Exploiting Mucosal Immunity for Antiviral Vaccines

Akiko Iwasaki

Howard Hughes Medical Institute, Department of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06520; email: akiko.iwasaki@yale.edu

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Abstract

Mucosal surfaces provide a remarkably effective barrier against potentially dangerous pathogens. Therefore, enhancing mucosal immunity through vaccines—strengthening that first line of defense—holds significant promise for reducing the burden of viral diseases. The large and varied class of viral pathogens, however, continues to present thorny challenges to vaccine development. Two primary difficulties exist: Viruses exhibit a stunning diversity of strategies for evading the host immune response, and even when we understand the nature of effective immune protection against a given virus, eliciting that protection is technically challenging. Only a few mucosal vaccines have surmounted these obstacles thus far. Recent developments, however, could greatly improve vaccine design. In this review, we first sketch out our understanding of mucosal immunity and then compare the herpes simplex virus, human immunodeficiency virus, and influenza virus to illustrate the distinct challenges of developing successful vaccines and to outline potential solutions.

INTRODUCTION

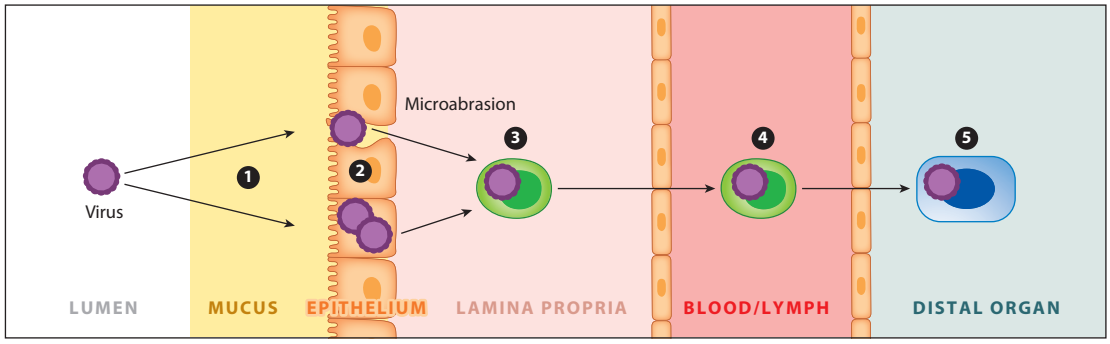
Over the past 200 years, vaccines have effectively limited the range and impact of many viral illnesses, such as polio, measles, and rubella. Vaccination can even be credited with eradicating smallpox, which had been a scourge of human populations for more than 10,000 years. As discussed in this review, however, viruses as a class present intricate challenges to the human immune system—and to our ability to counter them with vaccines.

This is not to say that we have not made great strides in understanding the mechanisms that initiate and maintain adaptive immunity. We now understand the viral sensors that prompt antigen-presenting cells to prime T and B cell responses (1, 2), and we have identified the effector cytokines and master transcription factors that govern T and B cell differentiation (3). These discoveries have enabled us to formulate better adjuvants, design epitopes to direct T and B cell responses, and target various dendritic cell (DC) populations to elicit a desired immunological outcome (4, 5). In parallel, the ability to manipulate viral genomes has given us the power to render viruses avirulent through deletions and to derive epitopes capable of eliciting robust and long-lasting T and B cell responses, many of which provide impressive protection in preclinical animal models of infectious diseases (6). Despite all of this progress, however, many preclinical successes fail in human trials; even experimental vaccines that elicit impressive immune responses in human subjects frequently fail to protect against viral infection or reduce disease burden. Why is it so difficult to generate effective vaccines for many clinically important viral pathogens?

Part of the answer is that viruses are ingenious at evading the immune response, either through mutation to escape B or T cell recognition or by avoiding effector mechanisms. Another facet of the problem is that, in many cases, we still lack sufficient understanding of the types of immune responses, natural or otherwise, that protect the host from a given virus. Vaccine development has historically focused on enhancing immunogenicity, i.e., the ability of a vaccine to elicit measurable levels of antibody (Ab) and T cell responses, which in humans can only be monitored in the peripheral blood. Immunogenicity, however, is no guarantee of protective immunity: an immune response capable of controlling a viral infection without causing host tissue damage (7). High levels of circulating Ab, antigen-specific T cells, and cytokines may fail to control viral invasion of a specific mucosal tissue simply because they lack access to it. Moreover, an overly exuberant immune response can itself drive pathology. The goal of this review is first to consider the immune mechanisms of protection against viral pathogens that infect the host through mucosal surfaces and next to consider the implications for ongoing efforts to develop vaccines for three specific viruses: herpes simplex virus 2 (HSV-2), human immunodeficiency virus 1 (HIV-1), and influenza A virus.

WHAT DOES IT TAKE FOR A VIRUS TO INFECT A HOST?

Humans are constantly exposed to and colonized by a vast array of fungi, parasites, bacteria, and viruses (8, 9). Most host-microbe interactions do not lead to disease either because mammalian hosts have evolved to tolerate or benefit from the microbes (commensal bacteria) or because the microorganisms never manage to invade the host and replicate. Even when infection occurs, it does not necessarily lead to disease, provided that the immune system efficiently handles the pathogen without causing immunopathology. Protective immune responses therefore can be defined as host countermeasures that prevent disease but not necessarily infection. A typical viral infection begins with exposure (through inhalation, ingestion, or sexual intercourse), followed by ① penetration of the virus through the mucosal layer (**Figure 1**). The virus must then ② enter the host, either by infecting epithelial cells or by gaining access to the submucosa through a microabrasion in the mucosal barrier, and ③ infect target cells in the lamina propria. Viruses can also ④ gain access to



	❶ Mucosal penetration	❷ Entry	❸ Local target infection	❹ Systemic spread	❺ Distal target infection
Innate effector	Coughing Sneezing Vomiting Diarrhea AMP pH IgM (Natural Abs)	Epithelial integrity	Type I IFNs ISGs NK cells Plasmacytoid DCs	IgM (Natural Abs) Plasmacytoid DCs	Type I IFNs ISGs NK cells Plasmacytoid DCs
Adaptive effector	sIgA IgG (Neutralizing Abs)		CD8 T_{RM}s (cytotoxicity) CD4 T_{RM}s (IFN-γ) Ab-dependent cytotoxicity Ab-dependent phagocytosis	IgM IgG	CD8 T_{RM}s (cytotoxicity) CD4 T_{RM}s (IFN-γ) Ab-dependent cytotoxicity Ab-dependent phagocytosis

Figure 1

Steps in viral infection and corresponding host counter mechanisms. Hosts have multiple layers of defense against viral infection. A virus must first ❶ cross the mucosal layer to enter the host. Some viruses infect epithelial cells; others ❷ pass through the epithelial layer through a microabrasion and ❸ infect target cells in the submucosa; still others gain access to the circulation through ❹ infecting migratory cells and ❺ become viremic. Each step of the infection process is counteracted by the host innate and adaptive immune responses indicated in the bottom half of the figure. Abbreviations: Ab, antibody; DC, dendritic cell; IFN, interferon; ISG, immune serum globulin; sIgA, secretory IgA; T_{RM}, tissue-resident memory T cell.

systemic circulation (through blood or lymph) and, in some cases, cause systemic viremia. Finally, some viruses establish infection of ⑤ distal target tissues, exemplified by hepatitis viruses infecting hepatocytes in the liver.

Not all viruses undergo all of these steps, of course. Unlike vector-borne viruses, which are injected directly into the systemic circulation, many viruses stop at step ③ and do not spread beyond the entry tissue. As the virus invades deeper, however, it meets both innate and adaptive host immune responses dedicated to counteracting its progress (**Figure 1**).

IMMUNE DEFENSES AT MUCOSAL SURFACES

Mucosal tissues mark the boundary between the environment and the body's interior and can be divided into two physiological types (**Figure 2**; 10). The type I mucosal surface is covered by simple columnar epithelia that perform vital functions, such as gas exchange in the respiratory tract, nutrient uptake in the GI tract, and regulation of fertility in the upper reproductive tract. Type II mucosal surfaces are much more robust. They consist of stratified squamous epithelia composed of multilayered keratinocytes; this offers greater protection against the mechanical stresses encountered by the cornea, mouth, esophagus, anus, ectocervix, and vagina. The two types of mucosal surfaces deploy distinct protective mechanisms, with respect to both innate and adaptive immune effector responses.

Constitutive and Inducible Antiviral Innate Defenses

Several layers of constitutive defensive barriers confront any virus that seeks to enter the host through mucosal surfaces (**Figure 2**). The first obstacle, for both type I and type II tissues, is the mucus layer. Differentiated columnar epithelial cells known as goblet cells secrete mucins, large glycoproteins responsible for the gel-like quality of mucus (11). This mucus enables the mucosa to intercept environmental antigens and protect the systemic compartment from overstimulation. Containment and clearance are largely physical processes. In the respiratory tract, for example, viruses trapped in the mucus layer are swept away by ciliated epithelial cells, and coughing and sneezing can expel large boli of mucus-entrapped materials. However, mucus also contains several antimicrobial peptides and natural Abs and is thus an active participant in viral defense. For example, cervical mucus varies in thickness, acidity, and hormonal composition according to the stage of the menstrual cycle and can either foster or hinder sperm (or virus) transport, and salivary glands secrete digestive enzymes as well as secretory IgA (sIgA), a powerful antimicrobial defense (12). In the GI tract, viruses must survive the low pH of the stomach as well as digestive enzymes secreted along the length of the tract. Paneth cells that reside in the crypts of Lieberkühn in

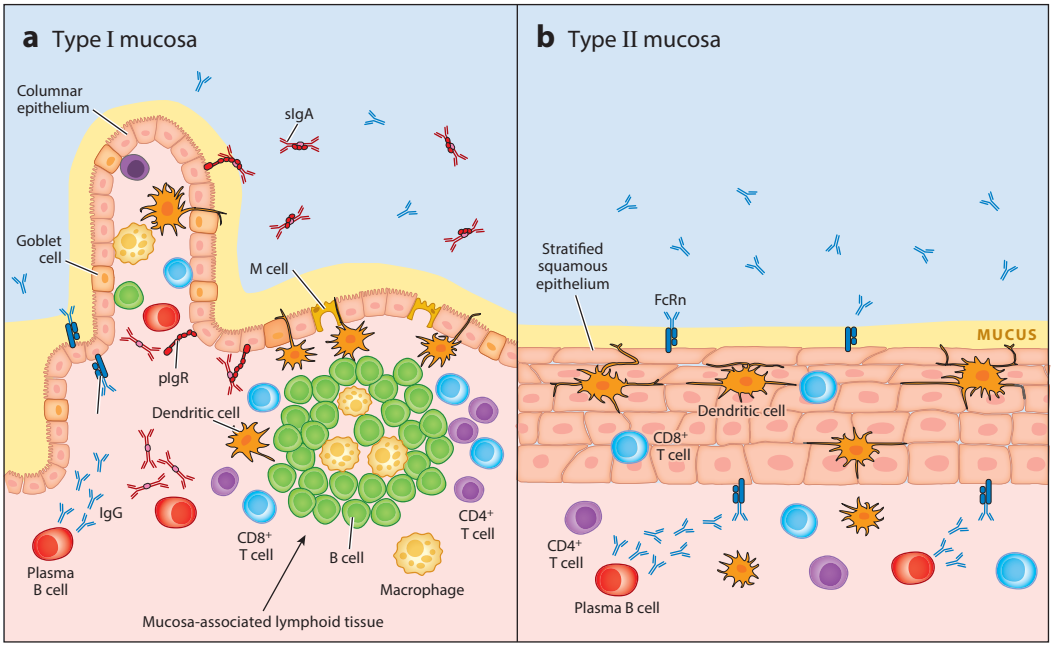


Figure 2

Type I and type II mucosal surfaces deploy distinct protective mechanisms. (*a*) Type I mucosae (e.g., gastrointestinal, respiratory, and upper female reproductive tracts) are covered by simple columnar epithelia. Goblet cells secrete mucus that covers the mucosal surface. Many type I mucosae contain mucosa-associated lymphoid tissue, which serves as a site for antigen presentation to naive T and B cells. Dendritic cells take up luminal antigen through their extended dendrites. M cells transport luminal antigens to the dendritic cells beneath. Type I epithelial cells express neonatal Fc receptor (FcRn), capable of transporting IgG across the epithelial cell layer. Type I mucosal epithelial cells also express polymeric Ig receptor, which allows transport of dimeric IgA secreted by plasma cells in the lamina propria to the apical side of the epithelial membrane. Cleavage of the pIgR-dIgA complex releases secretory IgA (sIgA) into the lumen of the type I mucosa. sIgA serves as the dominant protective isotype in type I mucosae. Tissue-resident memory T and B cells are found in the epithelial layer and the lamina propria. (*b*) Type II mucosae (e.g., corneal, oral, esophageal, and lower female reproductive tracts) are covered by squamous stratified epithelial cells. The mucus that covers these surfaces is secreted by nearby glands. Unlike type I mucosal epithelia, type II mucosal epithelia do not express pIgR but express FcRn, making IgG the predominant protective isotype.

the small intestine secrete antimicrobial peptides, and the lower GI tract is heavily colonized by bacteria that protect against viral infection in various ways (13).

If a virus invades the mucus layer, the next barrier to infection is the epithelial layer. Viruses can make their way through a breach in the epithelial barrier to directly infect the cells in the submucosa. For example, HIV-1 and human papillomavirus (HPV) enter the cervical tissue via microabrasions in the epithelial barrier, gaining direct access to CD4 T cells (14) or basal cells (15, 16), respectively. Alternatively, viruses can infect epithelial cells directly, replicate, and then infect other cell types. HSV-1 and HSV-2 employ this strategy: These viruses replicate in vaginal epithelial cells before infecting nearby innervating neurons, in which they establish latency to wait out the initial immune response.

Once a virus infects the epithelium or the cells beneath, it induces host defenses. One route feeds directly into the adaptive immune system: M cells within the epithelial layer not only serve as a site for gathering lymphocytes but also transport antigens from the lumen to the DCs beneath the mucosal layer. Plasmacytoid dendritic cells (pDCs) can also sense viruses through Toll-like receptors (TLRs) expressed in endosomes (17). Innate immunity is simultaneously activated: Most innate immune cells bear pattern recognition receptors (PRRs) that detect the unique features of viral infection, known as pathogen-associated molecular patterns (PAMPs) (18). Signaling through the PRRs activates transcription factors that induce expression of autocrine and paracrine antiviral mediators, such as type I interferons (IFNs).

DC PRRs engage viral PAMPs to activate a set of genes that promote pathogen uptake and antigen presentation on MHC class I and class II molecules (19). Viral engagement of DC PRRs also induces expression of the chemokine receptor CCR7, which promotes migration of the DCs to the draining lymph nodes to stimulate naive lymphocytes that carry cognate immune receptors. In some type I mucosal tissues, DCs do not have to migrate through the lymphatic vessel to encounter naive lymphocytes. Organized lymphoid tissues, such as the tonsils or Peyer's patches in the ileum, prime the T and B cell responses to antigen (**Figure 2*a***; 10). Activated T and B cells proliferate, differentiate, and migrate to the site of infection to help control primary viral infection. After an infection resolves, some of these lymphocytes become long-lived memory cells that protect against secondary infection with the same virus (see below).

If the virus infects mobile cells, it can enter systemic circulation and spread to target tissues at distal sites. In the blood, preexisting IgM binds viruses with broad specificity and blocks further spread. Viruses in circulation may also trigger pDCs in the spleen to secrete type I IFNs, but some viruses manage to evade these responses and gain access to target cells in distal tissues.

