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Chapter 15

Antiviral Vaccines: Challenges and Advances

Ali H. Ellebedy, Rafi Ahmed
Emory University School of Medicine, Emory Vaccine Center and Department of Microbiology and Immunology, Atlanta, GA, United States

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1 INTRODUCTION

Vaccination is the most effective means of preventing and controlling viral infections.[1] The eradication of smallpox and the significant progress made toward polio eradication are clear examples of the great impact of antiviral vaccines.[2,3] However, viral infections remain a major public health threat and a significant cause of death. Most of the antiviral vaccines introduced over the past century were empirically developed.[4] Poliomyelitis, measles, mumps, and rubella are examples of diseases that are now largely controlled thanks to these empirically developed vaccines.

The common factor among our most effective antiviral vaccines is that they were developed to mimic our natural immune response to the pathogen. For example, a single episode of measles confers lifelong immunity in the survivors. Hence, what we needed to do is induce a similar immune response.

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It is when we have to do better than "mother nature" that we have been facing substantial challenges in developing successful vaccines. For example, the immune response against viruses such as HIV, influenza, and respiratory syncytial virus (RSV) is either inadequate or outpaced by the pathogen's evolution. And while developing a broadly protective vaccine against such pathogens has been a colossal task, it is not impossible and similar missions have been successfully accomplished as in the case of anti-HBV and anti-HPV vaccines.

There is a growing list of emerging and reemerging viral infections against which an effective vaccine is yet to be developed. Recent technological advances in the areas of immunogen design, single cell transcriptomics, systems biology, gene delivery, epigenetics, nanoparticles, and adjuvants expanded our understanding of how vaccines work and provide potentially new platforms that could be harnessed to develop vaccines against challenging and emerging viral pathogens.[1]

2 TYPES OF CURRENTLY LICENSED ANTIVIRAL VACCINES

1. *Live viral vaccines*. Live virus vaccines are prepared from viral strains that have been attenuated, but retain their ability to replicate in the human host and thus their ability to induce protective immune responses.[5] Out of the 15 viruses against which antiviral vaccines are currently licensed in the United States, nine are live attenuated (Table 15.1). There are several immunological advantages for utilizing the live attenuated antiviral vaccine platform; (1) the replication of the attenuated vaccine strains in host cells allows for the potential activation of antigen-specific $CD8^+$ T-cell responses; (2) the potential of eliciting a mucosal immune response (eg, IgA), where the portal of entry for many viruses resides. Several methods have been used to attenuate virus strains in order to be safely used as human vaccines. One method depended on the use of viral strains that are specific to a different host as vaccine strain. The oldest example of such strategy is the use of cowpox virus to vaccinate humans against smallpox.[6] Another strategy relied on attenuation of the virus by passaging it in unnatural host or cells. Examples of this approach are the development of 17D, the yellow fever vaccine strain and polioviruses.[7] Introducing the virus via unnatural route is a strategy used to develop adenovirus Types 4 and 7 vaccine, which is given orally.[8] Finally, generation of temperature sensitive mutants such as the live attenuated influenza vaccines.[9]
2. *Inactivated whole viral vaccines*. Whole inactivated virus preparations are prepared by simply inactivating viral particles by heat, UV irradiation or by special chemical treatments. Formalin and beta-propiolactone are the most commonly used chemicals for this purpose. Vaccines against polioviruses and influenza were among the first to be prepared using this strategy.[10,11] Immunogenicity of these viral preparations is usually robust as they contain

TABLE 15.1 List of the Various Characteristics of Currently Licensed Antiviral Vaccines in the United States[a]

Virus	Number of serotypes included per disease	Platform	Adjuvant	Route of administration	Test used to measure the correlate of protection	Trade name
Adenovirus	2 (Types 4 and 7)	Live attenuated	No	Oral	Neutralization	No trade name, Barr Labs
Hepatitis A	1	Inactivated	Aluminum salts	Intramuscular	ELISA	Havrix, GSK
	1					VAQTA, Merck
Hepatitis A	1	Inactivated				Twinrix, GSK
Hepatitis B	1	VLP				
Hepatitis B	1	VLP	Aluminum salts	Intramuscular		Recombivax HB, Merck
	1					Engerix-B, GSK
Papillomavirus	4 (Types 6, 11, 16, 18)	VLP	Aluminum salts	Intramuscular		Gardasil, Merck
	9					Gardasil 9, Merck
	2 (Types 16 and 18)		AS04			Cervarix, GSK
Influenza	1 (2009 pH1N1)	Split	No	Intramuscular	HAI[b]	No trade name, CSL
		Live attenuated	No	Intranasal		No trade name, MedImmune
		Split	No	Intramuscular		No trade name, ID Biomedical
		Subunit	No			No trade name, Novartis
		Split	No			No trade name, Sanofi Pasteur

(Continued)

TABLE 15.1 List of the Various Characteristics of Currently Licensed Antiviral Vaccines in the United States[a] *(cont.)*

Virus	Number of serotypes included per disease	Platform	Adjuvant	Route of administration	Test used to measure the correlate of protection	Trade name
	1 (H5N1)	Split	No			No trade name, Sanofi Pasteur
		Split	AS03			No trade name, ID Biomedical
	3 (H1N1, H3N2, and type B)	Subunit	MF59			FLUAD, Novartis
		Split	No			Afluria, CSL
		Split	No			FluLaval, ID Biomedical
		Live attenuated	No	Intranasal		FluMist, MedImmune
		Split	No	Intramuscular		Fluarix, GSK
		Subunit	No	Intramuscular		Fluvirin, Novartis
		Subunit	No	Intramuscular		Agriflu, Novartis
		Split	No	Intramuscular or Intradermal		Fluzone, Sanofi Pasteur
		Subunit	No	Intramuscular		Flucelvax, Novartis
		Recombinant	No	Intramuscular		Flublok, Protein Sciences
	4 (H1N1, H3N2, and two type B strains)	Live attenuated	No	Intranasal		FluMist Quadrivalent, MedImmune
		Split	No	Intramuscular		Fluarix Quadrivalent, GSK
		Split	No	Intramuscular		Fluzone Quadrivalent, Sanofi Pasteur
		Split	No	Intramuscular		FluLaval Quadrivalent, ID Biomedical

