number of antigen-specific memory T lymphocytes that are generated.
- Following immune induction, about 10–15 cell divisions occur in antigen-responsive T lymphocytes at which time there is no further proliferation. After a "rest" of weeks to months, antigen-committed T cells may then be induced to proliferate again to produce an anamnestic immune response.
- For many viral infections, immunoglobulin and cellular effector systems can both participate in protective immunity, but their relative role varies for different viruses.
- Immune induction can be manipulated to favor either T_H1 (cellular) or T_H2 (antibody) responses, by formulation of immunogen, route of immunization, and the use of adjuvants.
- Adjuvants can enhance the immune response in a variety of ways, mediated by their induction of proinflammatory cytokines.
- Presentation of antigen to the mucosa-associated lymphoid system can induce local immunity, which may provide an effective barrier to viruses that invade via mucosal tissues.

virus replicates well in both sites. Attenuated OPV exhibits a different pattern of tropism than does wild-type poliovirus, since it replicates well in the gastrointestinal tract but poorly in the central nervous system (CNS). In contrast, wild-type virus replicates robustly in both sites.

Attenuated viruses used as vaccines depend for their efficacy on replication of the agent, which generates antibody and cellular immunity, as well as innate immune responses. In the case of measles, mumps, rubella, varicella, OPV, smallpox, and yellow fever vaccines it is mainly serum IgG antibody that prevents disease. Mucosal immune responses, particularly IgA but including transuded IgG, play the major role in protection afforded by rotavirus and LAIV. OPV prevents disease through elicitation of IgG serum antibodies, but also protects against intestinal infection through local induction of an IgA response. CD4+ T helper cells are critical to the B cell response, but cellular immune responses also contribute to protection from clinical measles, varicella, and smallpox if the wild virus infects.

Two current live attenuated vaccines are genetic reassortants: influenza and one of the rotavirus vaccines. These vaccines are made possible by the segmentation of the viral genomes. In the case of influenza, both live and inactivated, the RNA segments coding for hemagglutin and neuraminidase (of currently circulating strains) are reassorted with RNA segments coding for the six other viral proteins that are obtained from attenuated strains. Thus, the reassortant is attenuated but induces antibody responses against the two viral surface proteins. In the case of the rotavirus, there are also two surface proteins on the virus: VP4, the protease-cleaved protein (P) and VP7, the glycoprotein (G). The pentavalent rotavirus vaccine contains 10 double-stranded RNA fragments from a bovine rotavirus that is attenuated for humans and a single segment coding for both of the common P and G proteins, in order to induce antibodies that will protect by homotypic or heterotypic neutralization.

The search for an acceptable attenuated vaccine strain requires identification of variants that fall in a putative window of robust immunogenicity with minimal disease potential. In spite of diligent efforts to achieve complete safety, some attenuated vaccine viruses retain residual pathogenicity. For instance, OPV causes an occasional case of paralytic poliomyelitis, at a frequency of about 2 cases per 1,000,000 primary immunizations. In addition, some attenuated vaccine viruses may revert in virulence during passage in the primary vaccine recipient, which can be a problem if the virus is excreted. Thus, OPV often increases in virulence upon a single human passage due to revertant mutations. Type 3 OPV strains isolated from vaccine-associated poliomyelitis cases contain uracil to cytosine reversions at nucleotide 472 that restore neurovirulence. In the period 2000–present, more than 10 small outbreaks of poliomyelitis have been traced to reverted strains of vaccine virus that spread from person to person.

Another problem with live virus vaccines is that they may be inadvertently contaminated with adventitious

TABLE 1 Vaccine Modalities

	Live Attenuated Viruses	Inactivated or Subunit Viruses and Recombinant Proteins
Safety advantages	None	Avoids dangers of attenuated viruses
Safety disadvantages	Residual pathogenicity	Potential residual infectious pathogenic virus
	Reversion to increased pathogenicity	Safety tests difficult and expensive
	Unrecognized adventitious agents	Induction of unbalanced immune response
	Possible persistence	
Efficacy advantages	Local immunity at portal of entry	No viral interference
	Cellular and humoral immunity induction	
	Long-lasting immune response	Avoids limitations of attenuated viruses
	Herd immunity	
	Less expensive to manufacture	
Efficacy disadvantages	Interference between serotypes	No induction of local immunity
		Poor induction of cellular immunity
	Interference by adventitious viruses	May not mimic native epitopes for humoral immunity
	Loss of infectivity on storage	Short duration immunity (some products)
	Cold chain required to maintain infectivity	More expensive to manufacture

agents. For instance, yellow fever vaccine produced a massive epidemic of hepatitis B in the 1940s that was traced to a batch of vaccine that contained human serum obtained from an asymptomatic individual who was later shown to be a carrier of hepatitis B virus (HBV). Another contaminant of yellow fever vaccine was avian leukosis virus, acquired from the eggs used to prepare chick embryo cultures in which the vaccine virus was grown; this problem has now been eliminated by using leukosis-free eggs.

The extent of replication determines the level of the immune response. Increasing attenuation unfortunately is correlated with lower responses, presumably because of reduced antigen presentation and lower induction of innate immune responses. Thus, attenuation must establish a balance between safety and immunogenicity. For some candidate vaccines, this has been sought through making the agent replication-defective: in other words, permitting one cycle of replication to stimulate the immune response, but preventing the production of live virus. However, no replication-defective vaccine is yet licensed.

2.2 Inactivated Viruses

A number of chemical and physical methods can be used to inactivate viruses without destroying the integrity of the virus particle or much of its antigenicity. For instance, IPV is manufactured by treating the virus with dilute formalin (formaldehyde gas dissolved in water) at 37 °C for several weeks. The chemical treatment denatures the outer capsid protein sufficiently to prevent viral attachment and entry, while retaining epitopes that induce neutralizing antibodies. Beta propriolactone is another chemical that acts in a manner similar to formalin, and has been used to prepare inactivated rabies virus vaccines. An alternative is a so-called "split product" vaccine, produced by treatment of the virion with mild detergent or ethyl ether that dissociates the particle to yield a suspension of proteins and nucleic acids that are noninfectious but retain antigenicity. This method has been used to produce influenza virus vaccines.

Inactivated virus vaccines are often formulated from pathogenic virus strains, and their safety is contingent upon total inactivation. On occasion, failures in inactivation have caused cases of disease, such as occurred during the "Cutter incident" that confounded the introduction of IPV. A related problem is that "over-inactivation," done to insure safety, can compromise the immunogenicity of inactivated vaccines.

On rare occasions, inactivated vaccines can induce an "imbalanced" immune response that leads to untoward effects. For instance, inactivated measles virus elicited an immune response that resulted in enhanced disease. When children immunized in this manner were exposed to natural measles, they were not protected but developed "atypical" measles with unusual symptoms. Similarly, early trials of an inactivated vaccine against respiratory syncytial virus, an important respiratory virus of children, resulted in enhanced disease rather than protection.

