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## Vaccination against Viruses

**Harry B Greenberg**, Stanford University School of Medicine, Stanford, CA, USA

**Philip R Dormitzer**, Novartis Influenza Vaccines, Cambridge, MA, USA

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### Glossary

**Hemagglutination inhibition** The ability of an immune serum to block the clumping of red blood cells caused by cross-linking of the cells by multivalent viruses with receptors for red blood cell surface antigens.

**Heterotypic** Able to recognize more than one virus serotype.

**Jennerian** A vaccine approach in which an animal virus is used to protect humans from disease.

**Reassortment** The exchange of gene segments between viruses with multipartite genomes during coinfection.

**Split vaccine** A vaccine with antigens from chemically disrupted virus particles.

**Subunit vaccine** A vaccine with antigens that are purified virus or microbe components.

**Toll-like receptors** A type of receptor that recognizes conserved microbial components and triggers an innate immune response but does not undergo splicing recombination to generate diversity.

**Viremia** Presence of virus in the blood.

### Abstract

Most vaccines in use today are the result of empirical development. The mechanism of action of many vaccines in common use remains incompletely understood. Understanding how such vaccines protect is an ongoing subject of study using increasingly sophisticated immunological tools, such as B cell and T cell repertoire and transcriptome analysis. Such tools are also being applied to the design of vaccines against those viral targets that have evaded vaccine-mediated protection thus far. As basic immunological science intersects with the practicalities of assuring vaccine safety, tolerability, efficacy, and consistency in the clinic, the practical utility of more sophisticated immunological measures for vaccine development may be determined by whether they can be reduced to simply executed, highly standardized, reproducible assays with outcomes that have clear interpretations for vaccine development and use. Basic immunology, empirical vaccine testing, and regulatory science are all necessary contributors to developing the next generation of vaccines, including vaccines effective against the pathogens for which vaccines are not currently available.

### Introduction – Viral Vaccines Now in Use

Immunization against viruses has achieved some of the greatest successes of medicine, such as the eradication of smallpox worldwide and the control of polio in most of the world. Yet, there are major viral pathogens, such as the human immunodeficiency virus (HIV), hepatitis C virus (HCV), respiratory syncytial virus (RSV), and human cytomegalovirus, for which no effective vaccines are available, despite decades of research and development. **Table 1** lists the viral agents for which licensed vaccines are now available in the United States. With a singular exception, these viruses show limited antigenic diversity and have undergone little antigenic change over time. That exception, influenza virus, defines the current limits of our ability to immunize effectively in the face of substantial antigenic diversity and change.

On the list, there are few vaccines developed using the tools of modern molecular biology or sophisticated immunological methods. Many of the vaccine antigens (such as those in vaccines against influenza virus, Japanese encephalitis virus, rabies virus, and poliovirus) are produced by growing viruses

and chemically inactivating them. Other viral vaccines are produced by propagating live viruses that have been attenuated by experimental passage in culture or in nonhuman hosts (influenza virus, yellow fever virus, measles virus, mumps virus, and rubella virus), by using virus strains originating in nonhuman hosts ('Jennerian' vaccines – rotavirus, vaccinia virus), or by nonrecombinant genetic manipulation (genome segment reassortment through coinfection for rotavirus and influenza virus). Some preparations of vaccines still in use (against influenza virus and yellow fever virus) are produced by virus propagation in fertilized chicken eggs rather than in cell culture. Exceptions to the generally low technology approaches to producing vaccine antigens include recombinantly produced vaccine antigens for hepatitis B virus (HBV), human papillomavirus (HPV), and influenza virus. The HPV and HBV vaccine antigens, which are expressed in yeast or insect cells, self-assemble into particles that resemble those released from virus-infected cells (Zeltins, 2013).

Similarly, most vaccines in use today are either unadjuvanted or adjuvanted with alum, which has been used to enhance immune responses to vaccines since the 1920s