Japanese Encephalitis	1	Inactivated	Aluminum salts	Intramuscular	Neutralization	Ixiaro, Intercell Biomed
			No	Subcutaneous		JE-Vax, BIKEN-Osaka
Measles and mumps[c]	1	Live attenuated	No	Subcutaneous	Neutralization	M-M-Vax, Merck
Measles, mumps, and rubella	1	Live attenuated	No	Subcutaneous	Neutralization (measles and mumps) Immunoprecipitation (rubella)	M-M-R II, Merck
Measles, mumps, rubella, and varicella	1	Live attenuated	No	Subcutaneous	Neutralization (measles and mumps) Immunoprecipitation (rubella) FAMA gp ELISA (varicella)	ProQuad, Merck
Poliovirus	3 (Types 1, 2, 3)	Inactivated	No	Intramuscular or Subcutaneous	Neutralization	IPOL, Sanofi Pasteur
Rabies	1	Inactivated	No	Intramuscular		Imovax, Sanofi Pasteur
	1					RabAvert, Novartis
Rotavirus	1	Live attenuated	No	Oral	Serum IgA	ROTARIX, GSK
	5 [G1, G2, G3, G4, and P1A(8)]	Live attenuated	No	Oral		Rotateq, Merck
Smallpox	1	Live attenuated	No	Percutaneous	Neutralization	ACAM2000, Sanofi Pasteur
Varicella	1	Live attenuated	No	Subcutaneous	FAMA gp ELISA	Varivax, Merck
Yellow fever	1	Live attenuated	No	Subcutaneous	Neutralization	YF-Vax, Sanofi Pasteur
Zoster	1	Live attenuated	No	Subcutaneous	CD4 T cell Lymphoproliferation	Zostavax, Merck

[a] *Vaccines that have been licensed, but their production has been discontinued are not included.*
[b] *HAI stands for hemagglutination inhibition assay.*
[c] *Measles, mumps, and rubella are also licensed to be used in combination with other antibacterial and antipoliovirus vaccines under different trade names that are not included in this table.*

multiple pathogen-associated molecular patterns (PAMPs) that could engage several of the host innate immune receptors such as the toll-like receptors (TLRs).[12] For polio, an incident of incomplete inactivation of the vaccine preparation resulted in an outbreak of paralytic poliomyelitis in the United States, the so-called "Cutter Incident."[13,14] Hence, safety of such preparations has always been a concern.

3. *Subunit vaccines*. Due to the increased risk of reactogenicity associated with whole inactivated virus vaccine preparations, purified preparations that contain the main targets of protective immune responses were developed.[15] Subunit vaccines that contain the surface glycoproteins of influenza and hepatitis B viruses are currently licensed (Table 15.1). Subunit vaccines show an improved reactogenicity profile compared to whole inactivated virus preparations, but this is usually at the expense of the immunogenicity of the vaccine. When administered with adjuvants, immune responses to these vaccines can be significantly enhanced.[16]
4. *Recombinant viral proteins*. The advance in methods of protein manufacturing made it possible to express desired viral proteins on a large scale to be used as vaccine antigens. Bacterial, yeast, insect, and mammalian cell lines have been used for this purpose.[17] A recombinant vaccine that contains the main surface glycoprotein of influenza viruses, the hemagglutinin or HA, Flublok,[18] has recently been licensed in the United States (Table 15.1). As discussed later in the chapter, some recombinant viral proteins such as the surface antigen of hepatitis B viruses tend to form virus-like particles upon expression.
5. *Virus-like particles (VLPs)*. VLPs are multimeric structures assembled from viral structural proteins. They often display viral surface proteins in a high-density repetitive manner on their surface, which may play a role in the enhanced immunogenicity observed with this kind of vaccines compared to recombinant viral proteins.[19–22] In 1986, the first antiviral VLP vaccine (against hepatitis B) had been licensed.[23] The vaccine is based on the hepatitis B surface antigen or HBsAg, which upon expression in yeast forms spherical VLPs that are then adsorbed onto alum as adjuvant. Recently, another antiviral VLP vaccine against human papillomavirus has been licensed.[22]

3 HOW ANTIVIRAL VACCINES MEDIATE PROTECTION?

Viral infections can be broadly classified into three main categories depending on the nature of the infection:

1. Acute infections caused by antigenically stable viruses. Infection with- or vaccination against such viruses provides a lifelong immunity to clinical reinfection. Examples of such viruses include smallpox, yellow fever, measles, mumps, rubella, and polio. Developing effective vaccines against these viruses has been relatively a straightforward process.

2. Acute infections caused by rapidly mutating viruses. The immunity acquired against such viruses through infection or vaccination is usually short-lived because of the antigenic changes, and recurrent immunization is often required. The clearest example for such viruses is influenza.[24]
3. Chronic infections caused by rapidly mutating viruses. HIV and HCV are prime examples for such viruses. Developing vaccines against such viruses have proved to be a very daunting task.[25–27]

Two main effector arms of the adaptive immune response that are induced by antiviral vaccines mediate protection against viral infections: antibodies and T cells.[1,28] While we will briefly discuss these two arms later in the chapter, it is important to understand that other immune effectors such as cytokines secreted by innate immune cells activated by the vaccine itself or by coadministered adjuvants could also directly contribute to controlling the viral burden. Also, the initial innate immune recognition of the vaccines/adjuvants is essential not only for triggering the adaptive immune responses, but also for determining the quality and duration of such responses.[28]

3.1 Antibodies

Given the speed with which most viruses replicate, possessing protective levels of preformed antibodies is the best strategy to protect against most viral infections. Therefore, a major immunological goal for antiviral vaccines is to elicit high and durable levels of antigen-specific antibodies.[29] Preferably these antibodies are induced at the portal of virus entry. To date, all human vaccines that have shown considerable success in combating viral infections depend on antibodies as the primary mediators of protection.[1] The process of generating these antibodies starts when a vaccine antigen encounters and binds to its specific B cell. In the presence of cognate CD4 T-cell help, these vaccine specific B cells start to expand.[30] Some of the activated B cells differentiate into plasmablasts whose function is to secrete an early protective wave of antigen-specific antibodies.[30] In a primary vaccination, those early antibodies are mostly IgM and bind to the vaccine antigen with a relatively low affinity. A subset of the activated B cells will continue expanding forming specially organized structures in the secondary lymph nodes known as germinal centers (GCs).[30] GCs are where vaccine-specific B cells with the highest antigen binding affinity are preferentially selected and also where the majority of antibody isotype-switching from IgM to IgG and IgA occurs.[31]

Antibodies can protect against viral infections via several ways:

1. When induced to sufficient levels, antibodies prevent infection by blocking the binding of viruses to their receptors on host cells. These are called "neutralizing" antibodies, and their target epitopes lie primarily within the surface glycoproteins of enveloped viruses or the capsid proteins of non-enveloped ones. The target epitopes of neutralizing antibodies are usually conformational.