Effector Mechanisms of Immune Protection

Many mechanisms exist by which memory lymphocytes can block viral infection. In parallel, viruses have evolved numerous strategies to evade even the most effective of these mechanisms.

Neutralizing antibodies. Neutralizing Abs (nAbs), so called because they neutralize the biological effects of an antigen, inhibit viral attachment or entry. nAbs that are secreted into the mucosal lumen can bind to an incoming pathogen and prevent it from entering host target cells by blocking receptor binding or cellular uptake (**Figure 3*a***). Complement fixation by nAb kills viruses. Finally, nAbs provide sterilizing immunity at little cost to the host (**Figure 4**). Vaccines eliciting high titers of nAb in mucosal secretions are thus ideal for providing protective immunity in humans. Nevertheless, high levels of Ag present for an extended period of time may lead to the deposition of immune complexes in various tissues, which can cause immune pathology.

The nAb isotype best suited to providing protection in different mucosal tissues is determined by the ability of the Ab to cross the epithelial barrier into the lumen. Type I mucosal epithelia

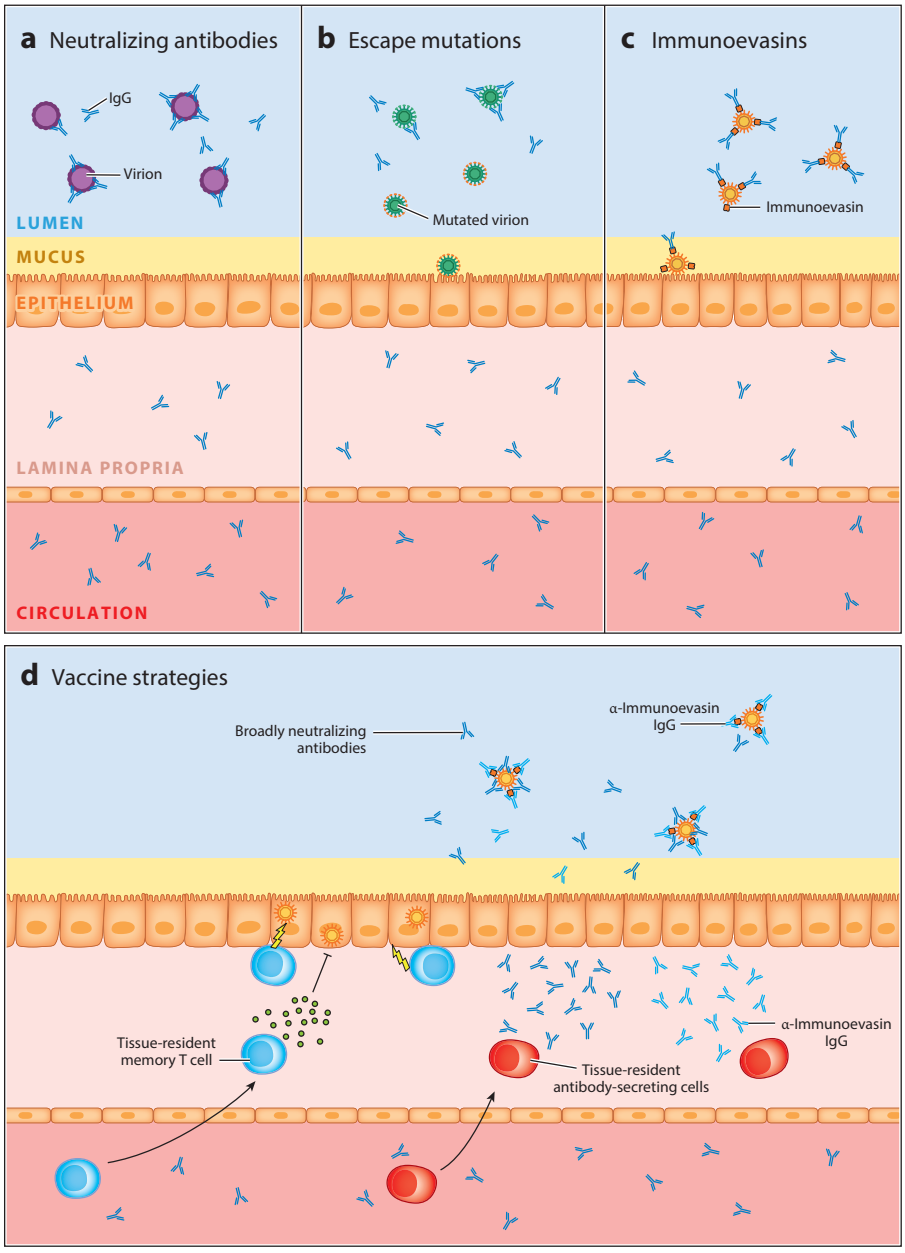


Figure 3

Overview of viral evasion pathways and possible vaccine strategies for protection against viruses. (*a*) An effective vaccine induces antibodies to neutralize viruses that invade the host through mucosal surfaces. (*b*) Mutations allow some viruses to escape detection by antibodies and successfully infect the host. (*c*) Other viruses incorporate immunoevasins on the surface of virions, blocking antibodies from neutralizing them. (*d*) Vaccine strategies that may be appropriate for evasive viruses include generating neutralizing antibodies against conserved, critical regions of viral surface proteins (*dark blue*); blocking immunoevasins with antibodies (*light blue*); establishing tissue-resident memory T cells (*blue*) that can block the virus at the site of entry; and establishing tissue-resident antibody-secreting cells (*red*) to increase the concentration of antibodies against the virus and its immunoevasins.

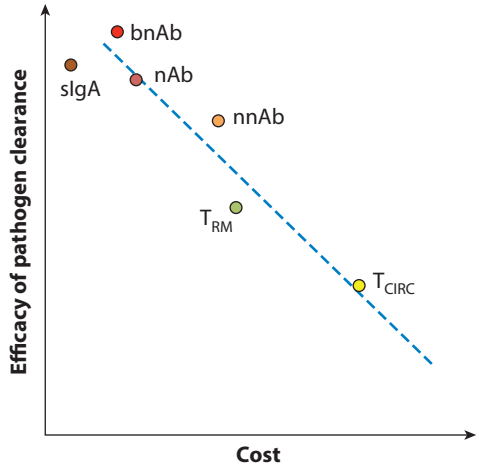


Figure 4

A theoretical model of efficacy and costs associated with distinct effector mechanisms. Secretory IgA (sIgA) and broadly neutralizing antibody (bnAb) and neutralizing antibody (nAb) in the mucosal lumen can bind to incoming virions and prevent viral entry altogether. These effector mechanisms are therefore highly efficient and impose very little tissue damage. Non-neutralizing antibody (nnAb) does not prevent infection of cells but clears infected cells by recruiting phagocytes and NK cells; this process may be accompanied by moderate levels of inflammation. Memory T cells mediate viral control through cytotoxicity and cytokine release, often resulting in inflammation. Tissue-resident memory T cells (T_{RM}s) are more effective than circulating memory T cells (T_{CIRC}) in controlling infection and spread. However, even T_{RM}s rely on recruiting inflammatory cells from the peripheral blood to mediate protection. T_{CIRC} can be protective, but they are less effective than T_{RM}s and carry high tissue damage costs, owing to the delay in pathogen control and the effector mechanisms employed. In some cases, tissue damage mediated by T cells, and not viral infection per se, results in the pathogenesis of viral diseases.

express polymeric Ig receptors (pIgRs) capable of transporting dimeric IgA; type II mucosal epithelia lack pIgR and are thus incapable of transporting dIgA. Both mucosal surfaces are capable of transporting IgG via neonatal Fc receptors (FcRns) (**Figure 2**; 20).

sIgA in the mucus layer of type I mucosa provides a formidable barrier to viral pathogens. Dimeric IgA secreted from plasma cells in the lamina propria binds to pIgRs at the basolateral surface of the type I epithelia and is transported to the lumen, where cleavage of the pIgR-dIgA complex releases sIgA into the lumen. sIgA is excellent at neutralizing viruses but poor at fixing complement or engaging FcRs on phagocytes or NK cells. sIgA therefore mediates little Ab-dependent cellular phagocytosis (ADCP) or Ab-dependent cellular cytotoxicity (ADCC) and produces very little damage to the host in comparison to IgG (**Figure 4**).

Despite the obvious appeal of stimulating nAbs as a vaccine strategy, several considerations render this approach unlikely to be feasible. First, certain pathogens are armed to disengage nAb-dependent defense mechanisms (21). For example, HSV-1 encodes gE/gI glycoproteins that act as FcR analogs to capture nAbs via their Fc domain (22, 23), rendering them incapable of neutralization. Second, nAbs cannot confer long-term protection if the surface glycoproteins expressed by the virus rapidly mutate away from recognition—this is why we need annual vaccination for influenza viruses. Third, even when broadly neutralizing Abs (bnAbs) are highly protective, they can be extremely difficult to elicit using current vaccine strategies. In the case of HIV-1, for example, we have, in rare cases, been able to isolate highly protective bnAbs from infected individuals, but we

Broadly neutralizing Abs (bnAbs): Abs that neutralize diverse strains of a particular infectious agent

have yet to develop a vaccine that can elicit such Abs in the general population (discussed below). When pathogens evade the protection conferred by nAbs, we must turn to non-nAb strategies.

Non-neutralizing antibodies. A non-neutralizing Ab (nnAb) is unable to block pathogen entry into a host organism but can engage other means of viral control. NK cells express FcR CD16, which binds to the Fc portion of a nnAb bound to the surface of an infected cell and mediates ADCC. By expressing phagocytic FcRs, nnAbs also mediate immediate uptake and destruction of pathogens bound by such Abs, leading to ADCP. Finally, nnAbs can destroy a pathogen by activating complement. All of these effector mechanisms require the recruitment of innate immune cells (NK cells, phagocytes) or effector molecules (complement system).

As discussed below, the different Ab isotypes engage distinct effector mechanisms, according to whether they fix complement, bind to FcRs, or are transported into the mucosal lumen (24). For example, human IgG1 is excellent at opsonization, whereas IgM and IgG3 are better at activating complement. Thus, nnAbs are efficient, but the inflammation associated with their effector strategy makes them more costly to the host than nAbs. Even so, many pathogens are able to evade nnAbs. For example, the HSV-1 envelope contains glycoprotein C (gC), which interferes with the C3 complement cascade (25). When a virus evades all Ab-dependent mechanisms, the next line of defense is local antigen-specific CD4 and CD8 T cells (**Figure 4**).

Memory T cells. Memory T cells provide the last line of defense. Circulating memory T cells are divided into central memory T cells (T_{CM}s), which survey secondary lymphoid organs, and effector memory T cells (T_{EM}s), which are capable of entering peripheral tissues (26). Circulating memory T cells can recognize cognate virus antigen in the lymph nodes draining the site of infection, expand, migrate to the site of infection, and help clear virus-infected cells. Because it requires time for T_{CM}s or T_{EM}s to proliferate, migrate, and mediate effector function at the site of infection, the virus has an opportunity to do quite a bit of damage while the body is mounting this response. Nevertheless, circulating memory T cells alone are capable of providing substantial protection against many viral infections (**Figure 4**).

Some T cells can establish residency within certain tissues and become tissue-resident memory T cells (T_{RM}s), which greatly accelerate the recognition and clearance of pathogens that have been previously encountered at that site. The cost of activating T_{RM}s in mucosal tissues might appear to be greater than that of Ab-based effector mechanisms; IFN-γ has a pleiotropic effect on various cell types, inducing IFN-stimulated gene expression, enhancing recruitment of leukocytes, and creating an inflammatory milieu. Because T_{RM}s curtail viral replication so efficiently, however, their inflammatory consequences are limited both anatomically and temporally.

Not all of these immune mechanisms are effective against all viruses. For instance, cytotoxic T cell responses are often ineffective against acute cytopathic viruses (27) because the viruses induce cell death faster than cytotoxic T lymphocytes (CTLs) can kill infected cells. Preventing further transmission of acute cytopathic viruses usually requires soluble antiviral effectors such as IFN-γ, which are better suited to inducing an antiviral state in neighboring cells. Conversely, memory CD8 T cells are often sufficient to control nonlytic viruses, such as lymphocytic choriomeningitis virus, or slowly replicating viruses, such as vaccinia virus (28). CTLs that kill infected cells before they can release viral progeny are typically effective at halting noncytopathic virus transmission.

Efficiency and Consequences of Different Effector Mechanisms

Each immune effector mechanism serves a distinct purpose, but in general, the first lines of defense offer more robust protection with less damage to the host than subsequent lines of defense

(**Figure 4**). For example, neutralizing Abs that block viral entry altogether not only are highly effective, but they also obviate a massive systemic immune response and do not allow pathogens time to do much damage. By contrast, when a virus has already slipped through multiple lines of immune defense, cytotoxic T cells can be very effective, but their activity is accompanied by inflammatory responses that can cause significant damage to the host. In addition, acutely cytopathic viruses, such as smallpox, poliovirus, and Ebola virus, cause tremendous pathology, directly and rapidly.

International units (IUs) of Ab per mL serum: Ab levels of a given individual measured against the of known titer

Vaccines that seek to elicit protective immunity must balance the need to stimulate the immune system against the need to minimize tissue damage. Although the vaccine field has historically focused on increasing immunogenicity, immune-mediated pathology itself can cause significant morbidity and mortality. This is particularly true for diseases caused by noncytopathic viruses, such as hepatitis B virus, hepatitis C virus, and HIV-1. The more rapidly a pathogen can be contained, the less pathology it or the immune response it provokes can cause; one advantage we may have with viruses that invade mucosa (as opposed to blood-borne pathogens) is the possibility of activating mucosal immunity to contain the virus, both anatomically and temporally.

An alternative to increasing host resistance to pathogens is promoting host tolerance of disease inflicted by them (29–31). The skin and various mucosae are, after all, already tolerant of many beneficial microorganisms. Increasing disease tolerance is an unexplored area of infectious disease biology that may prove useful in managing certain chronic infections.

IMMUNOLOGICAL CORRELATES OF VACCINE-MEDIATED PROTECTION

The successes and failures of vaccines in humans can provide a rich source of information on which immune responses simply reflect immunogenicity and which correlate with protective immunity. In this section, we consider what several successful human vaccines reveal about mechanisms of immunological protection.

B Cell–Mediated Protection

Virtually all successful human prophylactic vaccines rely on Abs (**Table 1**) (32). Understandably, serum Ab levels, expressed as endpoint titers or international units (IUs) per mL, have been used as correlates of the protection conferred by vaccines. Serum Ab levels are directly relevant for viruses that enter through the bloodstream. High levels of circulating IgG can immediately neutralize viruses, such as yellow fever virus and Japanese encephalitis virus, introduced through insect bites. In the case of viruses that enter through mucosal surfaces, however, serum Ab levels may not be relevant (**Table 1**). Protection against orally transmitted viruses, such as hepatitis A, rotavirus, and polio, for example, is conferred by high levels of neutralizing sIgA in the intestinal lumen (33). However, sometimes serum IgA measurements are useful for these viruses. For example, serum IgA is used as a surrogate for sIgA in the case of rotavirus vaccine. A meta-analysis of multiple cross-sectional studies in more than 30 countries found an inverse relationship between serum IgA titers and the level of child mortality from rotavirus infection, and Ab titers were highly predictive of vaccine efficacy: Serum IgA titers above 90 were correlated with significant protection (34).