2.3 Recombinant Proteins

A modern alternative to inactivated viruses is the preparation of a recombinant viral protein for use as an immunogen. Since the efficacy of the vaccine is based upon antibodies that target one or two of the viral proteins, there is no need to use the complete virion as an immunogen. However, recombinant proteins must retain their "native" conformation so that they elicit protective antibodies. For instance, the human papillomavirus (HPV) vaccine is based on in vitro synthesis of the major capsid protein, L1. Purified L1 proteins assemble into virus-like particles, which elicit antibodies that prevent attachment of the virus to the basement membrane of the mucosal epithelium. Another example is the recombinant hepatitis B vaccine that consists of the surface antigen of the virus produced in yeast or insect cells. However, industrial-scale production, purification, and stabilization of recombinant proteins are a daunting challenge, and such products are often expensive to manufacture.

2.4 Vectors

In the last few years, there has been a burst of research activity dedicated to novel modes of antigen presentation, sometimes called vectors or "platforms." These new approaches include recombinant viruses, replicons, and purified DNA.

2.4.1 Recombinant Viruses

Genetic engineering has allowed the development of vector-based strategies for immunization, in which the coding sequence for a protective protein is inserted into a nonpathogenic virus that expresses the protein of interest. Although many virus genomes can be manipulated to express foreign antigens, the largest viruses, such as poxviruses and herpesviruses, are most suitable for this purpose. Poxviruses have been used more frequently than other viruses, and vaccinia virus is the basis for some licensed animal vaccines, such as a rabies virus vaccine that has been deployed for the successful immunization of wildlife. A recombinant poxvirus was used in the HIV vaccine trial conducted in Thailand that provided the first evidence for modest efficacy in humans (see later section).

There are several considerations in selecting a replicating virus for use as a vaccine platform, including safety, immunogenicity, and prior immunity of the target population. Current safety standards make it much more acceptable to use a virus that has already had widespread use in the human populations, such as vaccinia virus or 17D, the attenuated vaccine strain of yellow fever virus. Even here, there

are safety problems, since vaccinia causes serious complications albeit at low frequency. Thus, certain attenuated strains of vaccinia virus, such as MVA (modified virus Ankara) or NYVAC are preferred to standard vaccinia virus.

The immunogenicity of a recombinant virus depends in part on the cells that it targets. Some viruses infect macrophages and dendritic cells, and this maximizes their ability to deliver proteins to professional APCs, thereby enhancing the immunogenicity of the recombinant proteins that they encode. Since many recombinant constructs are based on human viruses, vaccinees may have been previously infected with the wild-type counterpart, and this preexisting immunity can reduce the replication of the recombinant virus and compromise its immunogenicity. For instance, recombinant vaccinia viruses are somewhat less immunogenic in persons who were previously vaccinated than in vaccinia-naïve subjects. Recombinant adenoviruses have proven to be highly immunogenic vectors, but are less effective in subjects already immune to the serotype used in the vaccine construct.

2.4.2 Replicons

Replicons are virus-like particles that will enter a target cell, undergo limited transcription and translation to synthesize encoded proteins, but will not produce infectious progeny. Replicons consist of a virus genome that has been engineered to insert a new protein and to delete some of the genes of the parent virus. Such genomic constructs often lack the genes for their envelope spike, and are transfected into packaging cell lines that provide a viral envelope in trans. This permits the assembly of a virus-like particle with the cellular specificity associated with the envelope. Replicons cannot spread beyond the cells that they initially "infect," and are a lower risk platform than recombinant viruses. They can exploit the attributes of many wild-type viruses that would be unacceptable for use as an infectious recombinant virus.

The efficacy of replicons depends upon their ability to reach a sufficient number of target cells, to produce enough novel immunogen, and to deliver the immunogen to professional APCs. In addition, it may be difficult to produce certain replicons on the industrial scale needed for vaccine deployment. Finally, replicons must pass safety tests to ensure that they will not recombine with cellular sequences to reconstitute the potentially pathogenic viruses from which they are derived. Only future investigation will determine whether replicons are a practical platform for vaccine formulation.

2.4.3 DNA Vaccines

It was first discovered in the early 1990s that a DNA plasmid, encoding a protein, could be used as an immunogen by simple injection of the "naked" DNA. This novel technology is currently under active investigation. DNA vaccine plasmids usually use a promoter such as the cytomegalovirus (CMV) promoter, which is highly active in most eukaryotic cells, driving a genetic insert expressing the gene of interest, followed by a transcriptional terminator and a polyadenylation sequence. Modifications of the protein sequence, such as addition of a signal sequence or a transmembrane domain, can be used to influence how the protein is processed in APCs.

DNA constructs are usually administered intramuscularly using a hypodermic needle or into the epidermis using a gene gun, which bombards the skin with gold beads coated with DNA. To be immunogenic, the DNA-encoded protein must be presented by professional APCs. Proteins produced in epithelial cells would be taken up by APCs via the exogenous pathway, while proteins produced in APCs could enter the endogenous pathway. Gene gun injections induce responses with less DNA than is required for soluble DNA, but tend to induce T_H2 responses biased toward antibody. DNA immunogens may be enhanced by the use of adjuvants. For instance, unmethylated CpG motifs in plasmid DNA provide a T_H1-biased adjuvant effect through toll-like receptors (TLRs). Also, DNA can be adjuvanted with plasmids encoding cytokines such as IL-2. DNA-based immunogens have shown modest immunogenicity, but have been more effective when used to prime an immune response followed by boosting with another vaccine modality, a type of vaccination called heterologous prime/boost.

As a vaccine, DNA possesses several advantages. First, it represents a well-defined and stable immunogen that can be precisely characterized and controlled, and produced on a large scale at relatively low cost. It appears to be biologically safe, assuming that it is adequately purified, and it avoids some of the dangers intrinsic in attenuated viruses, inactivated viruses, and certain vectors. Also, DNA immunogenicity is not inhibited by preexisting immunity, a problem with some viral vectors such as recombinant adenoviruses.

2.5 Adjuvants

Adjuvants, sometimes called "the immunologist's dirty little secret," have long been known to enhance the immunogenicity of antigens, particularly foreign proteins. The classic adjuvant is Freund's complete adjuvant (FCA), an oil–water emulsion containing inactivated tubercle bacillus, and the selected foreign protein. However, FCA caused granulomas at the site of injection and is not acceptable for use in humans. Aluminum oxides (alum) are much less irritating and are used in some human vaccines. Recent understanding of the innate immune system (see Chapter 4) has illuminated the mechanisms by which adjuvants appear to operate. Most of them bind to one or more of the TLRs, thereby activating dendritic cells and increasing the

production of proinflammatory cytokines, as well as drawing macrophages to the site of antigen deposition. This amplifies the amount of the antigen that is bound by professional APCs and increases the number of antigen-specific T cells that respond to the antigen. Thus, adjuvants enhance innate immune responses that go on to initiate adaptive immune responses.

3. MECHANISMS OF PROTECTION BY ESTABLISHED VACCINES

A large number of viral vaccines have been developed, licensed, and are in use for the prevention of disease in humans (Table 2). These successful established products provide examples of the mechanisms of vaccine-conferred protection (Sidebar 1).