2. Opsonization and phagocytosis of viral particles by neutralizing and non-neutralizing antibodies that bind to the surface of viral particles.
3. Lysing infected cells that express viral antigens on their surface via the complement pathway or through antibody-dependent cellular cytotoxicity (ADCC). For ADCC, cells mediating the lysis of infected cells such as natural killer (NK) cells recognize the antibody labeling infected cells via Fc receptors.[32]

The cells responsible for the maintenance of antigen-specific serum antibody levels following vaccination and infection are long-lived plasma cells. These cells are generated during the germinal center reaction and reside mainly in the bone marrow.[33–36]

3.2 T Cells

The main two subsets of T cells are $CD4^+$ and $CD8^+$ T cells. Through at least one of these two subsets T cells participate in the protection mediated by all antiviral vaccines. The main function of T cells is to provide help to B cells ($CD4^+$) or clear the infection ($CD8^+$), and not to prevent the infection. In contrast to antibodies that recognize epitopes in 3-dimensional conformation, T cells recognize linear peptides from the infecting agent that are expressed on MHC molecules on the surface of virus-infected cells. Some of these peptides come from viral proteins that do not exhibit extensive antigenic variation making T cells an important mechanism of protection against rapidly evolving viruses.[37] $CD4^+$ T cells contribute to antiviral vaccine effectiveness in several ways; secreting cytokines such as IFN-γ and TNF and supporting the activation of B cells and $CD8^+$ T cells (Th1); secretion of IL-4, IL-5, IL-13, and other cytokines to support B-cell activation and differentiation (Th2); triggering the formation and maintenance of the GC reaction via the expression of CD40L and secretion of IL-21 (Tfh). $CD8^+$ T cells, on the other hand, clear virus infected cells by directly killing those cells (through the release of perforins and granzymes) or indirectly by secreting inflammatory cytokines. $CD8^+$ T-cells can control viral burden and thus limit the severity of the disease. In the United States, there is currently no licensed antiviral vaccine that works solely via the induction of T cells, but they significantly contribute to the protective effect of several antiviral vaccines such as those against the measles and zoster viruses.

4 MODERN APPROACHES TO STUDYING IMMUNE RESPONSES INDUCED BY ANTIVIRAL VACCINES

1. *Systems vaccinology*. Systems biology is the integrative analysis of all the components involved in a complex biological process.[38] It includes the analysis of the genes (eg, transcriptomics), the molecules (eg, proteomics) and cells (eg, multiparameter flow cytometry) that were "perturbed" in the course

of a certain biological process such as an active immune response.[38] Systems vaccinology refers to the use of systems biological approaches in analyzing human immune responses to vaccination.[38] Advantages of using systems vaccinology approaches include; (1) gaining new insights about the mechanisms of antiviral vaccine-mediated immunity; (2) defining new molecular signatures triggered by the immune response to various vaccines and adjuvants; (3) the potential use of those molecular signatures as alternative correlates of protection.[38] Moreover, applying systems biology approaches highlighted the important role played by the early innate immune responses in triggering adaptive immune responses to various antiviral vaccines. This role is usually overlooked when assessing the effectiveness of such vaccines using traditional correlates of protection (Table 15.1). Analysis of the immune response to the yellow fever YF-17D vaccine was one of the earliest examples of utilizing systems vaccinology and it provided a proof of concept for such approach.[39] The approach was later applied to other antiviral vaccines such as those against influenza.[40] In summary, these exciting advances highlight the potential of systems biology to transform our understanding of not only how antiviral vaccines work, but also the mechanisms of immune regulation in general.

2. *Multiparameter flow cytometry*. The introduction of flow cytometry has revolutionized how we analyze immune responses.[41] It allowed us to examine not only the physical parameters of various immune cells, such as cell size and granularity at different states, but also the expression levels of many proteins either on the cell surface or inside the cells simultaneously. These analyses provide us with enormous insights about a variety of biological processes that an immune cell experiences such as activation, proliferation, differentiation and death when responding to a foreign antigen. At the beginning the number of fluorescent dyes (which are conjugated to antibodies so that each dye could be assigned to one molecule) that could be used simultaneously was limited to one or two. This number has dramatically expanded (up to 18) over the past two decades.[41] These advances came from the introduction of novel fluorescent dyes that provided additional excitation and emission spectra to be used. Perhaps one of the most important insights that came from flow cytometric analyses is the defining of the multiple lineages of B and T cells that are elicited by various antiviral vaccines.[42] Also, how the differentiation status and fates of these lineages change over time.

The number of parameters that can be measured per cell has recently been expanded to more than 40 by the integration of mass spectrometry with single-cell fluidics (eg, CyTOF).[43] In CyTOF, antibodies are labeled with heavy metal ion tags instead of fluorochromes. Another major advantage of CyTOF is the elimination of signal interference resulted from spectral overlap of the various fluorescence dyes.[43] Reports using these new technologies are already revealing new insights about the complexity and interconnectedness of the different subsets of virus-specific T cells generated after infection.[44]