Neutralizing IgG in the vagina and neutralizing IgA secreted from the endocervix are predicted to block entry of viruses transmitted via the genital mucosa, such as HPV. Although anti-HPV16 IgG titers in cervical secretions are often less than 1% of those seen in the serum (titers of less than 10 IUs are typical in cervical secretions, whereas titers of ~3,000–4,000 IUs are typical in sera; 35), they are apparently sufficient to confer complete protection in vaccinated individuals. This may be because HPV enters through minor abrasions in the mucosa (15), which also likely

Table 1 Immunological correlates of protection for antiviral vaccines in humans[a]

Vaccine	Route of virus entry	Test	Level required	References
Hepatitis A	Oral	ELISA	10 mIU/mL	210
Hepatitis B	Blood, mucosal	ELISA	10 mIU/mL	211
Human papillomavirus	Genital, oropharyngeal	ELISA	ND	36, 37
Influenza	Respiratory contact	HAI	1/40 dilution (adults)	164–166
			1/100 dilution (children)	167
		Flow cytometry, PBMCs	granzyme B^{hi}, IFN-γ:IL-10 (>65 y)	191, 192
Japanese encephalitis	Mosquito bites	Neutralization	1/10 dilution	212
Measles	Respiratory contact	Neutralization	120–200 mIU/mL	213–215
Mumps	Respiratory contact	Neutralization	ND	216
Polio	Oral	Neutralization	1/4–1/8 dilution	217, 218
Rabies	Animal bites	Neutralization	0.5 IU/mL	219
Rotavirus	Oral	Serum IgA titer	>90	33, 34, 220, 221
Rubella	Respiratory contact	Immunoprecipitation	10–15 mIU/mL	222–225
Smallpox	Respiratory contact	Neutralization	1/20 dilution	226, 227
Varicella		gp ELISA	≥1/64 dilution; ≥5 IU/ml	228, 229
Yellow Fever	Mosquito bites	Log neutralization index	≥0.7	230, 231
Zoster	Reactivation from infected neurons	$CD4^+$ cells; ELISPOT–IFN-γ+, responder cell frequency (in individuals >60 y)	ND	40
		Fold increase in anti-gp Ab response (in individuals 50–59 y)	5.25-fold rise = 90% efficacy	41

Abbreviations: Ab, antibody; ELISA, enzyme-linked immunosorbent assay; gp, glycoprotein; ELISPOT, enzyme-linked immunospot; HAI, hemagglutinin inhibition; IFN, interferon; ND, not defined; PBMCs, peripheral blood mononuclear cells.

[a]Table is modified from References 232–235 and is also based on Reference 236. Green = Ab-mediated protection; pink = T cell–mediated protection.

allow serum IgG access to the mucosal tissue. Because of the complete efficacy of protection by the quadrivalent vaccine (targeting HPV types 6, 11, 16, 18) and the lack of breakthrough infection, the actual correlate of protection mediated by HPV cannot be ascertained (36). However, sustained generation of neutralizing anti-HPV Abs appears to explain the clinical efficacy of the quadrivalent vaccine (37).

In addition to secreting Abs, B cells facilitate DC–T cell interactions in lymph nodes, present antigens to effector T cells and T follicular helper cells, and produce cytokines that regulate innate and adaptive immunity to pathogens (38). Vaccines could use such Ab-independent effector functions of B cells to promote protective immunity by, for example, using B cells to present antigens to stimulate memory T cells.

T Cell–Mediated Protection

T cells help generate robust Ab responses, and they also mediate direct antiviral defenses by killing infected cells and secreting antiviral cytokines. The only human vaccine known to confer T cell–mediated protection, however, is the varicella-zoster virus (VZV) vaccine (**Table 1**).

Herpes zoster is caused by reactivation of latent VZV acquired originally as chicken pox during childhood. Most herpes zoster cases are seen in adults older than 50 years of age; reactivation is associated with a decline in T cell immunity. With increasing age, T cell responses to VZV undergo a precipitous decline, whereas Ab responses to the viral glycoproteins remain constant, even in subjects older than 79 years (39). In unvaccinated elderly individuals who have developed herpes zoster, higher numbers of responder T cells and IFN-γ+ cells one week after onset are associated with reduced severity and less postherpetic neuralgia (40). However, higher Ab titers are associated with increased herpes zoster severity and postherpetic neuralgia. These data indicate that the ability of memory T cells to rapidly expand in response to reactivating VZV correlates with protection in humans.

The live attenuated Oka VZV vaccine (Merck) is recommended for people older than 60 years. How does the vaccine confer protection? Presumably, VZV vaccine induces elevated T cell responses that mediate a rapid antiviral response to contain reactivation (39, 40). In a slightly younger age group (50–59 years), the magnitude of the rise in VZV Ab titers from the time before immunization to 6 weeks after immunization is a reliable correlate of protection (41). Notably, in vaccinees older than 60 years, the average increase in Ab titers is only 1.6-fold greater than placebo at 6 weeks post-vaccination (39). Collectively, these data indicate that the correlate of protection for VZV in adults 50–59 years old is the rise in Ab titers, whereas older adults rely on T cells for protection.

PROTECTION CONFERRED BY MUCOSAL B AND T CELLS

Even when immunogenicity correlates with protection, the precise mechanism of protection in the vaccinated host can remain unclear. For instance, a serum concentration of virus-specific IgG could reflect the concentration needed either to directly neutralize virus introduced into circulation or to effect protection within the mucosal lumen after transport by the FcRn. The serum IgG concentration could even reflect, for example, a mucosal Th1 response to a given virus. Animal studies suggest that the organization of memory cell types and molecules at the site of viral entry is critical for conferring antiviral defense.

Local Memory B Cells

Humoral immunity is conferred by circulating Abs and is thought to be systemic. Recent evidence, however, reveals the existence of local Ab-secreting B cells in mucosal tissues. For example, in mice vaginally immunized with HSV-2, a large number of IgG-secreting plasma cells is found in the vagina even 10 months after infection (42). Along similar lines, HIV-specific IgG concentrations in the cervicovaginal mucus of HIV-infected women are higher than those in serum, suggesting that HIV-specific IgG is produced locally (43, 44).

Evidence also exists that local Ab production provides viral control. For instance, nonhuman primates immunized intranasally with gp41-containing virosomes produce mucosal and circulating IgA and IgG Abs, whereas intramuscular immunization generates only serum Abs—and only mucosal Abs mediate protection (45). Intravenous vaccination of macaques with simian immunodeficiency virus (SIV)mac239Δnef causes plasma cells and ectopic lymphoid follicles to accumulate beneath the mucosal epithelium in the female reproductive tract (46). IgG produced by the local plasma cells is concentrated within the cervical reserve epithelium and vaginal epithelium by the action of FcRns. This local Ab production and delivery system correlates spatially and temporally to local protection against high-dose pathogenic SIV vaginal challenge (46). Collectively, these findings in humans, mice, and nonhuman primates suggest that

circulating Ab may not be sufficient to control certain viral infections at peripheral sites and that tissue localization of B cells or Ab is required for fully protective immunity (**Figure 3*d***). Future vaccine designs could employ strategies to increase local accumulation of virus-specific B cells.

Heterosubtypic immunity: a host immunized with antigens from one subtype of virus is immune to another subtype of the same virus

Tissue-Resident Memory T Cells

Circulating memory T cells do not patrol certain tissues, such as the skin, lung airways, vagina, or nervous system. Instead, restricted from memory patrol, these organs combat infections by recruiting memory T cells upon an inflammatory response (47). However, the restricted organs have the capacity to establish T_{RM}s after a primary viral infection, recruiting effector T cells in response to chemokines; when T_{RM}s establish residency within a given peripheral tissue, they undergo minimum exchange with the circulating pool and can reside in the tissue for quite some time (47–49).

Importantly, T_{RM}s are highly protective against local viral infections, effectively blocking viruses from infecting target cells in the lamina propria (step ③ in **Figure 1**). For example, following secondary HSV-1 challenge, previously infected areas of skin containing T_{RM}s show more rapid viral control than unimmunized skin (50). Delivery of antigens via epidermal disruption, as with immunization of mice via skin scarification with either vaccinia virus or the modified vaccinia Ankara virus (MVA), induces protection against skin challenge (51). In the lungs, CD8 T_{RM}s provide heterosubtypic immunity against influenza and far better protection than circulating memory T cells (52); a decline in CD8 T_{RM}s correlates with a loss of protection (53). Virus-specific CTLs near the bronchial tree mediate viral clearance (54). CD4 T_{RM}s isolated from the lung show greater lung tropism and better control of influenza virus than CD4 T cells isolated from the spleen (55). Finally, intravaginal immunization of mice with an attenuated HSV-2 virus protects the host, even upon lethal genital HSV-2 challenge, and CD4 T_{RM}s are responsible for this protection (56). Collectively, these studies show that T_{RM}s provide immediate control of viral pathogens that invade the host through mucosal tissues that is superior compared to T_{EM}s and T_{CM}s and that circulating T_{EM}s or T_{CM}s alone may not fully protect against infection at these sites. The superior ability of T_{RM}s to provide antiviral protection probably results from their ability to immediately respond to invading pathogens owing to their location, either through cell intrinsic protective mechanisms (cytolysis, cytokine secretion) or by recruiting circulating cell subsets (chemokine induction).

In humans, T_{RM}s are found in various mucosal tissues, including the gut and lung (57, 58). Although the importance of T_{RM}s in protecting against viral pathogens has not been definitively established in humans, evidence indicates that T_{RM}s provide protective immunity in certain cases. Mathematical modeling based on genital biopsies shows that the density of CD8 T cells at the site of HSV-2 release from the neuron to the epithelium predicts whether reactivation will lead to a genital ulcer or asymptomatic shedding, with higher density being more protective (59). HSV-induced CD8$\alpha\alpha$+ T cells that reside at the dermal-epidermal junction (DEJ) appear to promote virus control (60). These CD8 T cells rapidly contain viral spread: Infected cells are killed within hours (61). Once lesions have resolved, virus-specific CD8 T cells at the DEJ cluster around the nerve endings from which the virus was released (62). A spatial mathematical model by Schiffer (63) makes the intriguing prediction that high levels of CD8 T cells in the mucosa do not necessarily indicate a protective phenotype but rather a response to recent shedding. In a naturally infected individual, breakthrough shedding occurs under all sets of model parameter assumptions, as CD8 T_{RM} densities surpass a protective threshold in only small regions within the genital tract. If CD8 T cell densities are artificially increased in the vaginal tissue by 50% using an immunotherapeutic approach, better control of shedding is predicted to occur for at least a year (63). Therefore, a

vaccine strategy that increases CD8 T_{RM} density in susceptible organs may provide long-term protection against viruses.

Problems and Possible Solutions for Developing Antiviral Vaccines: Three Case Studies

To explore the gap that can emerge between immune responses to a vaccine and true protective immunity, we now consider various attempts to develop vaccines for specific viruses. In particular, we examine efforts to develop protection against a DNA virus (HSV-2), a retrovirus (HIV-1), and an RNA virus (influenza A virus). All three infect humans through mucosal tissues, but they do so via distinct routes and through distinct cell types, namely, epithelial (influenza, HSV-2), lymphoid (HIV-1), and neuronal (HSV-2) cells. These viruses cause either acute (influenza A virus) or chronic (HSV and HIV-1) infections, and each evades the immune response in a distinct way.

Genital Herpes

HSV-2 is one of the most common sexually transmitted viruses, with approximately 45 million people infected in the United States alone (64). This high rate of infection is a testament to the efficacy of the herpes virus life cycle, which alternates between lytic and latent stages. During the initial infection, HSV-2 enters the body primarily through the genital mucosal surface; the virus replicates and kills infected cells, eliciting a T cell response to the viral antigens. Some of the virus also migrates to the sacral ganglia, however, where it shuts down viral protein synthesis and enters a period of latency. Because the immune system remains unaware of latently infected cells that do not express viral antigens, the latent virus can wait out an acute immune response and thereby evade complete destruction. When the body is under stress, the virus reactivates and is transported back to the mucosa, where it can replicate in epithelial cells for a few days, until memory T cells find and contain infected cells. These intermittent rounds of viral replication make for a very successful virus. At present, no vaccines exist to prevent transmission, nor is there a cure.

Prior infection with HSV-2 provides significant though imperfect protection against exogenous HSV-2 infection. One study showed that 2 of 8 patients had HSV-2 isolates whose genomic DNA restriction digestion patterns varied, which suggests exogenous reinfection with a new viral strain (65); another study found no evidence of exogenous HSV infection in 45 patients (66); yet another analysis of viral isolates from recrudescent genital herpes found that only 2 of 65 subjects (3%) were reinfected with exogenous strains (67). It seems that the natural immunity acquired following infection with HSV-2 provides significant protection against superinfection, and basic understanding of naturally acquired protection can guide vaccine approaches.

Attempts to create vaccines for HSV-2. In mouse models, passive transfer of IgG specific for HSV glycoproteins into the vaginal lumen is highly protective against HSV-2 infection. Based on these results, most vaccine approaches against HSV-2 have sought to generate robust systemic IgG responses against viral glycoproteins. Unfortunately, human vaccine trials with recombinant HSV-2 glycoproteins have failed to establish protective immunity against HSV-2 genital infection (68–70). A recombinant bivalent gB2 and gD2 subunit vaccine with an oil/water emulsion adjuvant (Chiron) induces strong neutralizing serum Ab and circulating CD4 T cell responses in humans (71) but fails to prevent HSV-2 infection in discordant heterosexual couples. Similarly, a gD2 subunit vaccine given with an adjuvant containing alum and monophosphoryl lipid A (MPL, a TLR4 agonist; vaccine produced by GSK) induces both neutralizing serum Ab and circulating

CD4 T cell responses in HSV-2 seronegative subjects (72)—but largely fails to protect against HSV-2 infection (73, 74).

Immunoevasins: proteins encoded by pathogens that modulate host immune responses

One possible reason for the failure of the Ab-mediated genital herpes vaccines is that the concentration of IgG required to provide protection against HSV-2 within the vaginal mucosa is extremely high: Mouse studies indicate that the concentration of mouse monoclonal Ab (mAb) III-174 (IgG2a; 75) in the vaginal lumen required to block vaginal infection after passive vaginal Ab transfer is 50 μg/mL (76, 77). However, in a study using an intravenous injection of a large dose of ascites fluid containing mAbs specific for gB (15βB2) or gD (18βB3), the mAbs failed to enter vaginal secretions at all and offered no protection against genital HSV-2 challenge (although protection was conferred against systemic HSV-2 challenge; 78). Systemic IgG thus has little effect on vaginal infection, despite the presence of FcRns capable of transporting IgG to the lumen. Is it possible for a vaccine to achieve an intravaginal concentration of virus-specific IgG of 50 μg/mL? To put this number in context, in naive mice, the concentration of total IgG in the vagina is on the order of only 20 μg/mL (79). Even in mice vaginally immunized with attenuated thymidine kinase mutant HSV-2 and boosted with the wild-type (WT) virus, the concentration of HSV-specific IgG in the vaginal lumen is only 2.6 μg/mL and a protective level of IgG is not achieved (78). Naive mice given two rounds of recombinant gD2 immunization, intraperitoneally, followed by vaginal challenge with live WT HSV-2, produce polyclonal Ab capable of conferring protection (80). Protective IgG concentrations for HSV-2 are thus not impossible, although they are supraphysiological.

Why is such a high level of Ab required to protect against HSV-2? The vaginal levels of neutralizing IgG required to protect against a similar virus, SIV, are as low as 0.01 μg/mL (81). The answer probably has to do with the HSV-2 envelope being studded with glycoproteins that evade the effector function of Abs. Viral gC binds the complement component C3 and inhibits complement-mediated virus neutralization and the lysis of infected cells. In addition, the gE/gI complex acts as an FcR decoy on the viral envelope (25, 82–84). These immunoevasins (85) collectively render Abs ineffective in the control of HSV.