3.1 Poliovirus

The pathogenesis of poliovirus is understood at an organ level, although many of the specific cellular details have never been elucidated. When the virus is ingested, it invades via the tonsils and the lymphoid tissue of the small intestine, spreads to regional lymph nodes, and is transmitted through efferent lymphatics into the blood, where it circulates as a cell-free plasma viremia. Blood-borne virus invades the CNS either directly across the blood–brain barrier or indirectly by invading peripheral nerves or peripheral ganglia followed by neuronal spread to the CNS. Early studies demonstrated that after injecting a virulent wild-type virus into macaques, viremia is observed for about 1 week, followed by the appearance of neutralizing antibody, simultaneous with the disappearance of infectious virus.

These considerations, along with observations from the gamma globulin trial, led to the formulation by Jonas Salk of an inactivated preparation of poliovirus (IPV) as a candidate immunogen. The 1954 field trial of IPV provided an opportunity to test the hypothesis that neutralizing antibody could account for protection. There was a good correlation between the proportion of vaccinees who responded at a titer of 1:4 or greater and the estimated efficacy of the vaccine (~65%). This correlation suggests that a minimal level of neutralizing antibody can account for protection, not by preventing infection, but by preventing invasion of the CNS.

When attenuated strains of poliovirus (developed by Albert Sabin) were licensed as an OPV in the early 1960s, it became possible to compare IPV and OPV. IPV conferred minimal protection against enteric infection but OPV reduced fecal excretion significantly. It is likely that OPV generates local immunity by inducing antibody production by B cells in the gut-associated lymphoid tissue, although there is little direct evidence for this speculation.

Can the efficacy of poliovirus vaccines be attributed entirely to neutralizing antibody? It was noted above that OPV causes rare cases of poliomyelitis in vaccine recipients (about two per million vaccine recipients). Uniformly, these children have been diagnosed as hypo- or agammaglobulinemic. Strikingly, children with inherited T-cell defects (such as DiGeorge syndrome) do not seem to be at risk of vaccine-associated poliomyelitis. The absence of any data on the development of cellular immune responses to poliovirus vaccines precludes definitive conclusions, but there is little suggestion that CD8-mediated mechanisms play a role in protective immunity against poliovirus.

3.2 Rotavirus

Rotaviruses are an important cause of infant diarrhea and death, particularly in developing countries. These viruses have double-stranded 11-segmented RNA genomes, and genetic reassortants are readily obtained from mixed infections. The pathogenesis of rotavirus disease is not completely understood, but at least two mechanisms have been identified. The virus infects and kills epithelial cells at the tips of intestinal villi, and an internal protein, NSP4, acts as an enterotoxin. Rotaviruses have triple-layered virions, with two outer proteins, VP4 and VP7, both of which are targets for neutralizing antibody. These proteins also determine serotype; the most common VP7 serotypes are G1–G4 and G9 (G, glycoprotein) and the most common VP4 serotypes are P1 and P2 (P, protease sensitive). Vaccine trials (see below) suggest that there is some degree of immunological cross-protection between the different serotypes. Neutralizing antibody appears to be the most important determinant of protection against re-infection, while both T and B cells are important in recovery from primary infection.

Three live rotavirus vaccines have been developed, Rotashield (Wyeth), RotaTeq (Merck), and Rotarix (GlaxoSmithKline). Rotashield and RotaTeq are reassortant viruses, based on animal rotaviruses with VP4 and VP7 genes derived from human rotaviruses. Rotashield has a simian rotavirus and RotaTeq, a bovine rotavirus backbone. By contrast, Rotarix is a single human rotavirus (serotype G1 P1) that was attenuated by passage in cell culture. The ability of these three vaccine viruses to replicate in the human enteric tract varies considerably, and the dose used for immunizing human infants is highest for Rotateq and lowest for Rotarix. These vaccines are administered in two or three oral doses, beginning at age of 2 months. The vaccines elicit intestinal IgA and vaccine "takes" are usually determined by detection of virus-specific serum IgA. In large-scale trials, all three vaccines have been >80% efficacious at preventing severe rotavirus diarrheal disease in young infants. In developed countries, the vaccines are more than 90% effective, but in the tropics efficacy is much lower for reasons that may have to do with the microbiome.

Rotashield was the first of these vaccines to be licensed, but was withdrawn in 1999 (9 months after it became

TABLE 2 Commonly Used Viral Vaccines

Date of US Approval	Virus and Disease	Vaccine Modality and Route of Administration	Use in the United States
Before 1900	Variola	Attenuated	Only in the event of exposure
	Smallpox	Intradermal	
~1939	Yellow fever	Attenuated	Only in the event of exposure
		Subcutaneous	
1955	Polio	Inactivated	Yes
	Poliomyelitis	Intramuscular	All infants
1963	Polio	Attenuated	Yes
	Poliomyelitis	Oral	Special circumstances
1963	Measles	Attenuated	Yes
		Subcutaneous	All infants
1967	Mumps	Attenuated	Yes
		Subcutaneous	All infants
1969	Rubella	Attenuated	Yes
	German measles	Subcutaneous	All infants
1971	Influenza	Inactivated	Yes
		Intramuscular	High risk only
1980	Rabies	Inactivated	Yes
		Intramuscular	High risk only
1981	Hepatitis B	Inactivated	No
		Intramuscular	No longer made
1986	Hepatitis B	Recombinant HBs protein	Yes
		Intramuscular	All infants
1995	Varicella	Attenuated	Yes
	Chickenpox	Subcutaneous	All infants
~1996	Hepatitis A	Inactivated virus	Yes
		Intramuscular	High risk only
2006	Rotavirus	Attenuated	Yes
	Infant diarrhea	Oral	Infants
2006	Varicella	Attenuated	Yes
	Shingles	Subcutaneous	
2006	Human papillomavirus	Recombinant L1 and L2 proteins	Yes
		Intramuscular	

available) because it caused intussusception (a telescoping of the small intestine causing gangrene and peritonitis, requiring surgical intervention). The excess of intussusception cases (about 1 case per 10,000 vaccinees) occurred mainly during the first 2 weeks after the first dose of vaccine. Although the etiology of intussusception is not known, it has been speculated that the vaccine virus causes transient inflammation and swelling of Peyer's patches (lymphoid follicles in the intestinal wall) and that peristalsis leads to mechanical internalization of an intestinal segment. The

other two rotavirus vaccines have rarely been associated with intussusception. RotaTeq was licensed in the United States in 2006 and Rotarix in 2009.

Rotavirus vaccines raise provocative questions associated with mucosal immunity (see Chapter 5). In contrast to most ingested foreign proteins, why are the viral proteins immunogenic? This paradox is not completely understood, but it appears that there are several factors that favor immune induction. Rotaviral infection of the intestinal tract is an invasive process, in contrast to the passive presence of a foreign protein in the intestinal lumen. Rotavirions are taken up by activated dendritic cells in the intestinal epithelium, and invading viral RNA will bind to TLRs 3, 7, and 8, activating dendritic cells and facilitating immune induction. Antiviral IgA can be identified in the intestinal secretions of immunized infants and likely neutralizes ingested rotaviruses. However, rotaviruses also produce a transient viremia, and protection against severe disease may be partly due to circulating antiviral IgG. It is unclear whether cellular immune responses play a role in vaccine-induced protection against rotaviral disease.