3. *Single-cell transcriptomics*. While the aforementioned systems approaches have provided exceptional insights on how our immune system works, some of them measure only the "average" of the response from sometimes a highly heterogeneous cell population. Gene expression analyses for example are performed using total mRNA purified from highly heterogeneous cell populations. In the latter scenario, the end result of the analysis would probably be biased toward the most abundant fraction of the heterogeneous cell population because of their larger contribution to the overall RNA content. Therefore, identifying rare subsets of cells using such technologies is arguably impossible.[42] Single-cell RNA sequencing (RNA-seq) is an important extension of the gene expression arrays technologies. It enabled us to interrogate the genome-wide expression profile of individual cell mRNA in an unbiased way. In addition, single-cell RNA-seq revealed some other transcriptional features in single cells, such as splice variants, allele-specific expression, and the potential discovery of previously uncharacterized genes.[42]
4. *Epigenetic regulation of immune responses*. Epigenetics refer to histones and DNA modifications, which regulate the access of different transcription factors and polymerases to transcriptional regulatory elements in chromatin.[45] Such modifications regulate gene expression and provide cells with a mechanism to retain acquired transcriptional programs throughout cell division. Given the essential role of epigenetics in deciding and maintaining cell fate, a huge amount of interest has recently been given to studying the role of epigenetics in immune responses to viral infections and vaccines. There are several aspects through which immune responses to antiviral vaccines could be modified via epigenetics; (1) defining the epigenetic programs associated with memory B and T cells with optimal quality; (2) directing the differentiation of immune cells into the most desired fate (eg, Th1 vs. Th2 CD4 T cells); (3) reversing an undesirable fate of antigen-specific cells (eg, rejuvenation of exhausted CD8 T cells in chronic viral infections).[45] Characterization of the gene expression and epigenetic programs associated with antiviral vaccine-induced memory B and T cells will provide further insight into the protective quality of the poised effector recall response.[45]
5. *Next generation sequencing (NGS) of the B and T cell receptor repertoires*. Next generation sequencing (also referred to as deep sequencing) has significantly impacted how we analyze many biological phenomena;[46] immune response is no exception. In regard to immune responses to antiviral vaccines, deep sequencing has affected both sides of the equation: the virus/vaccine side and the adaptive immune side. Most RNA viruses such as HIV and influenza exist as quasispecies and the introduction of deep sequencing technology has afforded us a higher resolution look at such diversity instead of analyzing individual viruses.[1] Each clonal pool of antigen-specific B or T cell share a distinct junction region that is formed at the site of the B-cell receptor (BCR) heavy or T-cell receptor (TCR) beta gene segments ligation.[47]

Interrogating the B-cell repertoire by deep sequencing allowed us to study the diversity of B-cell responses to viral infections and vaccinations.[46] By diversity here we refer to how many distinct clonal pools are participating in the B- or T-cell response to a particular vaccine. This is particularly important when analyzing responses to vaccines against highly variable viruses such as influenza and HIV. Against such viruses it is better to have a polyclonal response that is directed against several epitopes than a focused response. Moreover, tracking B-cell clonal pools that secrete antibodies of desired specificity or quality has helped in studying the ontogeny and evolution of such responses.[48] Similar analyses have been performed on the alpha and beta chain of the TCRs.[49]

6. *Generation of human monoclonal antibodies (mAbs)*. More than a 100 years ago, Emil von Behring developed passive immunotherapy using serum to treat infections, such as diphtheria and tetanus and was awarded the Nobel prize in Physiology or Medicine in 1901.[50] The advent of hybridoma technology in the mid-1970s introduced the concept of generating a monoclonal antibody with a single defined specificity.[51] Over the past two decades, tremendous efforts have gone into developing technologies to generate human mAbs. The currently most widely used methodologies to generate human mAbs are:
   a. Phage display libraries: As the name indicates, phages are designed to express single-chain variable antibody fragments (scFvs) or antigen-binding fragments (Fabs) on their surface and screened for binding to the desired antigen. The libraries are constructed from the variable genes of B cells isolated from vaccinated individuals or from convalescent patients. This method has been successfully used to generate neutralizing mAbs against many viruses including West Nile, rabies, severe acute respiratory syndrome (SARS) virus, hepatitis A, HIV, Ebola, yellow fever, hepatitis C, measles and influenza.[52] A major drawback of this method is that it cannot be used to examine the repertoire or the immunodominance hierarchy of the antigen-specific B-cell response as the antibody fragments displayed were generated by random pairing of the BCR heavy and light chains and not from a naturally existing pairing.[53]
   b. B-cell immortalization: B cells can be immortalized by Epstein–Barr virus (EBV) mediated transformation.[54] Immortalized B cells can then be stimulated to secrete antibodies and those antibodies are screened for the desired specificity. B-cell pools secreting the desired antibody are then cloned by limiting dilution into single cells and the BCR genes are sequenced. This method has been used to generate mAbs against many viruses including influenza, HIV, SARS, dengue, and RSV.[55–58] While this method is effective in isolating mAbs from rare memory B cells,[59] it is labor-intensive, as it requires the screening of thousands of immortalized memory B cells in order to isolate few mAbs with the desired specificity.

c. Single cell cloning and expression of mAbs: This is the most recent technology and also the most efficient.[60,61] In this approach, the heavy and light chain genes of single-cell sorted B cells are amplified and cloned into antibody expression vectors. Single antigen-specific B cells can be sorted by flow cytometry based on their surface phenotype (eg, sorting of plasmablasts from blood following vaccination)[62] or based on their binding to a desired antigen.

Human mAbs have expanded our understanding of human B-cell responses to viral infections. Through the generation of mAbs following various viral infection and vaccination we were able to map the viral targets of our most protective immune responses. Most importantly, they revealed some of the subdominant epitopes within viral proteins that are now being extensively examined, as discussed later in the chapter, as targets for broadly neutralizing mAbs and potential cores for new immunogens.

5 NEXT GENERATION VACCINE PLATFORMS

1. *Structure-based immunogen design*. As mentioned earlier, the design an immunogen to be used as an antiviral vaccine has always been an empirical process. The immunogen was picked based on its ability to elicit a detectable protective immune response. While this process was sufficient for many viruses, for some challenging viruses such as HIV and influenza, a deeper analysis of the epitopes targeted by neutralizing antibodies was needed.[63] The traditional way of determining the amino acid residues within a viral protein that are recognized—and thus mediate virus neutralization—by a certain mAb is the generation of viral escape mutants. This method was instrumental in mapping the major neutralizing epitopes within the influenza HA molecule.[64] However, this method has several drawbacks; (1) some neutralizing mAbs fail to generate escape mutants such as the influenza broadly neutralizing mAbs recognizing the HA stem region (discussed later in the chapter) and therefore could not be mapped using this approach; (2) the increased risk and logistic difficulties associated with the generation of escape mutants against certain viruses, such as the highly pathogenic avian influenza viruses and Ebola; (3) given that the majority of neutralizing mAbs recognize conformational epitopes so identifying a single or few amino acid residues that contribute to binding does not provide a complete picture; (4) a change of an amino acid residue in an escape mutant does not necessarily mean that this residue is the point of contact between the viral protein and the mAb. A change of the epitope conformation induced by the change of an adjacent amino acid residue could also be responsible for the generation of an escape mutant.