Even high concentrations of IgG in vaginal secretions might not be sufficient to provide protection, however. Women receiving intramuscular injections of gB2 and gD2 in MF59 adjuvant (Chiron) generate concentrations of IgG against gB2 and gD2 in cervical secretions that are almost as high as the levels found in naturally HSV-2-infected women, but they are not protected against HSV-2 infection (86). Why does this highly immunogenic vaccine fail to protect humans? In naturally infected humans, HSV-2 induces not only IgG production but also robust local antiviral CD4 and CD8 T cell memory responses (87–90). Whether T_{RM}s provide protective immunity against genital HSV infection in humans remains to be proven, but studies in mouse models have shown T_{RM}s provide superior protection against viral challenge compared to circulating T cells (56, 91, 92). Although recombinant vaccines generate circulating memory T cells (71), they are unlikely to generate T_{RM}s in the genital mucosa. Even though CD4 T_{RM}s do not completely block primary infection, they dramatically reduce viral replication in the vaginal mucosa and disease caused by infection (93). In addition, CD8 T_{RM}s in the genital mucosa appear to be critical in blocking viral infection of the dorsal root ganglia (91) (see sidebar, Heterologous Protection Between HSV-1 and HSV-2).

Future approaches for an HSV-2 vaccine. As genital herpes involves infection of local cell types only (steps ❶ to ❸ in **Figure 1**), vaccine efforts should focus on generating robust local effector responses: Protective immunity against HSV-2 must be established to protect neurons from becoming infected. Harvey Friedman and colleagues (23, 94–96; **Figure 3*d***) have designed a clever vaccine strategy to counteract viral evasion by developing subunit vaccines

HETEROLOGOUS PROTECTION BETWEEN HSV-1 AND HSV-2

Although vaccines are designed to confer homologous protection, there appears to be considerable heterologous protection between the two HSV types. In women, HSV-1 seropositive status provides considerable protection against genital HSV-2 acquisition. In one study of discordant heterosexual couples, the HSV-2 attack rate for HSV-1 seropositive females was only 1.2%, whereas that for HSV-1 seronegative females was 11.9% (73), indicating that the preexisting immune response to HSV-1 confers protection against acquisition of genital HSV-2. In the follow-up Herpevax trial, women seronegative for both HSV-1 and HSV-2 were vaccinated with the gD2-alum MPL vaccine (GSK; 74). They developed no protection against HSV-2 but did develop an impressive level of protection against HSV-1 genital disease (58%) and HSV-1 infection (35%). Protection against HSV-1 was correlated with serum anti-gD2 IgG, with a mean titer of 6,875 found in protected individuals and 3,561 in unprotected individuals ($P = 0.04$). Cellular immune responses (CD4 T cell cytokines in response to gD2) did not correlate with cross-protection in gD2-immunized women. These studies indicate that pre-existing IgG against gD2 confers significant levels of protection against HSV-1. Therefore, considerable cross-protective immunity appears to exist between HSV-1 and HSV-2, which could be exploited for future HSV vaccines.

that incorporate immunoevasins as antigens. Simultaneous immunization with gD (immunogen), gC (immunoevasin), and/or gE (immunoevasin) leads to Ab production against all of these components, leading to Ab blockade of the inhibitory functions of gC and gE and enabling the anti-gD Ab to restrict viral infection.

Another vaccine approach is to induce local T_{RM}s through prime-and-pull (**Figure 3*d***). CD8 T_{RM}s specific to HSV-2 can be elicited by immunization followed by topical application of the chemokines CXCL9 and CXCL10 to the vaginal lumen during the primary immune response. These chemokines bind to CXCR3 expressed by effector CD8 T cells to recruit and maintain CD8 T_{RM}s (91). In a similar strategy, a chemical irritant such as 2,4-dinitrofluorobenzene or nonoxynol-9 is used to create local inflammation to recruit and maintain CD8 T_{RM}s (92). Once established, T_{RM}s can be restimulated to recruit circulating memory T cells to the genital mucosa, to amplify the antigen-specific memory T cell pool (97). Based on mathematical modeling, CD8 T_{RM}s must be established at a high enough density throughout the vaginal epithelium if they are to confer protection against HSV-2 replication (63). Therefore, an effective vaccine may need to establish high concentrations of T_{RM}s in the genital mucosa in addition to generating high levels of nAbs to prevent latent infection of neurons (**Figure 5**).

HIV-1

HIV-1 is a member of the lentivirus family of retroviruses. HIV-1 is transmitted through sexual contact (vaginal, penile, rectal), exposure to blood (including during pregnancy and the perinatal period), and breastfeeding. Since its discovery in the early 1980s, HIV-1 has claimed the lives of more than 25 million people and created 14 million orphans in sub-Saharan Africa alone (98). In endemic countries, HIV-1 deaths and the reduced fertility of HIV-positive women have dramatically altered population demographics (99). The pressing need for a vaccine against this terrible disease is, unfortunately, matched by the difficulty of the challenge. First, the virus shows a remarkable proclivity for mutation (both within a host and across the globe), and it establishes latency within only a few rounds of replication. Second, the natural immune response generated by HIV-1 infection often fails to protect against superinfection, except in rare humans who spontaneously control the infection. Therefore, we do not know the parameters of the gold standard protective

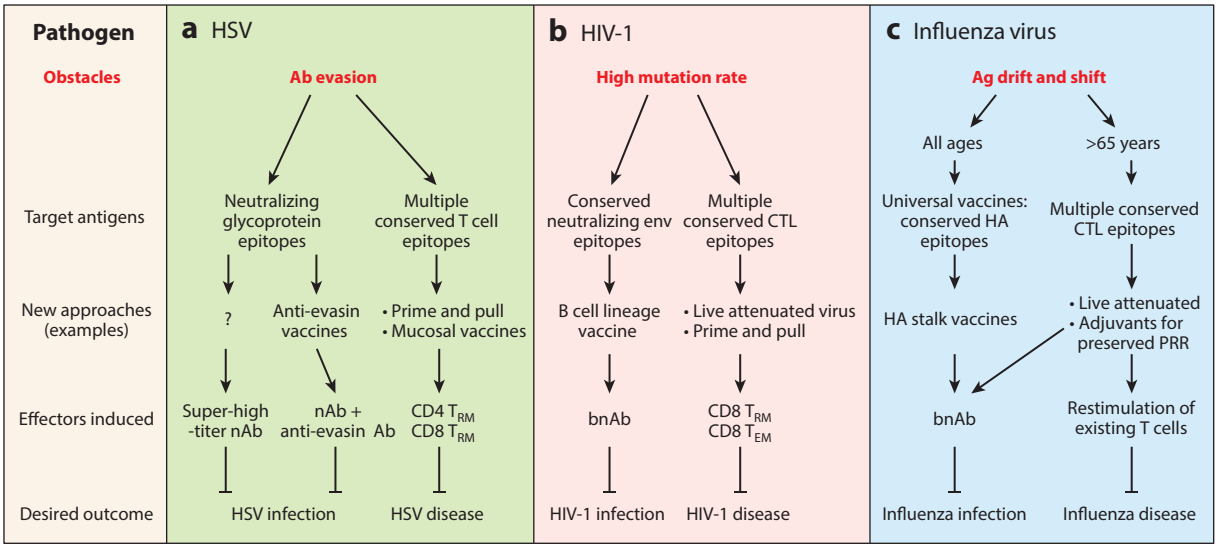


Figure 5

Obstacles and approaches to designing vaccines against viruses: three case studies. Viruses pose different obstacles (*red*) to vaccine-mediated protection. (*a*) In the case of herpes simplex virus (HSV) infection, the virus evades antibody (Ab) effector functions by expressing immunoevasins on its surface. To get around this problem, a vaccine could elicit super-high titers of neutralizing Ab (nAb) or could produce nAb in conjunction with anti-evasin Abs to enable Ab-dependent clearance of the virus. Alternatively, a vaccine could generate robust CD4 tissue-resident memory T cells (T_{RM}s) and CD8 T_{RM}s specific to conserved epitopes to block viral spread. (*b*) In the case of human immunodeficiency virus 1 (HIV-1), the obstacle is the high rate of mutation, which generates mutants that escape Ab detection. To overcome this obstacle, a vaccine that elicits broadly neutralizing Abs (bnAbs) against conserved epitopes on the viral surface glycoprotein would be desirable. In addition, CD4 T_{RM}s and CD8 T_{RM}s specific to conserved epitopes could block viral spread. Caution should be taken to ensure CD4 T_{RM}s do not serve as target cells for infection. (*c*) Antigen (Ag) drift and shift by the influenza A virus present obstacles to developing a universal vaccine. In younger vaccinees, a vaccine that generates bnAb to conserved regions of hemagglutinin (HA) may overcome this problem. In older vaccinees (>65 years), de novo Ab responses are nearly impossible to generate. Therefore, expansion of existing T cells against conserved epitopes may be needed to block viral spread. Caution should be taken to limit immunopathology caused by such a vaccine strategy. Abbreviations: CTL, cytotoxic T lymphocyte; PRR, pattern recognition receptor; T_{EM}, effector memory T cell.

immune responses to strive for. Third, even though bnAbs have been isolated from humans on rare occasions, we do not yet have a way to generate such Ab responses in human subjects with an immunogen.

Correlates of protection against HIV-1 acquisition in naturally exposed humans. Immune responses that develop naturally in people infected with HIV-1 generally fail to protect against superinfection, even with a closely related strain of HIV-1 (100); this is one way in which HIV-1 is quite different from HSV. Studies of those rare individuals who do resist disease have provided important insights into the pathogenesis of the virus as well as the host immune response. These individuals fall into two classes: those who are exposed to and/or acquire HIV-1 but control the infection at an undetectable viral load (controllers) and highly exposed, persistently seronegative (HEPSN) individuals who have a natural resistance to HIV-1 and do not seroconvert (101).

Phenotypic and genome-wide association studies indicate that HIV-1 controllers benefit from protective HLA class I alleles, including HLA-B*57, HLA-B*27, HLA-B*13, and HLA-B*58:01 (102–109). Specific amino acids in the HLA-B peptide–binding groove discriminate protective

from risk alleles. In addition, single nucleotide polymorphisms in HLA-C (102, 105–108) and HCP5 (105, 107) appear to suppress viremia in infected individuals. It thus appears that the ability of HLA class I molecules to bind to and present a certain set of viral peptides to CD8 T cells endows infected individuals with the ability to control viremia. The rectal mucosa of HIV controllers does, in fact, show robust, polyfunctional, HIV-specific CD8 T cell responses to conserved epitopes of the gag polyprotein (110). CD8 T cells from controllers also have higher levels of granzyme B, perforin, and IFN-γ than CD8 cells from non-controllers, and they are able to kill target cells more efficiently to suppress viral replication (111–113). nAbs are less frequently found in HIV controllers and are not associated with spontaneous protection against HIV-1 disease progression (114). Whether nnAbs mediate ADCC in HIV controllers as opposed to progressors remains unclear, as some reports support such a role (115–117), whereas others find no correlation (118, 119).

HEPSN groups include commercial sex workers, exposed healthcare providers, and the uninfected partners in serodiscordant couples. Three factors have been proposed to explain the resistance of HEPSN individuals to HIV-1: T cells, elevated numbers of NK cells, and mucosal IgA responses to HIV-1 (120). Some studies have found HIV-specific circulating T cell responses in the peripheral blood of HEPSN individuals (121–125): Healthcare workers that have been repeatedly exposed to HIV via needle sticks develop robust CD8 (123) and CD4 T cell responses to HIV, without detectable Ab responses (122). In a study of 12 potentially exposed but uninfected men who have sex with men, 4 had a detectable CD8 T cell response to gag and vif (121). Although HEPSN individuals, by definition, lack Ab responses to HIV, some display low but detectable HIV-specific CD4 T cell responses (126). Somewhat surprisingly, HEPSN individuals display decreased levels of T cell activation but increased levels of regulatory T cells (Tregs) (127–131). As with controllers, it remains uncertain whether HEPSN individuals enjoy protection via mucosal Abs. Some studies have found a correlation between mucosal IgA responses and HEPSN status (132). Six independent laboratories, however, measured HIV-1-specific IgG and IgA responses in the cervicovaginal mucosa of HEPSN women and found very few Abs to the virus—but virus-specific IgG Abs were detected in the cervicovaginal fluids of HIV-infected women (133). Another study, conducted in Vietnam, found that, compared with HIV-1-infected intravenous drug users, drug users exposed to but not infected with HIV-1 had elevated NK cell lytic function and cytokine secretion (134). These studies suggest that HEPSN individuals control HIV-1 infection independently of conventional T and B cell responses, possibly through elevated NK cell activity and enhanced Treg suppression of immune responses.

How can these immune correlates of spontaneous viral control and resistance help us design vaccines against HIV-1? Increasing CD8 and CD4 T cell responses to HIV-1 may be beneficial, but the quality of T cells must be sufficiently different from those elicited by vaccines (see below). Also, assuming that we cannot change the HLA genotype of the human host, identifying protective epitopes that are presented by controller HLA alleles may provide us with a basis to engineer a vaccine that mimics the features of such epitopes, a vaccine that can be engaged by more common HLA haplotypes.

Correlates of vaccine-induced control of HIV-1 acquisition in humans. In addition to looking for naturally protective immune responses in rare individuals, we can learn from studying correlates of protection in vaccine trials. There have been more than 200 human clinical trials (**http://iavireport.org/Trials-Database**), some of which have moved forward to Phase IIb and Phase III efficacy trials. Early efforts to elicit protective Ab responses with the AIDSVAX B/E (gp120) vaccines VAX003 and VAX004 induced significant IgG responses to gp120 but did not reduce the incidence of HIV-1 among vaccinees. VAX003 recipients were not protected from

acquisition of HIV-1 via the systemic route (e.g., intravenous drug use; 135). VAX004 did not protect against acquisition of HIV-1 among men who have sex with men or among women at high risk for heterosexual transmission of HIV-1 (136). In the STEP trial, a MRKAd5 HIV-1 gag/pol/nef vaccine elicited robust CD8 and CD4 T cell responses (137) but was not protective (138). These studies tell us that the parameters used to monitor vaccine-induced immune responses were not relevant for protective immunity.

The RV144 study tested the safety and efficacy of a prime-boost regimen in heterosexual individuals at various levels of risk for HIV infection; ALVAC-HIV (a canarypox vector expressing HIV-1 *env/gag/pro*) was the prime and AIDSVAX-gp120 B/E (recombinant gp120) was the boost. The vaccine elicited both T cell and Ab responses and achieved protection with 31% efficacy (139). Levels of IgG against variable regions 1 and 2 (V1V2) of the HIV-1 envelope (Env) protein correlated inversely with infection, whereas plasma IgA Ab binding to an Env panel correlated directly with infection (140). IgG avidity, ADCC, nAb, and Env-specific CD4 T cell responses were associated with a reduced risk of infection in vaccinees who showed low IgA responses, but none of these parameters correlated with protection in those who developed robust IgA responses. These data suggest that protective immunity against HIV may be conferred by IgG and that IgA may interfere with protective mechanisms elicited by vaccines.

Protection conferred by passive transfer of neutralizing antibodies. Studies of SIV in rhesus monkeys provide further clues about the immune response to HIV. Systemic transfer of SIV-specific IgG protects rhesus macaques from lethal vaginal challenge with SIV. For example, in one study, intravenous infusion of 15 mg/kg mAb 2F5 and/or mAb 2G12 (against surface gp120) blocked vaginal SIV transmission in most recipient monkeys. The concentration of mAb in the vaginal mucosa at the time of challenge was roughly 10–100 ng/mL (81). The serum concentration of the mAb at the time of challenge was 115–260 μg/mL, indicating that the level of Ab that reached the vaginal mucosa is 1/10,000 the circulating amount. Another study administered mAb b12 at 25 mg/kg, 5 mg/kg, or 1 mg/kg 6 hours prior to vaginal challenge with R5 simian-human immunodeficiency virus (SHIV; 141). The group that received the 25 mg/kg dose was completely protected. At the time of challenge, this group had vaginal mAb levels of 20–30 μg/mL and plasma mAb levels of 600–940 μg/mL. Similarly, passive transfer of a bnAb, PGT121, at 5 mg/kg or 1 mg/kg resulted in protection against vaginal challenge with $SHIV_{SF162P3}$ 24 hours later. Serum concentrations of 95 μg/mL and 15 μg/mL resulted in protection against high-dose viral challenge at relatively low vaginal Ab concentrations of 0.9 μg/mL and 0.2 μg/mL, respectively (142).