3.3 Rabies Virus

Rabies virus presents a special challenge for immunization because of its unusual pathogenesis, and it is one of the few infections where postexposure vaccination is frequently used. Rabies virus is often acquired through the bite of a rabid animal. Following injection into muscle or other peripheral site, the virus replicates locally, crosses the neuromuscular junction, and travels by the neural route to the CNS where it produces a fatal encephalomyelitis. Importantly, rabies virus never produces viremia.

One peculiar aspect of rabies pathogenesis is the variability in the incubation period. The virus may transit to the CNS within a few days or may be sequestered in an extraneural site for weeks to months before it invades the nervous system. This variability in the length of the rabies incubation period is determined by a variety of parameters, particularly the strain of virus. Thus, a neuro-adapted rabies virus, CVS (challenge virus standard), produces rabies with a high frequency and a short incubation period, whereas a freshly isolated wild-type strain (a so-called "street" virus) usually produces a lower frequency of infections and a much longer incubation period.

The long incubation period following exposure to street rabies virus provides the opportunity for postexposure prophylaxis. In the United States, preexposure vaccination is limited to veterinarians or others who are at occupational risk. Because the general population is not routinely immunized, postexposure prophylaxis is the major mode of rabies prevention. The protective mechanisms of pre- and postexposure prophylaxis are somewhat different and are considered separately.

3.3.1 Preexposure Prophylaxis

It appears that neutralizing antibody plays an important role in preexposure prophylaxis. Passive administration of antibody protects animals against subsequent challenge with rabies virus, the degree of protection being correlated with the titer of antibody, the timing of administration, and the strain and dose of rabies virus used for infection. Vaccinia recombinant viruses or DNA constructs that express only the rabies virus envelope glycoprotein provide excellent protection, which is proportional to the titer of neutralizing antibody. It is likely that antibody acts at several different levels, at the site of virus injection, at the neuromuscular junction, and even within the CNS. Specific depletion of antibody responses, by treatment with anti-μ antiserum, potentiates intracerebral infection with an attenuated nonlethal rabies virus, implying that antibody can even reduce *trans*-synaptic transmission within the CNS.

3.3.2 Postexposure Immunization

Active immunization, begun just after infection with street rabies virus, reduces overall mortality, and passive antibody synergizes this protective effect, reducing mortality even further. Passive antibody, given shortly after infection with street rabies virus, does not reduce overall mortality but does prolong the incubation period. Therefore, this synergistic effect is likely due to the ability of antibody to delay virus spread, thereby providing the host an advantage in the "race" between the virus and induction of an active immune response. A person exposed to rabies virus (and who has never been vaccinated) typically receives a dose of rabies immune globulin and four doses of rabies vaccine (made from inactivated rabies virus).

Rabies immunization also illustrates a much-discussed but probably rare phenomenon, immune-mediated disease enhancement by use of a vaccine. In the mouse model of postinfection vaccination, the number of long-incubation period cases is markedly reduced, but there is an absolute increase in short-incubation period cases following vaccination. The excess of short-incubation period cases implies immune enhancement, although the mechanism awaits elucidation.

3.4 Hepatitis B Virus

The pathogenesis of HBV is characterized by a number of unusual features. The timing of events suggests that HBV is not cytopathic and that the acute hepatitis is caused by the cellular immune response (see Chapter 6). The course of acute infection in adults is marked by replication in the

liver, rising levels of circulating hepatitis B surface antigen (HBsAg), the viral envelope protein, together with infectious virions (10^6 per ml of plasma). The resolution of infection is accompanied by acute hepatitis that ranges from subclinical to severe or even fatal. Concomitant with the resolution of infection, there is an immune response that leads to waning of liver infection and circulating HBsAg, and the appearance of anti-HBsAg antibodies.

Experimental evidence for immune-mediated viral clearance comes from a transgenic mouse model, in which mice express one or several HBV proteins in the liver. When these animals are adoptively immunized with HBsAg-specific T lymphocytes, the viral protein is cleared from hepatocytes, but treatment with anti-HBsAg antibody has no effect. CD8-initiated viral clearance is mediated by cytokines (IFNγ and TNF) secreted by effector cells that inhibit HBsAg expression, rather than by cytolysis, explaining how it occurs in the absence of overwhelming hepatitis. Thus infection of adult humans with HBV is an example of an immune response which both produces disease and clears the infection.

An alternative course of infection is seen frequently in infants infected during birth, who become persistent virus carriers, with high levels of virus in the liver and blood. Such persistent infections are not accompanied by acute hepatitis, strengthening the view that the virus infection alone does not cause hepatitis. However, neonatal infection carries a high risk of cirrhosis and hepatocellular carcinoma, which only develop decades later. It is likely that persistent infection represents a state of HBsAg immune tolerance due to "exhaustion" or "deletion" of HBsAg-reactive CD4+ or CD8+ T cells.

The HBV vaccine consists of a recombinant form of the HBsAg that induces "neutralizing" antibodies. Presumably these antibodies protect adults who are exposed to HBV, either through contaminated blood or blood products or (rarely) via sexual contact.

When infants born of mothers who are HBV carriers are immunized with the recombinant HBsAg vaccine at birth, a remarkable result is seen. A high proportion (~90%) of these infantile infections is "converted" from persistent to short duration, but without acute hepatitis. This is surprising, because the immune response to the vaccine only appears 1–3 months after birth (i.e., 1–3 months after infection). The sequence of events includes a transient HBs antigenemia. Since HBV can only replicate in hepatocytes, this implies that HBV infection is established in the liver and is subsequently cleared. Again, it is likely that a host cellular immune response, elicited by either the vaccine or by the active infection, plays a role in vaccine-induced protection. The synergistic cooperation of humoral and cellular immunity may therefore explain the efficacy of the HBV vaccine. Immune memory is also important in this case because half of vaccinees lose antibodies with time but are nevertheless protected by an anamnestic response.

3.5 Human Papillomavirus

HPV has evolved to replicate in a very specialized niche, that is the epithelium of skin and mucous membranes. There are over 100 HPV serotypes and a few of them (particularly types 16 and 18) are a significant cause of cervical cancer (see Chapter 8). Combining all serotypes worldwide, it is estimated that HPV causes at least 200,000 cervical cancer deaths annually.

The natural history of HPV is rather unusual. HPV is transmitted through sexual contact that deposits virions on the mucosal surface, and it invades through minute breaks in mucosal epithelia. Initially, the virions attach to the basal membrane that underlies the epithelial cell layers, and undergo an essential conformational change. The altered virions can now infect the basal stem cells that generate the overlying epithelia. The virus begins replication in these cells, and is carried within these differentiating cells toward the epithelial surface where mature virions are synthesized and released on the mucosal surface. Natural HPV infections persist for varying periods of time and cervical infections are often cleared in 1–2 years. In those infections with oncogenic types of HPV that do persist, cervical cancer develops in a series of steps progressing from initial infection, to persistent infection, to hyperplasia, to cervical intraepithelial neoplasia, to cervical cancer and metastatic spread. The whole process takes many years, but the early phases can be detected within 1–2 years of infection in some individuals.