   In 1990, the first crystal structure of a viral glycoprotein-antibody complex was published.[65] Solving such structures for many viral glycoproteins has allowed us to examine the binding of antibodies to their respective

epitopes at the atomic level. More importantly, it provided the basis for rationally designing viral immunogens that could—at least theoretically—induce an immune response enriched with antibodies with a desired specificity. This idea has recently been tested with partial success in the efforts to generate targeted antibody responses to HIV, RSV, and influenza.[1] These early experiments have also revealed that more work is needed to fully understand how these complex epitopes are recognized by B cells in vivo and how to minimally design an immunogen without interrupting its stability as a protein or the antigenicity of the epitope.[1]

2. *DNA- and RNA-based vaccines*. The concept of using naked DNA as a vaccine was introduced in the early 1990s.[66] It rapidly gained traction mainly due to its simplicity and versatility. While the early clinical trials demonstrated the safety of DNA vaccines, it also revealed that they were poorly immunogenic. The immunogenicity of DNA vaccines has been improved through different methods; (1) improving the efficiency of DNA delivery to enhance the cellular uptake of the plasmid DNA; (2) the use of adjuvants either in physical form or encoded on separate plasmids; (3) optimizing the sequence of the DNA vaccine to enhance the expression and immunogenicity of the encoded protein.[66] DNA vaccines against a variety of viruses are now being tested at different stages of clinical trials.

   Advances in the methods of mRNA synthesis and stabilization have paved the way for the possibility of using mRNA as vaccine platforms.[67] The ability of mRNA to stimulate several of the innate immune receptors (eg, TLR3 and TLR7/8) gives them an intrinsic adjuvant activity. The approach has been boosted by the recent introduction of self-amplifying RNA strategy, which works by delivering the alphavirus genes encoding the RNA replication machinery along with the recombinant viral target antigen resulting in enhanced antigen expression.[67]

3. *Vector-based vaccines*. Vectored-based vaccines could be considered a type of DNA vaccines where an attenuated virus or bacterium is used to introduce microbial DNA to host cells. The most commonly used virus vectors are adenoviruses, alphaviruses, and poxviruses.[68] As for bacteria, strains belonging to *Bacillus Calmette-Guerin, Listeria monocytogenes*, and *Salmonella typhi* are being tested as vectors for human vaccines.[69]

6 HARNESSING THE TECHNOLOGICAL ADVANCES TO DEVELOP VACCINES AGAINST CHALLENGING AND EMERGING VIRUSES

Viral pathogens against which an effective vaccine is yet to be licensed can be broadly grouped into two categories; challenging viruses and emerging viruses. Examples for challenging viruses are HIV, HCV,[70–72] RSV, CMV,[73] HSV-2,[74,75] EBV, and dengue. For a variety of reasons, developing an effective vaccine against these viral pathogens has been a formidable task despite the tremendous

TABLE 15.2 Some of the Antiviral Vaccine Candidates That are in Advanced Stage of Development (Phase 2 or Beyond)[a]

Virus	Name of vaccine candidate	Manufacturer or sponsor	Development phase	References
HCV	Ad6NSmut	GSK	Phase 1/2	[70–72]
	TG4040	Transgene	Phase 2	
	GI-5005	GlobeImmune	Phase 2	
CMV	ASP-0113	Astellas Pharma	Phase 3	[73]
SV-2	GEN-003	Genocea Biosciences	Phase 2	[74]
	HerpV	Agenus	Phase 2	[75]
HIV	AGS-004	Argos Therapeutics	Phase 2	[76–83]
	HIV recombinant	GSK	Phase 2	
	AIDSVAX	GeoVax	Phase 2	
	Vacc-4x	Bionor Pharma	Phase 2	
	VRC-hIVADV014-00-VP	GenVec/VRC	Phase 2	
RSV	RSV F Protein	GSK	Phase 2	[84,85]
	RSV F Nanoparticle	Novavax	Phase 3	
Dengue	Dengvaxia	Sanofi Pasteur	Phase 3 (approved in Brazil)	[86,87]
	DENVax	Inviragen	Phase 2	
Ebola	ChAd3-ZEBOV	GSK/PHAC	Phase 2/3	[89]
	VSV-EBOV	New Link Genetics/Merck	Phase 2/3	
Norovirus	G1-I/GII-4 VLP	Takeda Vaccines	Phase 2	[90]

[a]This list is not exhaustive. For example, it does not include vaccine candidates for viruses against which successful vaccines have already been licensed such as influenza, HPV, Zoster, and rabies.

efforts. Great amounts of resources have gone into developing a vaccine against HIV,[76–83] but this mission has proved to be the most arduous so far (challenges and prospects are discussed later in the chapter). As for influenza, effective vaccines against seasonal and potentially pandemic influenza virus strains have been licensed. However, these vaccines (as discussed later in the chapter) do not offer broad protection against these rapidly evolving viruses. Vaccine candidates against RSV[84,85] and dengue[86,87] have now entered advanced stages of clinical testing (Table 15.2).

Emerging viral pathogens include Ebola, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), hendra, nipah, Marburg, chikungunya, lassa, Crimean-Congo hemorrhagic fever, and zika viruses. Infections with most of these viruses are limited to certain endemic areas, which in turn make the decision of developing a vaccine against such viruses not an economically favorable one. However, in the wake of the 2014 massive Ebola outbreak that ravaged West Africa this perception could change. For Ebola, recent studies suggest that robust immune responses could be detected in convalescent patients,[88] indicating that developing a protective vaccine against this pathogen is doable. Indeed, many vaccine candidates have shown promising results in clinical trials.[89] Two of these candidates are ready for Phase 3 testing.[89] Other antiviral vaccines that are in advanced stages of clinical testing include vaccines developed against CMV and norovirus.[73,90] Later in the chapter we will discuss the challenges facing developing a vaccine against HIV, influenza (universal), and RSV, and how recent technological advances could help in overcoming such challenges.