Collectively, these studies show that vaginal concentrations of nAb as low as 0.01 μg/mL can protect against vaginal challenge with SIV or SHIV. The vaginal concentration of IgG is usually between 0.1% and 0.01% of that of the serum concentration. To reach a protective level in the vagina, the serum concentration of virus-specific IgG must be maintained, conservatively speaking, at approximately 100 μg/mL. A study using plasma from humans receiving passively transferred nAbs predicts that neutralizing titers higher than 1:200 are required to reach a 50% response rate, in terms of preventing acute infection with HIV-1 (143). This correlates with estimates of nAb titers needed to protect rhesus macaques from SHIV challenge (1:38 to 1:400; 141, 144, 145). These studies indicate that Ab-dependent protection against HIV-1 is efficiently mediated by neutralizing IgG at relatively low levels (0.01–20 μg/mL) in the vagina. To this end, a bnAb whose Fc portion has been modified to elicit greater affinity for the FcRn offers better protection within the rectal mucosa than unmodified bnAb, as the modified bnAb is more likely to reach the lumen (146). Therefore, the challenge of providing protective immunity against HIV-1 is *not* in generating supraphysiological levels of Ab but in eliciting bnAb.

Future approaches for HIV-1 vaccine development. Studies of HIV-1 controllers point to robust CD8 T cell immunity, perhaps based on epitopes presented by rare HLA haplotypes. The RV144 studies indicate that, in sufficient quantities, Env V1V2-specific IgG is protective as long as IgA responses are kept low. Passive immunization studies indicate that systemic bnAb (IgG) is highly effective in blocking HIV-1 transmission. Collectively, these data suggest that an ideal HIV vaccine should elicit a broadly neutralizing IgG response as well as generate specialized T cell responses (**Figure 5*b***). By enforcing the barriers to the first steps of HIV infection (**Figure 1**), we may be able to achieve protective immunity before the virus becomes latent in CD4 T cells.

The biggest hurdle in generating an Ab-based vaccine is finding ways to elicit bnAbs (147, 148). The conserved regions of Env are obscured by glycans that shield neutralizing epitopes from Ab recognition. In addition, it has been difficult to produce HIV-1 Env immunogens that retain their native conformation. In 10–25% of HIV-1 infected humans, a repertoire of modestly to broadly neutralizing Abs develops a few months to several years after infection (147). These bnAbs are characterized by extensive somatic hypermutation and long, variable heavy-chain third complementarity-determining regions (149). For example, rather than the ~5% V_H mutations accrued by high-affinity nAb against influenza HA, HIV-1 bnAb often accumulates ~15–30% V_H mutations (150). In addition, bnAb is often both polyreactive and autoreactive (151, 152). Most B cells bearing poly- or autoreactive receptors undergo negative selection in the bone marrow (153), however, so few such B cells exist in the periphery to be stimulated by a vaccine.

Haynes and colleagues (149) have proposed B cell–lineage vaccine design as a sophisticated, rational approach to generating bnAb. This strategy involves cloning and sequencing the V_H and V_L genes from bnAb. In this approach, the B cell receptor (BCR) sequences of intermediate ancestors and the unmutated ancestor that gave rise to the clone secreting the bnAb would be inferred computationally. Vaccine antigens that bind to the native BCR and to the BCR of successive generations of B cells would be designed using a combination of structural biological approaches, high-throughput screening, and computational methods. Vaccination would involve immunization with an antigen that binds strongly to the unmutated, naive B cell, followed by an antigen that binds to an intermediate descendent, and finally an antigen that drives the generation of bnAb.

To this end, the developmental pathway of a B cell lineage leading to the generation of V1V2 bnAb has been mapped within an HIV-infected individual (154). If the unmutated BCR and the BCRs of successive B cell generations leading to bnAb are conserved in humans, a B cell lineage vaccine could protect against HIV-1. In silico and experimental models of immunization predict that sequential immunization with antigen variants could elicit cross-reactive Abs to conserved epitopes (155). Only a minority of HIV infected humans naturally develop bnAb responses, however, suggesting that there may not be a universal BCR capable of giving rise to bnAb. Efforts to establish Ab-secreting cells within local mucosal tissue could be beneficial: One study showed that prime-and-pull with chemokine CCL28 or MPL, applied vaginally, modestly increased Ab-secreting B cells in the vaginal tissue after intranasal priming with gp140-MPL (156). Approaches to generate bnAb and increase local concentrations of bnAb should be combined to establish the most protective environment within the target tissue.

Despite overwhelming evidence that CD8 T cells protect against HIV-1, the STEP trial that induced high levels of systemic CD8 T cell immunity failed to protect vaccinees against HIV-1 infection. Based on analogy with other viruses, it may be that high levels of T_{RM}s rather than circulating T_{EM}s or T_{CM}s provide protective immunity against HIV-1. In support of this notion, after macaques were vaccinated with live, attenuated SIVmac239Δnef, which provides robust protection in this species, the greatest antiviral activity was found in mucosal, and not circulating, memory CD8 T cells (157). Protection conferred by live SHIV vaccine relies on CD8 T cells, and

the presence of CD8 T cells in the vagina at the time of SIV challenge and a modest expansion of local CD8 effector T cells correlates with protection (158). Virus-specific CD8 T cells form clusters just below the epithelial layer in animals immunized with SIVmac239Δnef as early as 2 weeks after immunization (159). Therefore, future vaccine approaches could benefit from including a pull approach to recruit and establish CD8 T_{RM}s in the genital and rectal mucosa. Alternatively, recombinant, live, attenuated rhesus cytomegalovirus (RhCMV) expressing SIV genes induces high-frequency CD8 T_{EM}s capable of early, complete control of intrarectal SIV challenge in approximately half of immunized animals (160). Moreover, CD8 T_{EM}s induced by the RhCMV-SIV vaccine ultimately clear SIV infection within infected animals following intrarectal or intravaginal challenge (161). Successful T cell–mediated protection from HIV-1 therefore requires high levels of mucosal memory CD8 T cells, and several preclinical models suggest ways of generating such a memory T cell population. Formation of local memory CD8 T cells may not be sufficient for protection against SIV challenge, however (159), so it seems more fruitful to combine bnAb and local T cell–based vaccine approaches to fortify protective immune barriers at the sites of HIV-1 entry (**Figure 3*d***).

Influenza Virus

We typically think of the flu as a rather innocuous annoyance, but the single most devastating disease epidemic in recorded history was the influenza pandemic of 1918, which killed between 20 and 40 million people. Even today, the influenza A virus causes seasonal epidemics around the world, regularly resulting in 250,000 to 500,000 deaths every year, primarily among the very young and the very old (162). The influenza A genome consists of eight single-stranded RNA segments. The internal nucleoprotein (NP) and matrix (M1) protein of the virus are highly conserved between strains and are recognized by CD8 T cells; it is the two major glycoproteins, hemagglutinin (HA) and neuraminidase (NA), on the surface of the virus that serve as the primary targets of Ab recognition. Each year, the HA sequence acquires drift mutations to escape Ab neutralization, and reassortment between different strains of influenza infecting the same cell gives rise to antigenic shift and pandemic flu strains (163). It is this mutability that requires the development of a new batch of vaccine every year, which in turn motivates the ambition to develop a universal influenza vaccine.

Immunological correlates of protection against influenza virus. The most common seasonal flu vaccine given to humans is the inactivated trivalent vaccine, which targets the three most representative virus types in circulation (two subtypes of influenza A and one influenza B virus). Quadrivalent inactivated flu vaccines, which include a second influenza B virus, have been recommended since 2013. Live attenuated influenza vaccines (LAIVs) (e.g., FluMist) made of temperature-attenuated reassortant virus also come in both trivalent (two subtypes of influenza A viruses and one B virus) and quadrivalent (with an additional B virus) forms. Inactivated flu vaccines are injected intramuscularly, and LAIVs are administered intranasally. For inactivated influenza vaccines, an HA inhibition titer of 1:40 has been established as an immunologic correlate of 50% protection in adults (164–166); children require 1:110 to achieve 50% protection (167; **Table 1**). The correlate of protection for adults over the age of 65, however, is unclear (168; see below). An immunological correlate of protection has not been defined for LAIVs.

Protection against influenza requires both Ab and T cell responses. Aside from T cells often being required to elicit robust, high-affinity Ab responses, T cells directly mediate antiviral protection, which becomes especially relevant if the circulating virus has HA and NA for which the population has no preexisting Abs. In this situation, preexisting T cells specific for influenza virus antigens mediate heterosubtypic protection from infection. A study in human volunteers

challenged with an influenza virus for which the subjects had no preexisting Abs showed that preexisting CD4 T, but not CD8 T, cell responses control viral replication and reduce illness (169). Memory CD4 T cells induce secretion of multiple cytokines and chemokines during influenza infection in the lung, contributing to protection from viral challenge (170). For example, in individuals born after 1957 who had no preexisting Ab response to H1N1 strains, protection against the 2009 pandemic H1N1 strain correlated with levels of IFN-γ–secreting CD8 T cells (171). In another study, pre-existing cytotoxic T cell levels above 10% (at 50:1 effector to target ratio in a ^{51}Cr-release assay) correlated with protection in human volunteers challenged with influenza virus (166). In sum, both CD4 and CD8 T cells specific to influenza provide important protective immunity in humans, especially in the absence of preexisting Ab responses.

Goals of creating a universal flu vaccine. The costs and logistical challenges of administering a seasonal flu vaccine each year make the idea of a universal influenza vaccine that would, in principle, protect against all strains of influenza after one immunization rather appealing (**Figure 5*c***). The main challenge in generating a universal flu vaccine is designing immunogens capable of eliciting Ab responses that are broadly neutralizing against multiple HA or NA subtypes (172). As noted above, the prime target of nAbs identified so far is the HA that decorates the surface of influenza virions. HA is synthesized as a precursor that is cleaved into two domains: HA1 forms the hypervariable globular head, whereas HA2 anchors the protein into the membrane with a relatively conserved stalk region. Most efforts to develop a universal flu vaccine focus on targeting the most conserved regions of HA, either the receptor binding site (RBS) within the globular head or the stalk region. The epitope recognized by several RBS-targeting bnAbs includes a highly conserved tryptophan residue within the floor of the RBS groove at HA1 position 153 (173). Because natural infection with influenza rarely elicits a cross-reactive nAb response (174), a vaccine must contain immunogens that are sufficiently different from those present during a natural infection with virus to generate nAbs. To this end, promising candidate vaccine approaches targeted to the conserved regions of HA are being tested in preclinical and clinical settings (173). Such approaches include immunization with headless HA (175, 176) and sequential immunization with HA with different head groups to focus Ab responses on the stalk region (177–179).

In addition to these approaches, empirical evidence indicates that certain prime-boost regimens and adjuvants can achieve nAb-based protective immunity. Animals primed with a DNA vaccine encoding HA and then given a seasonal inactivated vaccine boost develop protection against heterologous influenza strains (180). In addition, treatment with rapamycin during the first 28 days following intraperitoneal immunization with live influenza virus elicits stronger and more broadly cross-reactive protective nAb responses in mice (181). As for the mechanism of bnAb generation, an intriguing study shows that the germline-encoded BCR (V_H 1–69), which is shared by several bnAb clones, first binds the HA stem region as surface IgM. Affinity maturation of the BCR that leads to bnAb activity requires only seven amino acid changes from the germline sequence (182). Therefore, a vaccine strategy to elicit broadly cross-protective immunity to influenza viruses could benefit from targeting naive B cells expressing V_H 1–69 IgM. The next generation of influenza vaccine may take advantage of approaches to focus reactivity against the conserved HA epitope while exploiting the rare germline-encoded BCRs capable of giving rise to bnAbs.

Protecting the vulnerable—flu vaccines for the elderly. Adults over the age of 65 are particularly vulnerable to influenza infection; this group accounts for 90% of flu deaths in the United States (183). Innate and adaptive immune responses change with age (immunosenescence) and grow increasingly aberrant over time (184–186): Basal inflammatory responses increase, and antiviral cytokine responses change (184, 187–189). Immunosenescence not only increases the risk

of mortality associated with influenza, it also reduces the efficacy of influenza vaccination (168). Even with a good vaccine match and high viral circulation, the effectiveness of vaccines against influenza-like illness in elderly people is only 23% (95% CI 6–36%) and vaccination does not offer significant protection against influenza infection (RR 1.04, 95% CI 0.43–2.51) (190).

Immune correlates of protection in the elderly include high levels of granzyme B expression and a high ratio of IFN-γ to IL-10 expression in peripheral blood mononuclear cells (PBMCs) stimulated in vitro with influenza A virus antigen (191, 192). No difference in serum IgG titers against H3N2 exists between infected and uninfected elderly individuals vaccinated against this subtype, the most important cause of influenza disease in this population. Unlike young subjects, older adults immunized with inactivated flu vaccine fail to generated protective levels of Ab response against the vaccine antigen (193, 194). Moreover, the inactivated vaccine fails to induce any increase in CD8 T cell responses to flu in either young or old vaccinees (193).

In light of these studies, an approach to vaccine development for those over 65 years of age might include the following strategies: (*a*) eliciting protective levels of bnAb de novo, (*b*) inducing protective titers of Ab against the emerging influenza HA de novo, and (*c*) restimulating existing T cell immunity against conserved epitopes found in NP and M1. It may not be possible, however, to devise a vaccine capable of eliciting de novo Ab responses in older adults, as B cell development, differentiation, and function decrease with age (195). In an effort to overcome this consequence of immunosenescence, a high-dose trivalent vaccination (60 μg HA as opposed to the standard 15 μg; 196) has been approved for use in individuals older than 65 years. This approach has not achieved the levels of Ab stimulation seen in younger vaccinees, however, nor has the injection of intradermal trivalent vaccines, which randomized clinical trials suggest are more effective than intramuscular injections in adults older than 60 (197, 198). Given the impairment of TLR function in older populations (199), a more effective vaccine will require a proper adjuvant capable of inducing Ab responses, such as the TLR5 response: Unlike other TLRs, TLR5 expression and function are preserved in the monocytes of older individuals (194, 200). In addition, older mice have been successfully vaccinated with a fusion protein of flagellin (a TLR5 agonist) and HA1 (201).

Another strategy against influenza virus in the elderly involves the existing memory T cell pool (**Figure 5*c***). This situation is suited to influenza virus: Because most adults have existing T cell immunity to conserved epitopes, it may be more feasible to design a vaccine that can restimulate a preexisting pool of memory T cells to NP or M1 conserved epitopes than to generate immune responses de novo. FluMist is a temperature-sensitive LAIV capable of eliciting cross-reactive T cell immunity in mice (202), especially when booster immunizations are given (203). Several studies have shown that, in the elderly, FluMist exhibits modest efficacy alone or added efficacy in combination with an inactivated vaccine (204).

Alternative approaches, such as immunization with MVA expressing NP and M1, have demonstrated robust in vivo CD8 T cell stimulation in human volunteers aged 50–85 years (205). In vitro, glucopyranosyl lipid adjuvant–stable emulsion, a TLR4 agonist, serves as an adjuvant to restimulate the Th1 response to trivalent vaccine in PBMCs of older adults (206). A combination of vaccine and adjuvant is thus capable of restimulating existing T cell responses and can likely improve viral control in the elderly.