HPV vaccines have been formulated to prevent or ameliorate infection with HPV, and are not directed against the oncogenic proteins (E6 and E7) of the virus. Instead vaccines are focused on the L1 protein, a major component of the outer capsid. When L1 is expressed as a recombinant protein, the monomers self-assemble into virus-like particles, and these particles induce serum neutralizing IgG when administered as a parenteral immunogen. Neutralizing antibodies and protection are mainly type specific, so that vaccines are formulated as multivalent products.

There are two L1 vaccines, Gardasil by Merck (licensed in 2006) and Cervarix by GlaxoSmithKline (licensed in 2009). The vaccines induce circulating neutralizing antibodies in a high proportion of vaccinees, and they also prevent the earliest oncogenic changes. These vaccines have shown a high degree of efficacy, and that on the surface represent a paradox that "contradicts" vaccinology dogma; that is, that protection against a mucosal infection requires a mucosal—not a parenteral—vaccine. However, it is now known that serum antibodies leak into the small injuries that allow HPV to reach the basement membrane. In addition

to serum neutralizing IgG, circulating anti-HPV IgG antibodies also appear in the female genital tract at low levels, a process known as transudation. Genital tract antibodies reduce the frequency of infection, and if HPV infection is not prevented, these antibodies may reduce lateral spread of infection in the epithelium. These considerations provide at least a partial explanation for how a vaccine that induces serum IgG can provide effective protection against a mucosal infection acquired by sexual contact.

4. VACCINES MUCH NEEDED AND YET TO COME

Why do we not have certain needed vaccines? Despite the enormous success of vaccination since the time of Edward Jenner and Louis Pasteur, developing vaccines against some of today's pathogens is inhibited by several different problems. In some instances, the scientific challenges still elude solution. In other instances, the international community has not made a sufficient investment because the infection is considered a relatively rare "orphan" disease, or because it mainly impacts populations in low-income countries who do not represent an attractive market. We discuss a few prominent examples below.

4.1 HIV Vaccine: Why Do We Not Have One?

HIV was isolated and identified in 1983–84 as the cause of AIDS. Since that time, there has been a vast investment in the development of an effective vaccine, yet modern biomedical science is still being outwitted by 10,000 nucleotides. There are a number of reasons for the failure to develop an effective HIV vaccine, which illustrates some of the potential challenges in virus vaccinology:

- Most natural infections with wild viruses induce long-lasting, often life-long protection against a "second attack." They do not always produce "sterilizing" immunity against reinfection; but reinfections are reduced in magnitude and length, so that they are subclinical. In contrast, primary infections with HIV do not appear to prevent second infections or even ameliorate their magnitude. This is a poor augury for vaccine formulation.
- Viral diseases, even the most dreaded, cause less than 100% mortality (rabies is an exception), suggesting that there is a close balance between virus and host, a balance that could be tilted in favor of the host. HIV is a recent crossover from the chimpanzee, and that host has had a chance to evolve protection against it. In humans, HIV infections, if untreated, are 100% fatal. Another poor augury.
- For HIV, even a minimal inoculum involving a single infectious virion leads to a lethal infection. A protective vaccine, therefore, should provide "sterilizing" immunity. As noted above, this is a standard that few, if any, established effective viral vaccines meet.
- Although HIV infection does induce serum-neutralizing antibodies in the infected patient, these antibodies are "narrow"; that is, they will neutralize only the infecting virus strain, and few other HIV isolates. Furthermore, during the course of a single infection, neutralizing escape mutants are selected, so that the virus can continue to replicate in the face of an active immune response. These escape mutants are "fit," so that they can be transmitted to other uninfected individuals in the population. As a result, during the course of the AIDS pandemic, a very large number of antigenically distinct viruses have been generated. Among human patients, very few have raised antibodies capable of neutralizing this wide variety of mutants. This stands in contrast to most other human viruses, which are not capable of continuously generating new viable escape mutants. For instance, a single strain of measles vaccine virus, used for more than 50 years, will still induce antibodies that can neutralize current measles isolates from anywhere in the world.

Put together, these considerations constitute a set of daunting scientific challenges. One line of research seeks the "holy grail," that is, the development of an immunogen that can induce broadly neutralizing antibodies. Another effort uses gene therapy to endow recipient B cells with the ability to express rare antibody genes that will generate broadly neutralizing monoclonal antibodies. Researchers recently demonstrated that modification of HIV envelope-derived immunogens leads to preferential activation of B cells that produce broadly neutralizing antibodies over those that produce narrowly neutralizing antibodies.

Other research is more empirical, using trial and error to generate protective vaccine formulations. There have been six Phase III (efficacy) trials of candidate HIV vaccines (Table 3). Only one trial (the "Thai trial," RV144) has shown any inkling of success, about 30% protection versus placebo controls. A recombinant canarypox virus expressing the envelope protein of HIV was used to prime the immune system, followed by use of the envelope protein itself as a boost. Interestingly, protection appears to correlate with antibodies against two variable loops (V1 and V2) on the surface protein, rather than with serum-neutralizing activity. It is speculated that the anti-loop antibodies may have acted through antibody-dependent cellular cytotoxicity. However, in the Thai trial, only subjects who self-classified as "low or medium risk" (but not "high risk") showed evidence of protection, and protection appeared to wane after about 1 year. A repeat of this immunization regimen (with some modification) is under way, which will indicate if this empirical approach offers a pathway to success. There has also been a great deal of interest in a vaccine candidate that uses CMV as a vector because this strategy has been used

TABLE 3 Phase III Efficacy Trials of HIV Vaccines

Name of Trial	Vaccinees Risk of HIV Infection	Vaccine Construct	Efficacy	References
VAX003	High risk	AIDSVAXgp120	None	The rgp120 HIV Vaccine Study Group (2005)
VAX004	Injecting drug users	AIDSVAXgp120	None	Pitisuttithum (2006)
	High risk			
Step	High risk	Ad5-gag-pol-nef	None	Buchbinder (2008)
			Increased risk in some populations (see Section 5.3)	
Phambili	High risk	Ad5-gag-pol-nef	None	Gray (2011)
HVTN505	Mainly MSM	DNA-Ad5-env-gag-pol	None	Hammer (2013)
	High risk			
RV144	General community	ALVAC-AIDS-VAX	31%	Rerks-Ngarm (2009)
	Mixed risk			

to successfully abort SIV infection in nonhuman primates. A human version of the vaccine is currently being assessed in a phase I clinical trial in humans.

4.2 Dengue Virus

Dengue virus infections are transmitted mainly by *Aedes aegypti*, a peridomestic mosquito that is also the vector of urban yellow fever. Dengue fever is pandemic in many of the tropical parts of the world, with more than 50 million cases each year. It is an acute febrile infection with severe pain in muscles and joints (sometimes called "breakbone fever"). The majority of patients recover spontaneously, but a small proportion (less than 5%) develops hemorrhagic fever and shock syndrome (DHF/DSS), which has a fatality rate as high as 25%. Applied to the high incidence, this could result in as many as one million deaths annually.