6.1 The HIV Challenge

Efforts to develop a vaccine against HIV started in the mid-1980s[91] and the fact that there is still no licensed vaccine yet despite the plethora of resources invested shows the enormity of the task. The challenges that impede developing a vaccine against HIV stem from the following points:[92–94]

1. Like most RNA viruses, HIV viruses continually mutate and evolve leading to the emergence of new variants even within an infected individual. This necessitates that for any vaccine to be successful, it has to elicit an immune response with enough breadth to protect against such extensive diversity.
2. The correlates of protection against HIV infection are not well established. A common factor for viruses against which a vaccine has successfully been developed is that we know which immune effector mediates protection. Correlates of protection are usually defined by analyzing immune responses in individuals who have recovered from infection or showed less susceptibility to such infection. Complete recovery from HIV infection is not common occurrence, if at all. This is at least partially because the virus infects $CD4^+$ T cells, which orchestrate the two arms of adaptive immune responses: B cells and $CD8^+$ T cells.
3. There is a knowledge gap in regard to which protein/portion of the viral proteins is the most antigenic and immunogenic and thus best suited as a vaccine antigen. Also, whether a specific structural conformation is required for such protein to elicit a protective immune response is not clear.

The disappointing results of the early vaccines that were designed to solely induce $CD8^+$ T-cell responses [1] has refocused anti-HIV vaccine efforts on generating protective broadly neutralizing antibody responses. This notion was augmented by the modest success of the RV144 HIV vaccination trial conducted in

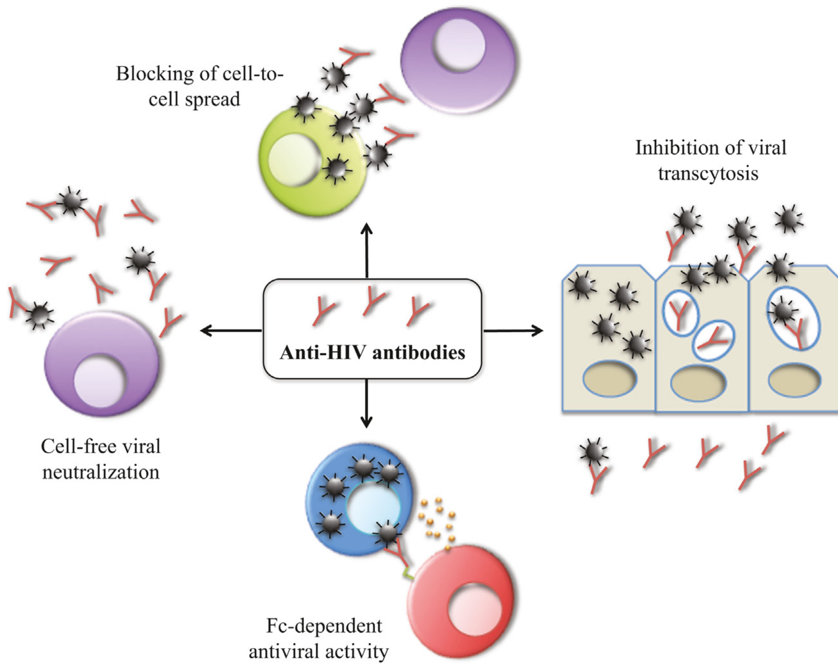


FIGURE 15.1 Potential mechanisms of neutralizing antibody-mediated protection against HIV. There are at least four different mechanisms by which anti-HIV neutralizing antibodies could block the virus. First, they can block the initial attachment of the virus to its receptor/coreceptor on the target cells. Second, they can block viral spread through cell-to-cell transmission. Third, they can aid in the clearance of infected cells through Fc-dependent mechanisms such as antibody-dependent cell-mediated cytotoxicity. Fourth, anti-HIV antibodies could inhibit the passage of HIV-1 from the lumen to the basolateral pole (HIV-1 transcytosis). *(Source: Adapted from Ref. [97].)*

Thailand in which subjects were primed with a replication-defective canarypox viral vector expressing HIV gp120, gag, and pol proteins, and then later boosted with the gp120 protein.[95] The rate of HIV infection among subjects who received the experimental vaccine was 30% lower than that in volunteers who received the placebo.[95] A correlation of protection with the antibody response to the V2 region of the HIV envelope protein was later established.[96]

In vivo, anti-HIV antibodies could protect in several ways; (1) neutralize cell-free virions; (2) block cell-to-cell transmission; (3) clear infected cells by antibody-dependent cell-mediated cytotoxicity or ADCC; (4) block transcytosis of the virions from the lumen to the basolateral side of mucosal cells (Fig. 15.1).[97] The RV144 trial observations resulted in a sharp increase in the number of broadly neutralizing anti-HIV human mAbs that are being isolated and characterized.[98–101] Sequence analysis of these antibodies and structural examination of their interactions with HIV antigens at the atomic level have strengthened our understanding of how these broadly neutralizing antibodies

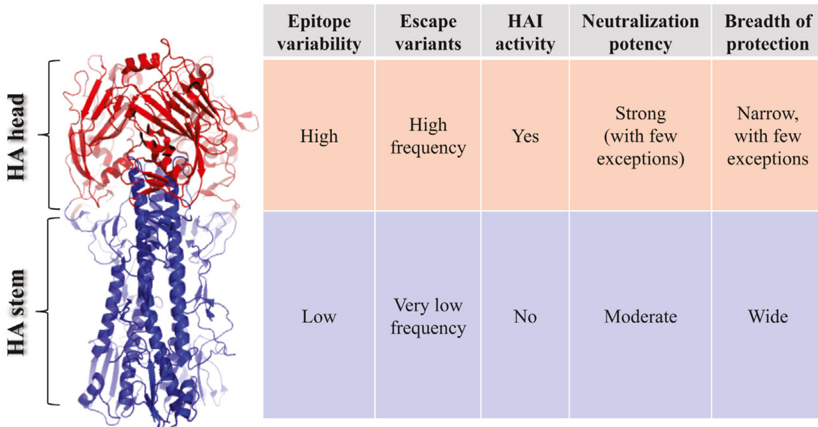
work. For example, we now know that broadly neutralizing antibodies are more likely to have either an unusually long CDR3 loop or an extremely high rate of somatic hypermutations.[102] Extensive research efforts are currently focused on learning how to elicit such antibodies by vaccination.[103] This target is being pursued from different angles:

1. The use of structurally inspired immunogens that mimic the epitopes of the broadly neutralizing mAbs. The hope is to get a B-cell immune response that is dominated by such antibodies. Early trials indicate that additional structural requirements may be needed for the desired epitopes to be recognized in vivo other than just presenting the epitopes in the right conformation.[104]
2. Possible need for a special immunization strategy that depends on sequentially exposing the immune system to different, but closely related HIV envelope proteins. The idea is to mimic how the immune system of chronically HIV-infected individuals experiences a rapidly evolving virus and ends up developing broadly neutralizing antibodies in a subset of them. Extensive deep sequencing analysis of the ontogeny of some of the broadly neutralizing antibodies has allowed us to dissect the affinity maturation steps required to attain such levels of reactivity.
3. Examining the evolution of the viral genome in infected individuals by deep sequencing. This approach would help us define the characteristics of the immunogens that should be used in the sequential immunization strategy discussed earlier.[1]

In summary, new technological advances have taught us that; (1); HIV exists as a swarm of viruses and evolves rapidly within infected individuals (deep sequencing analysis); (2) our immune system is capable of generating very potent and broadly neutralizing mAbs that could combat such viral diversity (generation human mAbs); and (3) we can design new immunogens to enrich B cell responses to HIV vaccines with the broadly neutralizing antibodies (structure-based immunogen design).

6.2 The Influenza Puzzle

The earliest trials to develop an influenza vaccine date back to the 1940s, shortly after the isolation of the first human influenza virus in 1933.[105] It was an inactivated whole virus preparation developed by the US military to be used in World War II.[10] Although these early vaccines were protective, controlling infections mediated by this respiratory pathogen continues to represent a formidable challenge. The dilemma stems from the ever-evolving nature of influenza viruses, which enables the viruses to escape preexisting immune surveillance.[106] Moreover, effective vaccines against influenza viruses work by eliciting antibody responses that primarily target the globular head of the HA, which is the most variable among virus proteins.[106] Therefore, it has been necessary to perform an annual revision of the antigens included in human seasonal influenza vaccines



	Epitope variability	Escape variants	HAI activity	Neutralization potency	Breadth of protection
HA head	High	High frequency	Yes	Strong (with few exceptions)	Narrow, with few exceptions
HA stem	Low	Very low frequency	No	Moderate	Wide

FIGURE 15.2 **Features of the epitopes within influenza HA globular head versus stem regions.** A structural view of influenza HA trimer with a listing of the features characterizing the epitopes within the HA globular head region versus those in the stem region.

to ensure that they match the circulating influenza strains. Currently, seasonal influenza vaccines include antigens from three (H1N1, H3N2, and an influenza B) or four (H1N1, H3N2, and two influenza B) human influenza virus strains. The need for a broadly protective influenza vaccine is clearly demonstrated by the occasional failure of these seasonal influenza vaccines to control the annual epidemics of influenza viruses, which result in about 3–5 million cases of severe illness, and up to 500,000 deaths worldwide.[106] In a more serious scenario, influenza viruses cause occasional pandemics when an antigenically novel virus spills into the human population or when, as in the case of the 2009 pandemic H1N1 virus, an influenza virus that has ceased to circulate among humans for decades reemerges.

The major neutralizing epitopes are located around the receptor-binding domain within the influenza HA globular head region (Fig. 15.2).[107] While antibodies targeting such epitopes are protective, they are mostly strain-specific and lack the broad neutralizing activity required to protect against different influenza subtypes. Structural analysis of a number of the recently isolated broadly neutralizing human HA-specific mAbs revealed that they bound to a conserved domain within the HA stem region (Fig. 15.2).[108–111] These mAbs were isolated by phage display libraries or by direct cloning of single cell sorted plasmablasts following influenza infection or vaccination. Unlike the case for HIV, influenza broadly neutralizing antibodies could be detected following infection and vaccination.[112] Therefore, it remains puzzling that despite being repeatedly exposed to such conserved domains of influenza HA either in the form of vaccination or natural infection that influenza remains a serious public health problem. One possible—among many—explanation for this puzzle is that the concentration of HA stem-specific antibodies is too low to prevent infection.[106]

This notion is supported by the observation that mAbs targeting the stem region are weaker in general than those targeting the head epitopes in terms of in vitro neutralization potency.

The question then is how to boost stem-specific responses to levels that are protective in vivo. Recent data suggest that conventional seasonal vaccines induced B-cell responses are dominated by those targeting the HA head region.[112] Therefore, new immunogens would be needed to refocus the responses on those targeting the HA stem region. The use of immunogens comprised of globular head region HA molecules derived from viruses which have not been widely circulating in the human population, such as H5N1 avian influenza viruses, combined with a stem region that is conserved among the strains can change the dominant immune response from head focused to stem focused.[112] The globular head region of H5 is significantly different from the circulating human viruses while the stem region is largely conserved. The idea is to engage stem-specific memory B cells with minimal interference of the head specific ones. While this strategy did indeed succeed in boosting the stem-specific responses, questions regarding whether this boost is enough to afford protection in vivo are yet to be addressed.[112] In addition, further boosting with H5 in the aforementioned trial restored the globular head immunodominance of B-cell responses suggesting that the globular head region might be intrinsically more immunogenic than its stem counterpart. Similar to HIV, efforts are now focused on designing structure-based immunogens that present the broadly neutralizing epitopes (those within the HA stem and the within the receptor-binding domain) to the immune system in the most relevant conformation.[113–116]

6.3 The Quest for a Vaccine Against RSV

As discussed earlier, the gold standard for designing a successful antiviral vaccine is to mimic natural infection. This scenario could not be applied in the case for RSV because natural RSV infection provides limited protection from reinfection.[117,118] Therefore, developing a protective vaccine against RSV has been a daunting task.[119] It has been a global public health priority for over 5 decades.[120,121] While antigenic variability is a major obstacle in developing a broadly protective vaccine against HIV and influenza, there are only two serotypes of RSV and cross-reactive antibodies could be readily detected in human sera.[1] The major hurdles that hindered the development of a licensed vaccine against RSV are:

1. Vaccine-enhanced disease: This phenomenon was first noted when a formalin-inactivated vaccine candidate (FI-RSV) was developed and tested in infants and children in the late 1960s and the immunogenicity results were promising.[118,122] However, upon natural RSV infection, 80% of the FI-RSV-vaccinated subjects were hospitalized, whereas only 5% of the control group required admission, and two children died.[118,122]