A word of caution is in order, however. Enhancing the immunogenicity of vaccines in the elderly may not be desirable if the immunopathological consequences are more harmful than the disease. The pathological consequences of vaccine strategies that rely on high-cost immunological mechanisms, inducing cytotoxic T cells, must be carefully examined in humans. It is also worth remembering that most flu-related deaths in this age group result from pneumonia, which is caused by secondary bacterial infections (207, 208). Primary influenza infection can cause loss of

disease tolerance to secondary bacterial infection (209), and elderly populations are particularly vulnerable. Understanding how to increase tolerance against subsequent disease caused by bacterial infection in flu-infected elderly people would illuminate a new way to approach medical care for this vulnerable group.

CONCLUDING REMARKS

As the world faces imminent threats from Zika virus, Ebola virus, Chikungunya virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and other particularly virulent viruses, new ways to approach vaccine design are becoming necessary. Vaccines have been generally successful against viral pathogens that do not mutate rapidly or that are devoid of structural immunoevasins. For such pathogens, nAbs are relatively easy to generate, and even low levels of nAb at the site of viral entry can inhibit infectious spread. Vaccines have not been successful against pathogens that block or escape Ab effector mechanisms of virus restriction, and such pathogens are among the most devastating causes of disease.

Looking forward, the tactic taken in vaccinology for the latter class of pathogens must deviate from the traditional approach, which seeks to generate high levels of serum Ab titers. High serum Ab levels are likely insufficient to protect against viruses capable of immune evasion. The obstacles are specific to each pathogen, and the immune effector mechanisms that are capable of overcoming viral evasion tactics are distinct. Vaccine design would benefit from considering the stage of virus infection that should be targeted to create the most robust protection with the least amount of collateral damage to the host, and from focusing on the appropriate effector mechanism (**Figure 1**). Above all, we need to understand the pathogenesis of a given viral infection. If the mechanism by which a pathogen evades T and B cell immune responses can be delineated at the molecular level, a strategy can be designed to counteract its evasive maneuvers.

Future vaccines could also take advantage of recent gains in our understanding of the biology of tissue-resident memory lymphocytes. The right memory cell types at the portals of pathogen entry will provide better protection than circulating memory cells, with less cost to the host. Establishing tissue-resident T follicular helper cell populations will stimulate local Ab secretion, which could be enhanced by promoting the expression and function of the pIgR (for type I mucosa) and the FcRn (for both types of mucosal surface). Although vaccine approaches that modify these facets of mucosal immunity have not yet been developed, basic research will provide clues for how to get there.

SUMMARY POINTS

1. Protective immunity controls a pathogen without causing excess harm to the host.
2. Every effector mechanism is associated with a different means of pathogen control—and the potential to damage the host.
3. Serum Ab titers do not always predict protective immunity for a given virus, especially when the virus enters through a localized mucosal site.
4. Designing effective vaccines requires an understanding of the mechanisms by which a given pathogen evades the immune response.
5. Future vaccines could benefit from research determining which stage of viral infection can be interrupted to give the highest levels of host protection.

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LITERATURE CITED

1. Palm NW, Medzhitov R. 2009. Pattern recognition receptors and control of adaptive immunity. *Immunol. Rev.* 227:221–33
2. Iwasaki A, Medzhitov R. 2010. Regulation of adaptive immunity by the innate immune system. *Science* 327:291–95
3. Kanno Y, Vahedi G, Hirahara K, Singleton K, O'Shea JJ. 2012. Transcriptional and epigenetic control of T helper cell specification: molecular mechanisms underlying commitment and plasticity. *Annu. Rev. Immunol.* 30:707–31
4. Banchereau J, Steinman RM. 1998. Dendritic cells and the control of immunity. *Nature* 392:245–52
5. Kawai T, Akira S. 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11:373–84
6. Liniger M, Zuniga A, Naim HY. 2007. Use of viral vectors for the development of vaccines. *Expert Rev. Vaccines* 6:255–66
7. Iwasaki A, Medzhitov R. 2015. Control of adaptive immunity by the innate immune system. *Nat. Immunol.* 16:343–53
8. Virgin HW, Wherry EJ, Ahmed R. 2009. Redefining chronic viral infection. *Cell* 138:30–50
9. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature* 489:220–30
10. Iwasaki A. 2007. Mucosal dendritic cells. *Annu. Rev. Immunol.* 25:381–418
11. Linden SK, Sutton P, Karlsson NG, Korolik V, McGuckin MA. 2008. Mucins in the mucosal barrier to infection. *Mucosal Immunol.* 1:183–97
12. Cone RA. 2009. Barrier properties of mucus. *Adv. Drug Deliv. Rev.* 61:75–85
13. Wilks J, Beilinson H, Golovkina TV. 2013. Dual role of commensal bacteria in viral infections. *Immunol. Rev.* 255:222–29
14. Haase AT. 2010. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature* 464:217–23
15. Frazer IH. 2004. Prevention of cervical cancer through papillomavirus vaccination. *Nat. Rev. Immunol.* 4:46–54
16. Moody CA, Laimins LA. 2010. Human papillomavirus oncoproteins: pathways to transformation. *Nat. Rev. Cancer* 10:550–60
17. Gilliet M, Cao W, Liu YJ. 2008. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat. Rev. Immunol.* 8:594–606
18. Goubau D, Deddouche S, Reis e Sousa C. 2013. Cytosolic sensing of viruses. *Immunity* 38:855–69
19. Hou B, Reizis B, DeFranco AL. 2008. Toll-like receptors activate innate and adaptive immunity by using dendritic cell–intrinsic and –extrinsic mechanisms. *Immunity* 29:272–82
20. Rath T, Baker K, Pyzik M, Blumberg RS. 2015. Regulation of immune responses by the neonatal Fc receptor and its therapeutic implications. *Front. Immunol.* 5:664
21. Hangartner L, Zinkernagel RM, Hengartner H. 2006. Antiviral antibody responses: the two extremes of a wide spectrum. *Nat. Rev. Immunol.* 6:231–43

22. Para MF, Goldstein L, Spear PG. 1982. Similarities and differences in the Fc-binding glycoprotein (gE) of herpes simplex virus types 1 and 2 and tentative mapping of the viral gene for this glycoprotein. *J. Virol.* 41:137–44
23. Awasthi S, Huang J, Shaw C, Friedman HM. 2014. Blocking HSV-2 glycoprotein E immune evasion as an approach to enhance efficacy of a trivalent subunit antigen vaccine for genital herpes. *J. Virol.* 88:8421–32
24. Ackerman ME, Alter G. 2013. Opportunities to exploit non-neutralizing HIV-specific antibody activity. *Curr. HIV Res.* 11:365–77
25. Lubinski J, Wang L, Mastellos D, Sahu A, Lambris JD, Friedman HM. 1999. In vivo role of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. *J. Exp. Med.* 190:1637–46
26. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401:708–12
27. Zinkernagel RM. 1996. Immunology taught by viruses. *Science* 271:173–78
28. Wherry EJ, Teichgraber V, Becker TC, Masopust D, Kaech SM, et al. 2003. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat. Immunol.* 4:225–34
29. Medzhitov R, Schneider DS, Soares MP. 2012. Disease tolerance as a defense strategy. *Science* 335:936–41
30. Raberg L, Sim D, Read AF. 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318:812–14
31. Ayres JS, Schneider DS. 2012. Tolerance of infections. *Annu. Rev. Immunol.* 30:271–94
32. Zeitlin L, Cone RA, Moench TR, Whaley KJ. 2000. Preventing infectious disease with passive immunization. *Microbes Infect.* 2:701–8
33. Franco MA, Angel J, Greenberg HB. 2006. Immunity and correlates of protection for rotavirus vaccines. *Vaccine* 24:2718–31
34. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. 2013. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. *J. Infect. Dis.* 208:284–94
35. Nardelli-Haefliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, et al. 2003. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. *J. Natl. Cancer Inst.* 95:1128–37
36. Frazer I. 2007. Correlating immunity with protection for HPV infection. *Int. J. Infect. Dis.* 11(Suppl. 2):S10–16
37. Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, et al. 2006. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. *Vaccine* 24:5571–83
38. Leon B, Ballesteros-Tato A, Misra RS, Wojciechowski W, Lund FE. 2012. Unraveling effector functions of B cells during infection: the hidden world beyond antibody production. *Infect. Disord. Drug Targets* 12:213–21
39. Levin MJ, Oxman MN, Zhang JH, Johnson GR, Stanley H, et al. 2008. Varicella-zoster virus-specific immune responses in elderly recipients of a herpes zoster vaccine. *J. Infect. Dis.* 197:825–35
40. Weinberg A, Zhang JH, Oxman MN, Johnson GR, Hayward AR, et al. 2009. Varicella-zoster virus-specific immune responses to herpes zoster in elderly participants in a trial of a clinically effective zoster vaccine. *J. Infect. Dis.* 200:1068–77
41. Gilbert PB, Gabriel EE, Miao X, Li X, Su SC, et al. 2014. Fold rise in antibody titers by measured by glycoprotein-based enzyme-linked immunosorbent assay is an excellent correlate of protection for a herpes zoster vaccine, demonstrated via the vaccine efficacy curve. *J. Infect. Dis.* 210:1573–81
42. Parr EL, Parr MB. 1998. Immunoglobulin G, plasma cells, and lymphocytes in the murine vagina after vaginal or parenteral immunization with attenuated herpes simplex virus type 2. *J. Virol.* 72:5137–45
43. Belec L, Tevi-Benissan C, Lu XS, Prazuck T, Pillot J. 1995. Local synthesis of IgG antibodies to HIV within the female and male genital tracts during asymptomatic and pre-AIDS stages of HIV infection. *AIDS Res. Hum. Retrovir.* 11:719–29
44. Belec L, Dupre T, Prazuck T, Tevi-Benissan C, Kanga JM, et al. 1995. Cervicovaginal overproduction of specific IgG to human immunodeficiency virus (HIV) contrasts with normal or impaired IgA local response in HIV infection. *J. Infect. Dis.* 172:691–97

45. Bomsel M, Tudor D, Drillet A-S, Alfsen A, Ganor Y, et al. 2011. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. *Immunity* 34:269–80
46. Li Q, Zeng M, Duan L, Voss JE, Smith AJ, et al. 2014. Live simian immunodeficiency virus vaccine correlate of protection: local antibody production and concentration on the path of virus entry. *J. Immunol.* 193:3113–25
47. Shin H, Iwasaki A. 2013. Tissue-resident memory T cells. *Immunol. Rev.* 255:165–81
48. Mueller SN, Gebhardt T, Carbone FR, Heath WR. 2012. Memory T cell subsets, migration patterns, and tissue residence. *Annu. Rev. Immunol.* 31:137–61
49. Cauley LS, Lefrancois L. 2013. Guarding the perimeter: protection of the mucosa by tissue-resident memory T cells. *Mucosal Immunol.* 6:14–23
50. Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR. 2009. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat. Immunol.* 10:524–30
51. Liu L, Zhong Q, Tian T, Dubin K, Athale SK, Kupper TS. 2010. Epidermal injury and infection during poxvirus immunization is crucial for the generation of highly protective T cell–mediated immunity. *Nat. Med.* 16:224–27
52. Hogan RJ, Zhong W, Usherwood EJ, Cookenham T, Roberts AD, Woodland DL. 2001. Protection from respiratory virus infections can be mediated by antigen-specific Cd4$^+$ T cells that persist in the lungs. *J. Exp. Med.* 193:981–86
53. Liang S, Mozdzanowska K, Palladino G, Gerhard W. 1994. Heterosubtypic immunity to influenza type A virus in mice. Effector mechanisms and their longevity. *J. Immunol.* 152:1653–61
54. Wu T, Hu Y, Lee YT, Bouchard KR, Benechet A, et al. 2014. Lung-resident memory CD8 T cells (T_{RM}) are indispensable for optimal cross-protection against pulmonary virus infection. *J. Leukoc. Biol.* 95:215–24
55. Teijaro JR, Turner D, Pham Q, Wherry EJ, Lefrançois L, Farber DL. 2011. Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. *J. Immunol.* 187:5510–14
56. Iijima N, Iwasaki A. 2014. A local macrophage chemokine network sustains protective tissue-resident memory CD4 T cells. *Science* 346:93–98
57. Farber DL, Yudanin NA, Restifo NP. 2014. Human memory T cells: generation, compartmentalization and homeostasis. *Nat. Rev. Immunol.* 14:24–35
58. Thome JJ, Yudanin N, Ohmura Y, Kubota M, Grinshpun B, et al. 2014. Spatial map of human T cell compartmentalization and maintenance over decades of life. *Cell* 159:814–28
59. Schiffer JT, Abu-Raddad L, Mark KE, Zhu J, Selke S, et al. 2010. Mucosal host immune response predicts the severity and duration of herpes simplex virus-2 genital tract shedding episodes. *PNAS* 107:18973–78
60. Zhu J, Peng T, Johnston C, Phasouk K, Kask AS, et al. 2013. Immune surveillance by CD8$\alpha\alpha^+$ skin-resident T cells in human herpes virus infection. *Nature* 497:494–97
61. Schiffer JT, Swan D, Sallaq RA, Magaret A, Johnston C, et al. 2013. Rapid localized spread and immunologic containment define herpes simplex virus-2 reactivation in the human genital tract. *eLife* 2:e00288
62. Zhu J, Koelle DM, Cao J, Vazquez J, Huang ML, et al. 2007. Virus-specific CD8$^+$ T cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. *J. Exp. Med.* 204:595–603
63. **Schiffer JT. 2013. Mucosal HSV-2 specific CD8$^+$ T-cells represent containment of prior viral shedding rather than a correlate of future protection. *Front. Immunol.* 4:209**
64. Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, et al. 1997. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N. Engl. J. Med.* 337:1105–11
65. Buchman TG, Roizman B, Nahmias AJ. 1979. Demonstration of exogenous genital reinfection with herpes simplex virus type 2 by restriction endonuclease fingerprinting of viral DNA. *J. Infect. Dis.* 140:295–304
66. Schmidt OW, Fife KH, Corey L. 1984. Reinfection is an uncommon occurrence in patients with symptomatic recurrent genital herpes. *J. Infect. Dis.* 149:645–46