Dengue virus is a flavivirus that occurs in four distinct serotypes (1–4). Infection with a specific serotype confers long-term immunity against that serotype but not against other serotypes. Immune protection appears to be conferred by circulating neutralizing antibodies, but the role of cellular immunity is unclear. The pathogenesis of dengue hemorrhagic fever is poorly understood, but it appears to be immune-mediated in part, since most severe cases occur in persons who are immune to at least one serotype.

The challenge for a safe and effective dengue vaccine is to induce protective antibodies against all four serotypes. A vaccine that induces antibodies against some but not all serotypes might not only fail to protect against the "missing" serotypes, but might enhance the risk of dengue DHF/DSS. A chimeric vaccine has been developed based on the live, attenuated 17D yellow fever virus. The premembrane and envelope genes from 17D have been deleted and replaced by those of each of the four dengue viruses, creating a quadrivalent replicating vaccine. It was thought that antibodies against the envelope of each virus would provide a highly effective vaccine, but when phase II and III studies were performed, efficacy against type 2 virus, and to a lesser extent type 1, was considerably less than efficacy against types 3 and 4. The reasons for these differences have not been fully elucidated, but it appears that the conformations of the types 1 and 2 envelopes in the chimera are significantly different from those in the native virus and thus the induced antibodies do not always neutralize the viruses injected by mosquitoes.

4.3 Ebola Virus

Ebola hemorrhagic fever is caused by a filovirus and is a prime example of an emerging viral disease (see Chapter 16). Although the mortality rate is above 50%, Ebola has historically been considered an "orphan" disease. There have been more than 25 outbreaks of Ebola disease in Africa since the 1970s. Prior to 2014, outbreaks had been relatively small (the largest no more than several hundred cases) and all had been controlled by quarantine. Several laboratories had been working on candidate immunogens as vaccine candidates, but there was little incentive for a full-blown vaccine development program, in either the public or private sector. All that changed with the 2014 epidemic in West Africa, which by the end of that year had caused over 10,000 cases with more than 5000 deaths. Furthermore, the importation of a few cases into high-income countries lifted Ebola to a global health problem.

Two Ebola vaccine candidates were moved into human trials in 2014. Each candidate is based on a recombinant vector (either adenovirus or vesicular stomatitis virus, VSV) that expresses the Ebola virus glycoprotein. When tested in nonhuman primates, both of these candidates provide 100% protection against a potentially lethal challenge with wild Ebola virus. As of August 2015, interim results from a phase 3 trial indicate that the VSV-based vaccine is highly efficacious. Absent untoward events, it is likely that a vaccine will become available by 2016.

5. SYSTEMS APPROACHES TO VACCINOLOGY

In addition to the examples described above, more general impediments to vaccine development include our limited understanding of the following:

- how vaccines induce a specific, potent, broad, and long-lived immune response;
- which pathogen-specific antigens are needed to confer protective immunity;
- the differences between naïve and immunized hosts in responses to infection; and
- ways in which to maximize vaccine efficacy in heterogeneous populations.

Systems vaccinology aims to address these challenges by characterizing the complexity of the host response to vaccination and by facilitating predictions of vaccine efficacy (Figure 1). Although systems approaches have only been applied to vaccinology in the past decade, they have already improved our understanding of how some vaccines provide protection. The majority of this work has been done with yellow fever and influenza vaccines, but the responses to HIV vaccines are also being explored, as are efforts to develop pan-vaccination signatures.

5.1 Yellow Fever Virus

The 17D vaccine is one of the most efficacious vaccines ever created and is considered a gold standard for vaccine development. Although this live-attenuated vaccine has been in use for over 50 years, the reason for its effectiveness was fully understood only recently. Transcriptional profiling of whole blood and peripheral blood mononuclear cells (PBMCs) from human vaccinees revealed that 17D activates multiple aspects of innate and adaptive immunity. The key to its success lies in its ability to activate dendritic cells through multiple TLRs leading to a mixed Th1/Th2 T-cell response. An early transcriptional signature that includes expression of complement gene C1qB and eukaryotic translation initiation factor 2 (EIF2AK2) is predictive of a strong CD8+ T-cell response. Another signature that includes expression of TNFRS17, a B-cell growth factor, is predictive of the strength of the humoral response. These data suggest that vaccines that can elicit these early signatures may activate protective immune responses.

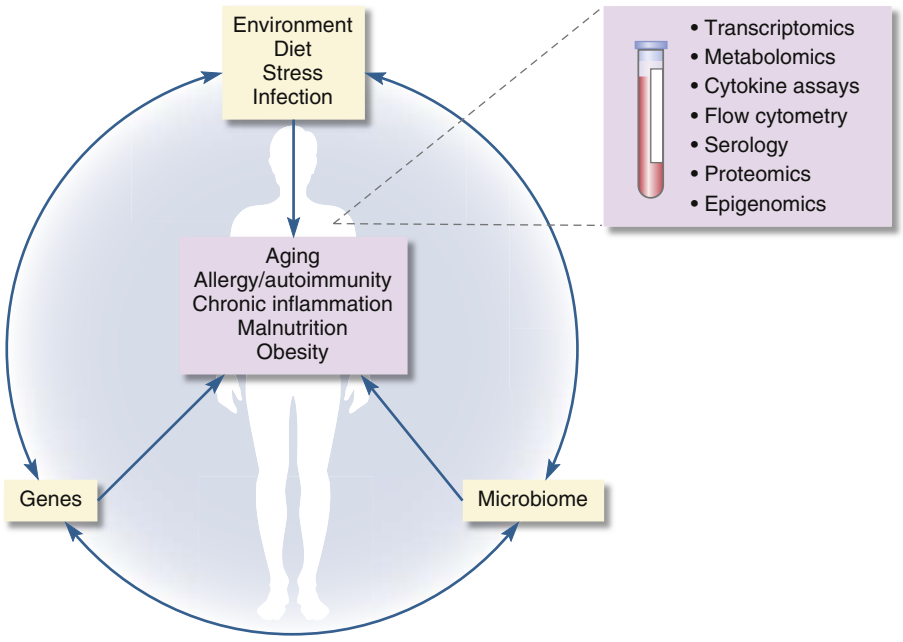

FIGURE 1 Systems vaccinology. Genes, the environment, and the microbiome are interdependent determinants of human physiology. Variations in each of these factors impact aging, immunity, inflammation, and nutritional status. Systems vaccinology seeks to understand the complexity and diversity of host determinants and immune responses to enable the rational design of vaccines. *Adapted from Pulendran (2014).*

5.2 Influenza Virus

There are two types of seasonal influenza vaccines, live attenuated and inactivated. LAIV contains replication-competent viruses of low pathogenicity that are administered intranasally. Inactivated influenza vaccine (IIV) is administered intramuscularly and consists of viruses that have been chemically denatured. LAIV and IIV come in trivalent or tetravalent formulations.