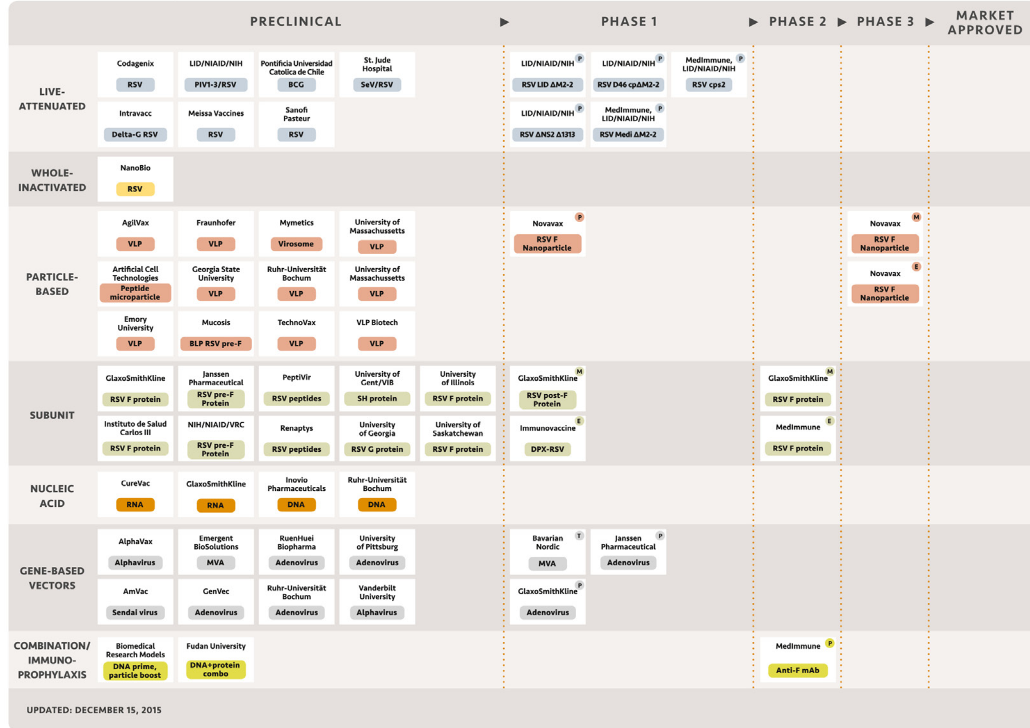


FIGURE 15.3 **RSV vaccines currently in pipeline.** *(Source: This RSV vaccine snapshot is reproduced with permission from PATH and can be found at http://sites.path.org/vaccinedevelopment/respiratory-syncytial-virus-rsv/.)*

2. The formidable task of getting the right balance between vaccine safety and immunogenicity/efficacy especially in the most vulnerable and highest priority target population, infants.[1]
3. The lack of ideal animal models making clinical trails—which are expensive and time consuming—an imperative measure to assess candidate vaccines. Additionally, the clinical endpoints in evaluating an RSV vaccine efficacy are not very specific as the disease symptoms are shared with many other respiratory viral infections.[1]

Based on the epidemiology and burden of RSV disease there are four target populations for RSV vaccination; (1) infants (<6 months); (2) children (6–24 months); (3) pregnant women; (4) the elderly.[117,118] Each of these populations presents its own challenges in terms of how their immune systems respond to various vaccine candidates. There are several types of RSV vaccines that have been developed and many others are in the pipeline (Fig. 15.3). Many of these vaccines are still in the preclinical stage. Four types of vaccine platforms are being tested in Phase 1, 2, or 3 trials (Fig. 15.3); live attenuated virus vaccines; particle-based vaccines (VLPs); subunit vaccines; and vector-based vaccines.[123] Each of these vaccine platforms has advantages and disadvantages,[117,118] but they mostly share that they focus on eliciting antibody response to RSV F glycoprotein.[123] Palivizumab, which is a humanized mAb specific to the F glycoprotein, is a proof of principle that neutralizing antibodies could provide protection against hospitalization.[124,125] Palivizumab is licensed to be used in infants at high risk of severe disease.[124,125]

Recently, the structural insights gained by solving the atomic structure of the pre- and postfusion forms of the F proteins have invigorated RSV vaccine development efforts.[126–130] We now know that many of the neutralizing F-specific antibodies that did not bind the postfusion form of the F protein are actually specific to the prefusion form. Moreover, a novel antigenic site, termed Ø, was revealed. Antibodies targeting this epitope show a more potent neutralization capacity compared to palivizumab.[1] The efforts are now focused on preparing physically stable vaccine candidates comprised of the native F protein trimer in its prefusion form.[131]

7 SUMMARY

There are multiple factors that contribute to the lack of a licensed protective vaccine against any particular virus. Most of these factors originate from the nature of the virus itself. Examples include: (1) viruses that exhibit extensive genetic variations (eg, influenza); (2) viruses that evolved multiple mechanisms for evading host immune detection and response (eg, HCV and HSV); (3) viruses that integrate their genomes in that of the host (eg, HIV); (4) viruses that are capable of developing latency (HSV). Some factors stem from the inability of the host to protect against infection (and reinfection) due to the inadequacy of the immune response (eg, RSV). Other factors that are unrelated to both virus and

host include the lack of animal models that are suitable for evaluating the efficacy of vaccine candidates and economical considerations. Early antiviral vaccine success stories (eg, smallpox, rabies, and yellow fever) were based on empirical efforts in which vaccines were derived from either a live attenuated form of the virus or inactivated whole virus. It is clear that such approaches are not enough to address the current challenges. Developing an effective vaccine against many of the currently challenging viruses would require an integrative effort from scientists representing various disciplines. For example, public health studies would be needed to assess the disease burden in different populations in order to define the most vulnerable ones. Virological studies would be important for generating viral vaccine strains that lack immune evasion capacity, for example. Immunologists would define which arms of the immune response are needed to achieve protection. Bioinformaticians would need to work very closely with immunologists in order to transform high-throughput data and analyses into concrete, useful knowledge. Structural analyses would help in identifying important immune targets and how to rationally design immunogens that elicit maximal immune responses to such targets. Vaccinologists would then assess vaccine candidates in preclinical and clinical trials. Finally, engaging the pharmaceutical industry would be essential for scaling up the production of the final product.

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