63. Mathematical modeling predicts that increasing CD8 T cell density will lead to better control of viral shedding.

67. Sakaoka H, Aomori T, Gouro T, Kumamoto Y. 1995. Demonstration of either endogenous recurrence or exogenous reinfection by restriction endonuclease cleavage analysis of herpes simplex virus from patients with recrudescent genital herpes. *J. Med. Virol.* 46:387–96
68. Awasthi S, Friedman HM. 2014. Status of prophylactic and therapeutic genital herpes vaccines. *Curr. Opin. Virol.* 6C:6–12
69. Hofstetter AM, Rosenthal SL, Stanberry LR. 2014. Current thinking on genital herpes. *Curr. Opin. Infect. Dis.* 27:75–83
70. Dropulic LK, Cohen JI. 2012. The challenge of developing a herpes simplex virus 2 vaccine. *Expert Rev. Vaccines* 11:1429–40
71. Corey L, Langenberg AG, Ashley R, Sekulovich RE, Izu AE, et al. 1999. Recombinant glycoprotein vaccine for the prevention of genital HSV-2 infection: two randomized controlled trials. *JAMA* 282:331–40
72. Bernstein DI, Aoki FY, Tyring SK, Stanberry LR, St. Pierre C, et al. 2005. Safety and immunogenicity of glycoprotein D–adjuvant genital herpes vaccine. *Clin. Infect. Dis.* 40:1271–81
73. Stanberry LR, Spruance SL, Cunningham AL, Bernstein DI, Mindel A, et al. 2002. Glycoprotein-D–adjuvant vaccine to prevent genital herpes. *N. Engl. J. Med.* 347:1652–61
74. Belshe RB, Heineman TC, Bernstein DI, Bellamy AR, Ewell M, et al. 2014. Correlate of immune protection against HSV-1 genital disease in vaccinated women. *J. Infect. Dis.* 209:828–36
75. Para MF, Parish ML, Noble AG, Spear PG. 1985. Potent neutralizing activity associated with anti-glycoprotein D specificity among monoclonal antibodies selected for binding to herpes simplex virions. *J. Virol.* 55:483–88
76. Whaley KJ, Zeitlin L, Barratt RA, Hoen TE, Cone RA. 1994. Passive immunization of the vagina protects mice against vaginal transmission of genital herpes infections. *J. Infect. Dis.* 169:647–49
77. Sherwood JK, Zeitlin L, Whaley KJ, Cone RA, Saltzman M. 1996. Controlled release of antibodies for long-term topical passive immunoprotection of female mice against genital herpes. *Nat. Biotechnol.* 14:468–71
78. McDermott MR, Brais LJ, Evelegh MJ. 1990. Mucosal and systemic antiviral antibodies in mice inoculated intravaginally with herpes simplex virus type 2. *J. Gen. Virol.* 71:1497–504
79. Parr EL, Bozzola JJ, Parr MB. 1998. Immunity to vaginal infection by herpes simplex virus type 2 in adult mice: characterization of the immunoglobulins in vaginal mucus. *J. Reprod. Immunol.* 38:15–30
80. Chu CF, Meador MG, Young CG, Strasser JE, Bourne N, Milligan GN. 2008. Antibody-mediated protection against genital herpes simplex virus type 2 disease in mice by Fc γ receptor–dependent and -independent mechanisms. *J. Reprod. Immunol.* 78:58–67
81. Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, et al. 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat. Med.* 6:207–10
82. Nagashunmugam T, Lubinski J, Wang L, Goldstein LT, Weeks BS, et al. 1998. In vivo immune evasion mediated by the herpes simplex virus type 1 immunoglobulin G Fc receptor. *J. Virol.* 72:5351–59
83. Lubinski JM, Lazear HM, Awasthi S, Wang F, Friedman HM. 2011. The herpes simplex virus 1 IgG Fc receptor blocks antibody-mediated complement activation and antibody-dependent cellular cytotoxicity in vivo. *J. Virol.* 85:3239–49
84. Hook LM, Lubinski JM, Jiang M, Pangburn MK, Friedman HM. 2006. Herpes simplex virus type 1 and 2 glycoprotein C prevents complement-mediated neutralization induced by natural immunoglobulin M antibody. *J. Virol.* 80:4038–46
85. Gewurz BE, Gaudet R, Tortorella D, Wang EW, Ploegh HL. 2001. Virus subversion of immunity: a structural perspective. *Curr. Opin. Immunol.* 13:442–50
86. Ashley RL, Crisostomo FM, Doss M, Sekulovich RE, Burke RL, et al. 1998. Cervical antibody responses to a herpes simplex virus type 2 glycoprotein subunit vaccine. *J. Infect. Dis.* 178:1–7
87. Zhu J, Hladik F, Woodward A, Klock A, Peng T, et al. 2009. Persistence of HIV-1 receptor–positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat. Med.* 15:886–92
88. Schiffer JT, Abu-Raddad L, Mark KE, Zhu J, Selke S, et al. 2010. Mucosal host immune response predicts the severity and duration of herpes simplex virus-2 genital tract shedding episodes. *PNAS* 107:18973–78

89. Peng T, Zhu J, Phasouk K, Koelle DM, Wald A, Corey L. 2012. An effector phenotype of $CD8^+$ T cells at the junction epithelium during clinical quiescence of herpes simplex virus 2 infection. *J. Virol.* 86:10587–96
90. Zhu J, Peng T, Johnston C, Phasouk K, Kask AS, et al. 2013. Immune surveillance by $CD8\alpha\alpha^+$ skin-resident T cells in human herpes virus infection. *Nature* 497:494–97
91. Shin H, Iwasaki A. 2012. A vaccine strategy that protects against genital herpes by establishing local memory T cells. *Nature* 491:463–67
92. Mackay LK, Stock AT, Ma JZ, Jones CM, Kent SJ, et al. 2012. Long-lived epithelial immunity by tissue-resident memory T (T_{RM}) cells in the absence of persisting local antigen presentation. *PNAS* 109:7037–42
93. Iijima N, Iwasaki A. 2014. A local macrophage chemokine network sustains protective tissue-resident memory CD4 T cells. *Science* 346:93–98
94. Awasthi S, Zumbrun EE, Si H, Wang F, Shaw CE, et al. 2012. Live attenuated herpes simplex virus 2 glycoprotein E deletion mutant as a vaccine candidate defective in neuronal spread. *J. Virol.* 86:4586–98
95. Awasthi S, Lubinski JM, Shaw CE, Barrett SM, Cai M, et al. 2011. Immunization with a vaccine combining herpes simplex virus 2 (HSV-2) glycoprotein C (gC) and gD subunits improves the protection of dorsal root ganglia in mice and reduces the frequency of recurrent vaginal shedding of HSV-2 DNA in guinea pigs compared to immunization with gD alone. *J. Virol.* 85:10472–86
96. Awasthi S, Lubinski JM, Friedman HM. 2009. Immunization with HSV-1 glycoprotein C prevents immune evasion from complement and enhances the efficacy of an HSV-1 glycoprotein D subunit vaccine. *Vaccine* 27:6845–53
97. Schenkel JM, Fraser KA, Vezys V, Masopust D. 2013. Sensing and alarm function of resident memory $CD8^+$ T cells. *Nat. Immunol.* 14:509–13
98. UNAIDS, WHO (U.N. Prog. HIV/AIDS, World Health Organ.). 2007. *AIDS Epidemic Update 2007*. Geneva: UNAIDS
99. WHO (World Health Organ.). 2008. *Report on the Global AIDS Epidemic*. Geneva: WHO
100. Altfeld M, Allen TM, Yu XG, Johnston MN, Agrawal D, et al. 2002. HIV-1 superinfection despite broad $CD8^+$ T-cell responses containing replication of the primary virus. *Nature* 420:434–39
101. Walker BD, Yu XG. 2013. Unravelling the mechanisms of durable control of HIV-1. *Nat. Rev. Immunol.* 13:487–98
102. Pereyra F, Jia X, McLaren PJ, Telenti A, de Bakker PI, et al. 2010. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. *Science* 330:1551–57
103. Pereyra F, Addo MM, Kaufmann DE, Liu Y, Miura T, et al. 2008. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. *J. Infect. Dis.* 197:563–71
104. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, et al. 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *PNAS* 97:2709–14
105. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, et al. 2007. A whole-genome association study of major determinants for host control of HIV-1. *Science* 317:944–47
106. Pelak K, Goldstein DB, Walley NM, Fellay J, Ge D, et al. 2010. Host determinants of HIV-1 control in African Americans. *J. Infect. Dis.* 201:1141–49
107. Limou S, Le Clerc S, Coulonges C, Carpentier W, Dina C, et al. 2009. Genomewide association study of an AIDS-nonprogression cohort emphasizes the role played by *HLA* genes (ANRS Genomewide Association Study 02). *J. Infect. Dis.* 199:419–26
108. Carrington M, Nelson GW, Martin MP, Kissner T, Vlahov D, et al. 1999. *HLA* and HIV-1: heterozygote advantage and *B*35-Cw*04* disadvantage. *Science* 283:1748–52
109. Kaslow RA, Carrington M, Apple R, Park L, Munoz A, et al. 1996. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat. Med.* 2:405–11
110. Ferre AL, Hunt PW, Critchfield JW, Young DH, Morris MM, et al. 2009. Mucosal immune responses to HIV-1 in elite controllers: a potential correlate of immune control. *Blood* 113:3978–89
111. Hersperger AR, Pereyra F, Nason M, Demers K, Sheth P, et al. 2010. Perforin expression directly ex vivo by HIV-specific $CD8^+$ T-cells is a correlate of HIV elite control. *PLOS Pathog.* 6:e1000917

112. Killian MS, Johnson C, Teque F, Fujimura S, Levy JA. 2011. Natural suppression of human immunodeficiency virus type 1 replication is mediated by transitional memory $CD8^+$ T cells. *J. Virol.* 85:1696–705
113. Akinsiku OT, Bansal A, Sabbaj S, Heath SL, Goepfert PA. 2011. Interleukin-2 production by polyfunctional HIV-1-specific CD8 T cells is associated with enhanced viral suppression. *J. Acquir. Immune Defic. Syndr.* 58:132–40
114. Bailey JR, Lassen KG, Yang HC, Quinn TC, Ray SC, et al. 2006. Neutralizing antibodies do not mediate suppression of human immunodeficiency virus type 1 in elite suppressors or selection of plasma virus variants in patients on highly active antiretroviral therapy. *J. Virol.* 80:4758–70
115. Ahmad R, Sindhu ST, Toma E, Morisset R, Vincelette J, et al. 2001. Evidence for a correlation between antibody-dependent cellular cytotoxicity-mediating anti–HIV-1 antibodies and prognostic predictors of HIV infection. *J. Clin. Immunol.* 21:227–33
116. Baum LL, Cassutt KJ, Knigge K, Khattri R, Margolick J, et al. 1996. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. *J. Immunol.* 157:2168–73
117. Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, et al. 2009. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *AIDS* 23:897–906
118. Dalgleish A, Sinclair A, Steel M, Beatson D, Ludlam C, Habeshaw J. 1990. Failure of ADCC to predict HIV-associated disease progression or outcome in a haemophiliac cohort. *Clin. Exp. Immunol.* 81:5–10
119. Smalls-Mantey A, Doria-Rose N, Klein R, Patamawenu A, Migueles SA, et al. 2012. Antibody-dependent cellular cytotoxicity against primary HIV-infected $CD4^+$ T cells is directly associated with the magnitude of surface IgG binding. *J. Virol.* 86:8672–80
120. Miyazawa M, Lopalco L, Mazzotta F, Lo Caputo S, Veas F, Clerici M. 2009. The 'immunologic advantage' of HIV-exposed seronegative individuals. *AIDS* 23:161–75
121. Erickson AL, Willberg CB, McMahan V, Liu A, Buchbinder SP, et al. 2008. Potentially exposed but uninfected individuals produce cytotoxic and polyfunctional human immunodeficiency virus type 1–specific $CD8^+$ T-cell responses which can be defined to the epitope level. *Clin. Vaccine Immunol.* 15:1745–48
122. Clerici M, Levin JM, Kessler HA, Harris A, Berzofsky JA, et al. 1994. HIV-specific T-helper activity in seronegative health care workers exposed to contaminated blood. *JAMA* 271:42–46
123. Pinto LA, Sullivan J, Berzofsky JA, Clerici M, Kessler HA, et al. 1995. ENV-specific cytotoxic T lymphocyte responses in HIV seronegative health care workers occupationally exposed to HIV-contaminated body fluids. *J. Clin. Investig.* 96:867–76
124. Rowland-Jones SL, Dong T, Fowke KR, Kimani J, Krausa P, et al. 1998. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. *J. Clin. Investig.* 102:1758–65
125. Stranford SA, Skurnick J, Louria D, Osmond D, Chang SY, et al. 1999. Lack of infection in HIV-exposed individuals is associated with a strong $CD8^+$ cell noncytotoxic anti-HIV response. *PNAS* 96:1030–35
126. Ritchie AJ, Campion SL, Kopycinski J, Moodie Z, Wang ZM, et al. 2011. Differences in HIV-specific T cell responses between HIV-exposed and -unexposed HIV-seronegative individuals. *J. Virol.* 85:3507–16
127. Begaud E, Chartier L, Marechal V, Ipero J, Leal J, et al. 2006. Reduced CD4 T cell activation and in vitro susceptibility to HIV-1 infection in exposed uninfected Central Africans. *Retrovirology* 3:35
128. Jennes W, Evertse D, Borget MY, Vuylsteke B, Maurice C, et al. 2006. Suppressed cellular alloimmune responses in HIV-exposed seronegative female sex workers. *Clin. Exp. Immunol.* 143:435–44
129. Card CM, McLaren PJ, Wachihi C, Kimani J, Plummer FA, Fowke KR. 2009. Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of $CD4^+CD25^+FOXP3^+$ regulatory T cells. *J. Infect. Dis.* 199:1318–22
130. Camara M, Dieye TN, Seydi M, Diallo AA, Fall M, et al. 2010. Low-level $CD4^+$ T cell activation in HIV-exposed seronegative subjects: influence of gender and condom use. *J. Infect. Dis.* 201:835–42
131. Koning FA, Otto SA, Hazenberg MD, Dekker L, Prins M, et al. 2005. Low-level $CD4^+$ T cell activation is associated with low susceptibility to HIV-1 infection. *J. Immunol.* 175:6117–22
132. Mazzoli S, Trabattoni D, Lo Caputo S, Piconi S, Ble C, et al. 1997. HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. *Nat. Med.* 3:1250–57

133. Mestecky J, Wright PF, Lopalco L, Staats HF, Kozlowski PA, et al. 2011. Scarcity or absence of humoral immune responses in the plasma and cervicovaginal lavage fluids of heavily HIV-1–exposed but persistently seronegative women. *AIDS Res. Hum. Retrovir.* 27:469–86
134. Scott-Algara D, Truong LX, Versmisse P, David A, Luong TT, et al. 2003. Cutting edge: increased NK cell activity in HIV-1–exposed but uninfected Vietnamese intravascular drug users. *J. Immunol.* 171:5663–67
135. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, et al. 2006. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J. Infect. Dis.* 194:1661–71
136. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF. 2005. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J. Infect. Dis.* 191:654–65
137. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, et al. 2008. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet* 372:1894–905
138. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, et al. 2008. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 372:1881–93
139. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, et al. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N. Engl. J. Med.* 361:2209–20
140. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, et al. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N. Engl. J. Med.* 366:1275–86
141. Parren PW, Marx PA, Hessell AJ, Luckay A, Harouse J, et al. 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. *J. Virol.* 75:8340–47
142. Moldt B, Rakasz EG, Schultz N, Chan-Hui PY, Swiderek K, et al. 2012. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. *PNAS* 109:18921–25
143. Trkola A, Kuster H, Rusert P, von Wyl V, Leemann C, et al. 2008. In vivo efficacy of human immunodeficiency virus neutralizing antibodies: estimates for protective titers. *J. Virol.* 82:1591–99
144. Nishimura Y, Igarashi T, Haigwood N, Sadjadpour R, Plishka RJ, et al. 2002. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. *J. Virol.* 76:2123–30
145. Mascola JR. 2002. Passive transfer studies to elucidate the role of antibody-mediated protection against HIV-1. *Vaccine* 20:1922–25
146. Ko SY, Pegu A, Rudicell RS, Yang ZY, Joyce MG, et al. 2014. Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature* 514:642–45
147. Kwong PD, Mascola JR, Nabel GJ. 2011. Rational design of vaccines to elicit broadly neutralizing antibodies to HIV-1. *Cold Spring Harb. Perspect. Med.* 1:a007278
148. Walker LM, Burton DR. 2010. Rational antibody-based HIV-1 vaccine design: current approaches and future directions. *Curr. Opin. Immunol.* 22:358–66
149. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. 2012. B-cell–lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat. Biotechnol.* 30:423–33
150. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, et al. 2010. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* 329:856–61
151. Mouquet H, Scheid JF, Zoller MJ, Krogsgaard M, Ott RG, et al. 2010. Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. *Nature* 467:591–95
152. Haynes BF, Fleming J, St. Clair EW, Katinger H, Stiegler G, et al. 2005. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. *Science* 308:1906–8
153. Meffre E. 2011. The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. *Ann. N. Y. Acad. Sci.* 1246:1–10
154. Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, et al. 2014. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature* 509:55–62
155. Wang S, Mata-Fink J, Kriegsman B, Hanson M, Irvine DJ, et al. 2015. Manipulating the selection forces during affinity maturation to generate cross-reactive HIV antibodies. *Cell* 160:785–97