LAIV and IIV protect against influenza virus by different mechanisms and elicit vastly different transcriptional profiles in the blood of vaccinees. CamkIV and E2F2 expression are negatively associated with the magnitude of the humoral response to IIV and STAT1 expression is positively associated. Type-I IFN responses dominate with LAIV, whereas genes enriched in antigen secreting cells are important for IIV-induced immunity. A predictive signature has not been identified for LAIV, because the correlates of protection are less defined. However, serum antibody and IgA mucosal antibody both correlate with protection by LAIV. The US Food and Drug Administration defines seroconversion as an HAI titer of 1:40 or an at least fourfold increase in antibody titer after vaccination, but these numbers are rarely reached after LAIV vaccination. Since LAIV does not induce high antibody titers, it would be deemed inferior to IIV if it were judged solely on this basis. Thus, other correlates of protection need to be identified.

Systems approaches have also been used to compare the host responses of naive and vaccinated macaques to a wild-type influenza virus challenge. Protective vaccines do not necessarily induce sterilizing immunity, but they do alter the course of infection with a wild-type virus such that the infection is often subclinical. When naïve macaques and macaques immunized with a live influenza vaccine (attenuated through the truncation of the viral nonstructural 1 protein) were challenged with virulent influenza virus, the responses in vaccinated and naïve animals were drastically different. The lungs of the vaccinated animals had lower virus levels, less pathology, and lower expression of innate immune response and cytokine genes.

5.3 Human Immunodeficiency Virus

The failure of the MRKAd5/HIV vaccine (and possible enhancement of HIV infection) has been examined through a systems lens. This vaccine is comprised of a replication-incompetent adenovirus-serotype-5 vector expressing HIV gag, pol, and nef. In 2007, a clinical trial for MRKAd5/HIV efficacy was halted prematurely when data indicated that MRKAd5/HIV vaccination increased HIV-1 acquisition rates in vaccine recipients with high levels of antibodies against the Ad5 vector. Transcriptional profiling revealed that PBMCs isolated from Ad5-seropositive patients display an attenuated innate immune signature to MRKAd5/HIV compared to that of Ad5-seronegative patients. Down-regulation of RANTES and up-regulation of IFNλ2 are associated with induction of strong CD8+ T-cell responses in Ad5-seronegative patients, but these are muted in Ad5-seropositive patients. However, blood cell transcriptional profiling can only explain some differences, and additional work is needed to understand the possible enhancement of infection in Ad5-positive vaccinees.

5.4 Developing Pan-Vaccination Signatures

It is clear that different vaccines elicit different responses in the blood, but are there common signatures that could be predictive across vaccine types? To attempt to answer this question, one study has used publicly available human blood transcriptomic data from multiple vaccine trials. These data were used to generate gene co-expression networks and to form different gene expression modules. Correlating antibody titers with changes within a module increases prediction sensitivity because large changes in the expression of individual genes are not necessary for efficacy. Integrative network modeling of PBMC responses to LAIV, IIV, 17D, and two meningococcal vaccines (MCV4 and MPSV4) identified early transcriptional signatures that determine the magnitude of the antibody responses to these vaccines. MPSV4 and MCV4 elicit similar protection as measured by serum bactericidal activity even though they elicit different amounts of IgG. This is a common theme in vaccinology; antibody levels are not necessarily predictive of vaccine efficacy. There are numerous similarities and differences in the host response to LAIV, IIV, 17D, MCV4, and MPSV4, but there is currently no single gene signature that predicts responses to multiple vaccines (Figure 2).

5.5 Population Heterogeneity

One of the biggest challenges vaccine developers face is ensuring that vaccines will be effective in heterogeneous populations. Sex, age, ethnicity, and microbiota have a large impact on the host response to vaccination (Figure 3). Males and females respond differently to the yellow fever vaccine with more women than men reporting adverse events (AE). When the responses of male and female vaccinees were compared, it was found that 10-fold more genes are differentially expressed in the blood of female vaccinees. These genes are enriched for innate and adaptive immunity functions, suggesting that the increased incidence of AE in females is due to a more robust inflammatory response. Women also consistently have more AE in response to influenza vaccinations, and produce more robust antibody responses to IIV. In one study on sex-dependent differences in vaccination, researchers identified a cluster of lipid metabolism genes that are likely modulated by testosterone and whose expression

FIGURE 2 Transcriptional profiling of whole blood reveals distinct mechanisms of antibody response. Li et al. compiled over 30,000 human blood transcriptomes from over 500 studies to extract modules that contained genes that were co-expressed. These modules were then used to correlate the transcriptomic programs and antibody responses elicited by different vaccines. Each vaccine data set is shown as one of six segments on the circular plot. In each segment, the inner circular bands show an ordered list of all blood transcriptional modules, layered by histograms of modules significantly correlated to the antibody response, red for positive correlation and blue for negative correlation. Modules that are common between vaccines are linked by a color curve in the center. *From Li et al. (2014).*

correlate with the higher antibody-neutralizing response to IIV observed in females. Testosterone may act by decreasing expression of transcription factors such as FOS, JUNB, and JUND that, in turn, repress the expression of lipid metabolism genes that encode immunosuppressive activities. In fact, women develop antibody responses that are equal to those of men when given only half the standard vaccine dose, suggesting that vaccine regimens may need to be tempered in women (or boosted in men) to achieve equal efficacy or reduced AE.

Other factors, such as ethnicity and country of origin, also affect vaccination outcomes. This is likely due to a combination of environmental and genetic factors. A study of responses to 17D vaccination in subjects from Switzerland and Uganda found that 17D-induced B- and T-cell responses were significantly lower in Ugandan vaccinees. The Ugandan volunteers had higher frequencies of differentiated T- and B-cell subsets, proinflammatory monocytes, and exhausted and activated NK cells. This suggests that Ugandan patients had an activated immune microenvironment, and this is supported by the fact that 17D replicated to lower levels in this cohort. These finding suggest that 17D vaccine regimens might need to be boosted in African populations to achieve efficient immunity.

The effects of the host microbiome on vaccination outcome are also being explored. It was recently shown that

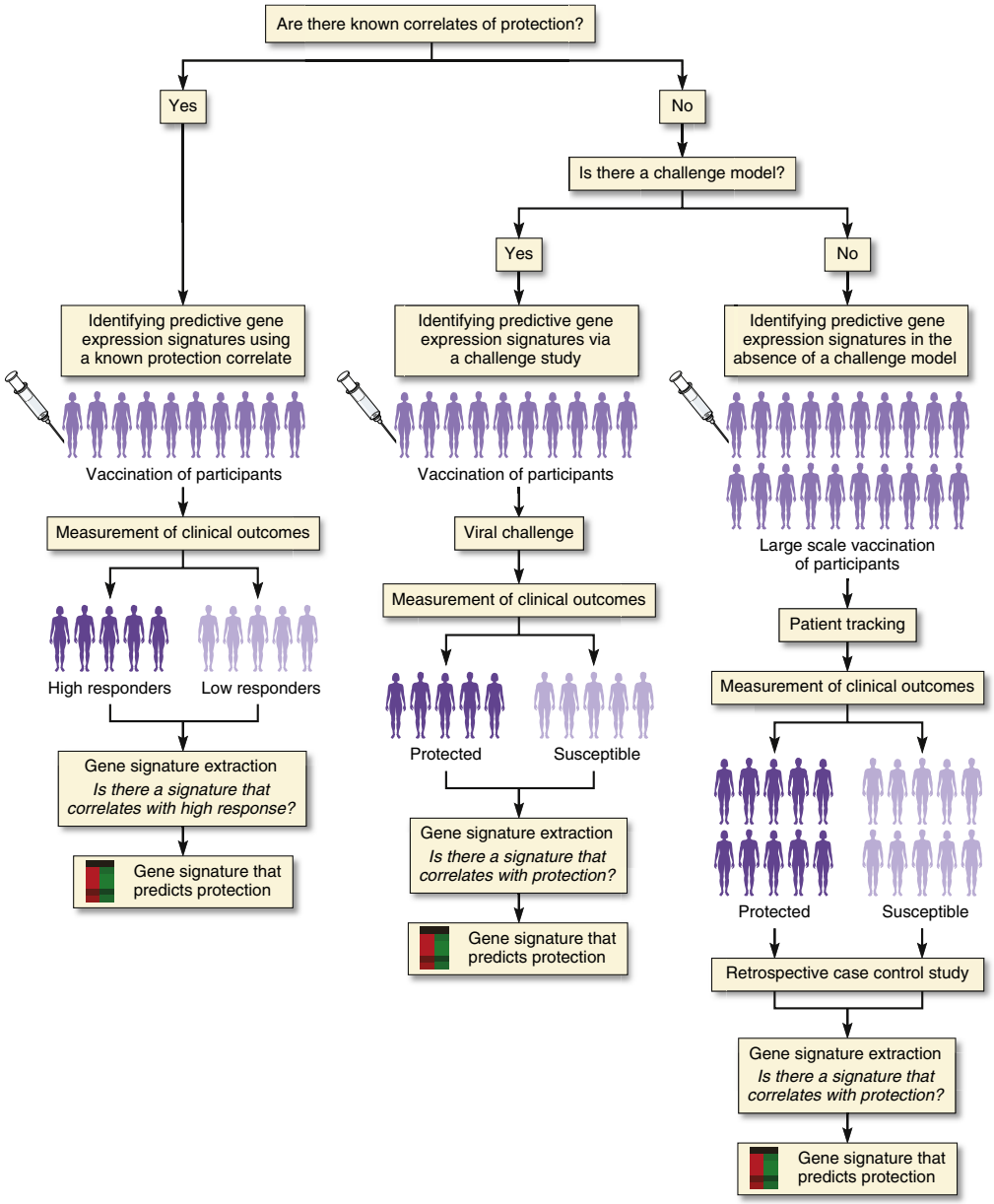

FIGURE 3 Systems approaches can be used to identify gene expression signatures predictive of vaccine protection. The ease of gene signature identification depends on whether correlates of protection from a disease are known and whether there is a human challenge model. When no established correlate of protection and no human challenge model exist, systems approaches can be applied to retrospective studies to identify novel correlates. *Adapted from Pulendran (2014).*

antibiotic-treated mice have a defect in the production of virus-specific humoral and cellular responses to influenza A virus infection. Neomycin-sensitive bacteria may be required for optimal lung immunity because they effectively "prime" the expression of proinflammatory cytokines, which in turn promote robust immunity to influenza. TLR5, a sensor of bacterial flagellin, also appears to have a role in vaccine immunity. TLR5 expression on day 3 after IIV vaccination correlates with the magnitude of the antibody response in humans, and TLR5$^{-/-}$ mice have weaker antibody responses to IIV vaccination than wild-type mice. Apparently, intestinal microbiota stimulate TLR5 leading to an enhancement of IIV immunity. Similar results were found for rotavirus vaccination where antibiotic treatment before vaccination results in a more durable rotavirus antibody response in mice.

5.6 The Future of Systems Vaccinology

Systems vaccinology studies generally rely upon taking measurements hours or days after vaccination, but it

is possible that the quality of a vaccine response can be predicted using prevaccination information. When PBMC transcriptomic data, serum titers, cell subpopulation frequencies, and B-cell responses are assessed before and after vaccination in patients vaccinated with IIV, the frequency of various cell populations on day 1 can be used to predict the response.

Recent studies using a BALB/C mouse model of influenza infection suggest that peptide microarrays may also hold promise for predicting vaccine performance. Sera from mice vaccinated with live or killed influenza virus were screened by peptide microarray. The mice were then challenged with wild influenza virus to determine whether they were protected. Immunosignatures derived from peptide microarrays were more predictive of vaccine efficacy than ELISA, and could be used to identify the protective epitopes within a vaccine. This approach may potentially identify pathogen-specific antigens that are needed to confer protective immunity.

6. VACCINES AND PUBLIC HEALTH

There are about 20 safe and effective viral vaccines available for use throughout the world. This armamentarium represents one of the most cost-effective tools in public health and preventive medicine. Nevertheless, viral vaccines are underutilized in many parts of the world, mainly due to the absence of health systems for well-child care. For instance, it is estimated that worldwide more than 100,000 children die each year from measles, a totally preventable disease with a safe and inexpensive vaccine.

In developed countries, there are small groups of individuals who refuse to have their children immunized. These vaccine "refuseniks" are motivated by several different imperatives. Some base their attitudes upon religious beliefs and others upon the view that vaccines are a risk factor for diseases such as autism. Although there is strong scientific evidence against most purported vaccine-associated disease risks, the antivaccine movement remains robust.

7. REPRISE

The mechanisms whereby immunization protects against viral disease depend upon the pathogenesis of the specific infection. In some instances, preformed neutralizing antibody intercepts invading virus at the portal of entry and partially or (rarely) totally inactivates the viral inoculum. In other instances, circulating antibodies neutralize virus entering the blood, preventing dissemination to or within key target organs or tissues. In some instances, CD4+ or CD8+ T cells, B cells, and perhaps other lymphoreticular elements cooperate to provide vaccine-induced protection that is more effective than that mediated by any single component of the immune response. In most vaccine-protected individuals, exposure to wild-type virus usually initiates a mild infection that is rapidly cleared through CD8+ effector lymphocytes and antibody. However, there are many variants in the mechanisms by which effective vaccines protect, and some vaccines violate the general "rules" of vaccinology.

There are a number of modalities that have been used to formulate vaccines. Most human vaccines now in use are based on attenuated live virus strains, inactivated viruses, or viral proteins. However, other platforms can be used to present antigens, including recombinant viruses, replicons, and naked DNA. Undoubtedly, some of these will be used for future vaccines. Multiple parameters determine optimal vaccine modalities, including immunogenicity, safety, route of administration, public acceptability, and ease and cost of production. Experience suggests that different modalities will be best suited for different vaccines.

In the past, vaccine development has depended upon an empirical strategy involving repetitious trial and error; a cumbersome and inefficient process. Systems biology offers a new and important addition to the evaluation of candidate vaccines. Recent studies have developed omics profiles for successful existing vaccines, and these offer guidance for vaccines now under development. Systems approaches can also identify early innate responses, which are critical for an effective adaptive immune response. These new approaches and technologies therefore provide a potentially more efficient approach to vaccine development, which is badly needed for vaccines yet-to-be formulated, such as those against HIV or dengue. Such efforts are imperative, since vaccines are arguably the most effective approach to controlling the viral diseases of mankind and animals.

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