140. Vaccine protects against HIV-1 through IgG, whereas IgA may interfere with protection.

156. Tregoning JS, Buffa V, Oszmiana A, Klein K, Walters AA, Shattock RJ. 2013. A "prime-pull" vaccine strategy has a modest effect on local and systemic antibody responses to HIV gp140 in mice. *PLOS ONE* 8:e80559
157. Greene JM, Lhost JJ, Burwitz BJ, Budde ML, Macnair CE, et al. 2010. Extralymphoid $CD8^+$ T cells resident in tissue from simian immunodeficiency virus SIVmac239Δnef-vaccinated macaques suppress SIVmac239 replication ex vivo. *J. Virol.* 84:3362–72
158. Genesca M, McChesney MB, Miller CJ. 2009. Antiviral $CD8^+$ T cells in the genital tract control viral replication and delay progression to AIDS after vaginal SIV challenge in rhesus macaques immunized with virulence attenuated SHIV 89.6. *J. Intern. Med.* 265:67–77
159. Sasikala-Appukuttan AK, Kim HO, Kinzel NJ, Hong JJ, Smith AJ, et al. 2013. Location and dynamics of the immunodominant CD8 T cell response to SIVΔnef immunization and SIVmac251 vaginal challenge. *PLOS ONE* 8:e81623
160. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, et al. 2011. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* 473:523–27
161. Hansen SG, Piatak M Jr, Ventura AB, Hughes CM, Gilbride RM, et al. 2013. Immune clearance of highly pathogenic SIV infection. *Nature* 502:100–4
162. WHO (World Health Organ.). 2014. *Influenza Fact Sheet.* **http://www.who.int/mediacentre/factsheets/fs211/en**
163. Palese P. 2004. Influenza: old and new threats. *Nat. Med.* 10:S82–87
164. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. 2010. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a Bayesian random-effects model. *BMC Med. Res. Methodol.* 10:18
165. Hobson D, Curry RL, Beare AS, Ward-Gardner A. 1972. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J. Hyg.* 70:767–77
166. McMichael AJ, Gotch FM, Noble GR, Beare PA. 1983. Cytotoxic T-cell immunity to influenza. *N. Engl. J. Med.* 309:13–17
167. Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, et al. 2011. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. *Pediatr. Infect. Dis. J.* 30:1081–85
168. McElhaney JE. 2011. Influenza vaccine responses in older adults. *Ageing Res. Rev.* 10:379–88
169. Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, et al. 2012. Preexisting influenza-specific $CD4^+$ T cells correlate with disease protection against influenza challenge in humans. *Nat. Med.* 18:274–80
170. Strutt TM, McKinstry KK, Dibble JP, Winchell C, Kuang Y, et al. 2010. Memory $CD4^+$ T cells induce innate responses independently of pathogen. *Nat. Med.* 16:558–64
171. Sridhar S, Begom S, Bermingham A, Hoschler K, Adamson W, et al. 2013. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat. Med.* 19:1305–12
172. Yewdell JW. 2013. To dream the impossible dream: universal influenza vaccination. *Curr. Opin. Virol.* 3:316–21
173. Krammer F, Palese P, Steel J. 2014. Advances in universal influenza virus vaccine design and antibody mediated therapies based on conserved regions of the hemagglutinin. *Curr. Top. Microbiol. Immunol.* 386:301–21
174. Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, et al. 2011. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science* 333:850–56
175. Sagawa H, Ohshima A, Kato I, Okuno Y, Isegawa Y. 1996. The immunological activity of a deletion mutant of influenza virus haemagglutinin lacking the globular region. *J. Gen. Virol.* 77(Pt. 7):1483–87
176. Steel J, Lowen AC, Wang TT, Yondola M, Gao Q, et al. 2010. Influenza virus vaccine based on the conserved hemagglutinin stalk domain. *mBio* 1:e00018–10
177. Krammer F, Pica N, Hai R, Margine I, Palese P. 2013. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. *J. Virol.* 87:6542–50
178. Margine I, Krammer F, Hai R, Heaton NS, Tan GS, et al. 2013. Hemagglutinin stalk-based universal vaccine constructs protect against group 2 influenza A viruses. *J. Virol.* 87:10435–46

169. In humans without cross-reactive antibodies, preexisting CD4, but not CD8, T cells control viral replication and disease.

178. This study, along with Krammer et al. (179), illustrates the benefit of targeting the HA stalk region as a strategy for a universal flu vaccine.

179. This study, along with Margine et al. (178), illustrates the benefit of targeting the HA stalk region as a strategy for a universal flu vaccine.

179. Krammer F, Hai R, Yondola M, Tan GS, Leyva-Grado VH, et al. 2014. Assessment of influenza virus hemagglutinin stalk–based immunity in ferrets. *J. Virol.* 88:3432–42

180. Wei CJ, Boyington JC, McTamney PM, Kong WP, Pearce MB, et al. 2010. Induction of broadly neutralizing H1N1 influenza antibodies by vaccination. *Science* 329:1060–64

181. Keating R, Hertz T, Wehenkel M, Harris TL, Edwards BA, et al. 2013. The kinase mTOR modulates the antibody response to provide cross-protective immunity to lethal infection with influenza virus. *Nat. Immunol.* 14:1266–76

182. Lingwood D, McTamney PM, Yassine HM, Whittle JR, Guo X, et al. 2012. Structural and genetic basis for development of broadly neutralizing influenza antibodies. *Nature* 489:566–70

183. Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, et al. 2003. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 289:179–86

184. Shaw AC, Goldstein DR, Montgomery RR. 2013. Age-dependent dysregulation of innate immunity. *Nat. Rev. Immunol.* 13:875–87

185. Weng N-p. 2006. Aging of the immune system: How much can the adaptive immune system adapt? *Immunity* 24:495–99

186. Maue AC, Yager EJ, Swain SL, Woodland DL, Blackman MA, Haynes L. 2009. T-cell immunosenescence: lessons learned from mouse models of aging. *Trends Immunol.* 30:301–5

187. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, et al. 2000. Inflamm-aging: an evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 908:244–54

188. Murasko DM, Jiang J. 2005. Response of aged mice to primary virus infections. *Immunol. Rev.* 205:285–96

189. Stout-Delgado HW, Yang X, Walker WE, Tesar BM, Goldstein DR. 2008. Aging impairs IFN regulatory factor 7 upregulation in plasmacytoid DCs during TLR9 activation. *J. Immunol.* 181:6747–56

190. Jefferson T, Rivetti D, Rivetti A, Rudin M, Di Pietrantonj C, Demicheli V. 2005. Efficacy and effectiveness of influenza vaccines in elderly people: a systematic review. *Lancet* 366:1165–74

191. In elderly people, prevaccine T cell responses, and not Ab, correlate with protection against influenza virus.

191. McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, et al. 2006. T cell responses are better correlates of vaccine protection in the elderly. *J. Immunol.* 176:6333–39

192. McElhaney JE, Ewen C, Zhou X, Kane KP, Xie D, et al. 2009. Granzyme B: correlates with protection and enhanced CTL response to influenza vaccination in older adults. *Vaccine* 27:2418–25

193. Wagar LE, Gentleman B, Pircher H, McElhaney JE, Watts TH. 2011. Influenza-specific T cells from older people are enriched in the late effector subset and their presence inversely correlates with vaccine response. *PLOS ONE* 6:e23698

194. Panda A, Qian F, Mohanty S, van Duin D, Newman FK, et al. 2010. Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. *J. Immunol.* 184:2518–27

195. Allman D, Miller JP. 2005. B cell development and receptor diversity during aging. *Curr. Opin. Immunol.* 17:463–67

196. Falsey AR, Treanor JJ, Tornieporth N, Capellan J, Gorse GJ. 2009. Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. *J. Infect. Dis.* 200:172–80

197. Holland D, Booy R, De Looze F, Eizenberg P, McDonald J, et al. 2008. Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial. *J. Infect. Dis.* 198:650–58

198. Lambert ND, Ovsyannikova IG, Pankratz VS, Jacobson RM, Poland GA. 2012. Understanding the immune response to seasonal influenza vaccination in older adults: a systems biology approach. *Expert Rev. Vaccines* 11:985–94

199. Shaw AC, Goldstein DR, Montgomery RR. 2013. Age-dependent dysregulation of innate immunity. *Nat. Rev. Immunol.* 13:875–87

200. Qian F, Wang X, Zhang L, Chen S, Piecychna M, et al. 2012. Age-associated elevation in TLR5 leads to increased inflammatory responses in the elderly. *Aging Cell* 11:104–10

201. Leng J, Stout-Delgado HW, Kavita U, Jacobs A, Tang J, et al. 2011. Efficacy of a vaccine that links viral epitopes to flagellin in protecting aged mice from influenza viral infection. *Vaccine* 29:8147–55

202. Sun K, Ye J, Perez DR, Metzger DW. 2011. Seasonal FluMist vaccination induces cross-reactive T cell immunity against H1N1 2009 influenza and secondary bacterial infections. *J. Immunol.* 186:987–93
203. Slutter B, Pewe LL, Lauer P, Harty JT. 2013. Cutting edge: rapid boosting of cross-reactive memory CD8 T cells broadens the protective capacity of the Flumist vaccine. *J. Immunol.* 190:3854–58
204. Ambrose CS, Luke C, Coelingh K. 2008. Current status of live attenuated influenza vaccine in the United States for seasonal and pandemic influenza. *Influenza Other Respir. Viruses* 2:193–202
205. Antrobus RD, Lillie PJ, Berthoud TK, Spencer AJ, McLaren JE, et al. 2012. A T cell–inducing influenza vaccine for the elderly: safety and immunogenicity of MVA-NP+M1 in adults aged over 50 years. *PLOS ONE* 7:e48322
206. Behzad H, Huckriede AL, Haynes L, Gentleman B, Coyle K, et al. 2012. GLA-SE, a synthetic Toll-like receptor 4 agonist, enhances T-cell responses to influenza vaccine in older adults. *J. Infect. Dis.* 205:466–73
207. Beadling C, Slifka MK. 2004. How do viral infections predispose patients to bacterial infections? *Curr. Opin. Infect. Dis.* 17:185–91
208. McCullers JA. 2006. Insights into the interaction between influenza virus and pneumococcus. *Clin. Microbiol. Rev.* 19:571–82
209. Jamieson AM, Pasman L, Yu S, Gamradt P, Homer RJ, et al. 2013. Role of tissue protection in lethal respiratory viral-bacterial coinfection. *Science* 340:1230–34
210. Nalin DR, Kuter BJ, Brown L, Patterson C, Calandra GB, et al. 1993. Worldwide experience with the CR326F-derived inactivated hepatitis A virus vaccine in pediatric and adult populations: an overview. *J. Hepatol.* 18(Suppl. 2):S51–55
211. Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. 1999. What level of hepatitis B antibody is protective? *J. Infect. Dis.* 179:489–92
212. Hombach J, Solomon T, Kurane I, Jacobson J, Wood D. 2005. Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, WHO, Geneva, 2–3 September, 2004. *Vaccine* 23:5205–11
213. Chen RT, Markowitz LE, Albrecht P, Stewart JA, Mofenson LM, et al. 1990. Measles antibody: reevaluation of protective titers. *J. Infect. Dis.* 162:1036–42
214. Samb B, Aaby P, Whittle HC, Seck AM, Rahman S, et al. 1995. Serologic status and measles attack rates among vaccinated and unvaccinated children in rural Senegal. *Pediatr. Infect. Dis. J.* 14:203–9
215. WHO (World Health Organ.). 1993. *The Immunological Basis for Immunization Series*. Geneva: WHO
216. Weibel RE, Buynak EB, McLean AA, Hilleman MR. 1975. Long-term follow-up for immunity after monovalent or combined live measles, mumps, and rubella virus vaccines. *Pediatrics* 56:380–87
217. Faden H, Modlin JF, Thoms ML, McBean AM, Ferdon MB, Ogra PL. 1990. Comparative evaluation of immunization with live attenuated and enhanced-potency inactivated trivalent poliovirus vaccines in childhood: systemic and local immune responses. *J. Infect. Dis.* 162:1291–97
218. Marine WM, Chin TD, Gravelle CR. 1962. Limitation of fecal and pharyngeal poliovirus excretion in Salk-vaccinated children. A family study during a type 1 poliomyelitis epidemic. *Am. J. Hyg.* 76:173–95
219. WHO (World Health Organ.) 2007. Rabies vaccines: WHO position paper. *Wkly. Epidemiol. Rec.* 82:425–35
220. Jiang B, Gentsch JR, Glass RI. 2002. The role of serum antibodies in the protection against rotavirus disease: an overview. *Clin. Infect. Dis.* 34:1351–61
221. McNeal MM, Broome RL, Ward RL. 1994. Active immunity against rotavirus infection in mice is correlated with viral replication and titers of serum rotavirus IgA following vaccination. *Virology* 204:642–50
222. Matter L, Kogelschatz K, Germann D. 1997. Serum levels of rubella virus antibodies indicating immunity: response to vaccination of subjects with low or undetectable antibody concentrations. *J. Infect. Dis.* 175:749–55
223. Skendzel LP. 1996. Rubella immunity. Defining the level of protective antibody. *Am. J. Clin. Pathol.* 106:170–74
224. Plotkin SA. 1995. Rubella vaccine. *J. Infect. Dis.* 171:1690–92
225. Fogel A, Gerichter CB, Barnea B, Handsher R, Heeger E. 1978. Response to experimental challenge in persons immunized with different rubella vaccines. *J. Pediatr.* 92:26–29

226. Mack TM, Noble J Jr, Thomas DB. 1972. A prospective study of serum antibody and protection against smallpox. *Am. J. Trop. Med. Hyg.* 21:214–18
227. Sarkar JK, Mitra AC, Mukherjee MK. 1975. The minimum protective level of antibodies in smallpox. *Bull. World Health Organ.* 52:307–11
228. White CJ, Kuter BJ, Ngai A, Hildebrand CS, Isganitis KL, et al. 1992. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. *Pediatr. Infect. Dis. J.* 11:19–23
229. Clements DA, Armstrong CB, Ursano AM, Moggio MM, Walter EB, Wilfert CM. 1995. Over five-year follow-up of Oka/Merck varicella vaccine recipients in 465 infants and adolescents. *Pediatr. Infect. Dis. J.* 14:874–79
230. Mason RA, Tauraso NM, Spertzel RO, Ginn RK. 1973. Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. *Appl. Microbiol.* 25:539–44
231. Monath TP, Nichols R, Archambault WT, Moore L, Marchesani R, et al. 2002. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF-VAX) in a phase III multicenter, double-blind clinical trial. *Am. J. Trop. Med. Hyg.* 66:533–41
232. Plotkin SA. 2001. Immunologic correlates of protection induced by vaccination. *Pediatr. Infect. Dis. J.* 20:63–75
233. Plotkin SA. 2008. Vaccines: correlates of vaccine-induced immunity. *Clin. Infect. Dis.* 47:401–9
234. Plotkin SA. 2010. Correlates of protection induced by vaccination. *Clin. Vaccine Immunol.* 17:1055–65
235. Plotkin S, Orenstein WA, Offit P. 2012. *Vaccines*. Amsterdam: Elsevier
236. Amanna IJ, Messaoudi I, Slifka MK. 2008. Protective immunity following vaccination: How is it defined? *Hum. Vaccin.* 4:316–19