**Table 1** Viruses for which vaccines are licensed and available in the United States<sup>a</sup>

<i>Virus</i>	<i>Type(s) of Vaccines</i>
Adenovirus types 4 and 7	Oral live attenuated, bivalent
Hepatitis A virus	Inactivated virus, alum adjuvanted
Hepatitis B virus	Recombinant, self-assembling antigen, alum adjuvanted
Human papillomavirus types 6, 11, 16, 18, 31, 33, 45, 52, 58	Recombinant virus-like particles; adjuvanted with alum or with alum and monophosphoryl lipid A; bivalent, quadrivalent, or 9-valent
Influenza virus (seasonal)	Live attenuated, recombinant, or inactivated split or subunit; trivalent or quadrivalent
Influenza virus (H1N1 pandemic)	Live attenuated or inactivated split or subunit, monovalent
Influenza virus (H5N1 prepandemic)	Inactivated split, unadjuvanted or adjuvanted with an oil-in-water emulsion, monovalent
Japanese encephalitis virus	Inactivated unadjuvanted or adjuvanted with alum
Measles virus	Live attenuated (in combination with mumps and rubella with or without varicella)
Mumps virus	Live attenuated (in combination with measles and rubella with or without varicella)
Rubella virus	Live attenuated (in combination with measles and mumps with or without varicella)
Poliovirus	Inactivated, trivalent
Rabies virus	Inactivated
Rotavirus	Live attenuated, monovalent or pentavalent
Smallpox virus	Live attenuated, Jennerian (bovine virus)
Varicella virus	Live attenuated (two dosages – low for prevention of chicken pox in children and high for prevention of shingles in the elderly)
Yellow fever virus	Live attenuated

<sup>a</sup>Information from US Food and Drug Administration (2015).

(Baylor et al., 2002). There are a few exceptions. One licensed HPV vaccine includes a Toll-like receptor agonist adjuvant based on monophosphoryl lipid (MPL) A, and some influenza vaccines available in the European Union and other countries include oil-in-water emulsion adjuvants (Del Giudice and Rappuoli, 2015; Garçon et al., 2011).

### Viral Vaccine Mechanism of Action and Correlates of Protection

Vaccines are more complex in their mechanism of action than almost all other pharmaceutical products. Rather than acting directly through a well-defined, single ligand–receptor interaction, vaccine antigens and adjuvants have a series of complex interactions with multiple components of the immune system at or near the site of immunization and

sometimes at systemic sites as well. These interactions prime the host for an anamnestic response to a subsequent infectious challenge, often at another anatomic site in the host, at a time remote from the immunization. The antigens in the viral challenge may have differences from those in the vaccine. In some cases, the host may have experienced infection with an antigenically related virus prior to immunization, adding an additional element of complexity to the vaccine immune response. The complex immunology that underlies protection from disease by immunization makes it a fascinating subject for scientific investigation. However, for practical vaccine development, straightforward, definitive, easily executed, reproducible, and readily interpreted immunological assays are needed to characterize and define the response to vaccines more fully. The intersection between the rich complexity of vaccine immunology and the practical need for simplicity in regulatory science contributes to a gap between the science and practice of vaccinology.

For some vaccines, simple models of the determinants of protection have proven sufficient for practical decision making. For example, if the HBV vaccine elicits a postvaccination-binding titer against the HBV surface antigen (SAg)  $\geq 10$  mIU/ml, a subject is considered to be protected (Jack et al., 1999). Even if the anti-SAg titer falls to  $< 10$  mIU/ml after having been  $\geq 10$  mIU/ml, substantial levels of protection persist (West and Calandra, 1996). Similarly, for the yellow fever vaccine, a plaque neutralization serum antibody titer of 0.9 neutralization index (equivalent to the difference in titer,  $\log_{10}$ , between pre- and post-vaccination serum) is strongly associated with protection (Mason et al., 1973). Neutralization of virus by antibody is generally considered the dominant mode of protection against those viruses whose pathogenesis requires viremia. Examples include poliovirus, smallpox virus, yellow fever virus, HBV, varicella zoster virus (for chicken pox, not zoster), measles virus, mumps virus, and rubella virus. This is likely an oversimplification of the true situation, but it has proved a useful simplification for vaccine development and monitoring (Plotkin, 2013).

Assigning reliable correlates of protection can be more challenging for vaccines against viruses that predominantly infect and replicate at a mucosal surface. For inactivated influenza vaccines, a serum hemagglutination inhibition (HI) titer of 1:40 has long been accepted as a correlate of protection, based primarily on studies in young adults (de Jong et al., 2003). However, considerably higher antibody levels may be required to protect children, with HI titers of 1:110 conferring 50% protection after immunization with an adjuvanted, inactivated influenza vaccine (Black et al., 2011). In the elderly, measures of cell-mediated immunity, such as granzyme B levels in virus-stimulated peripheral blood mononuclear cells, may correlate better than serum antibody titers with vaccine-elicited protection (McElhaney et al., 2009). In addition, the correlates of protection after immunization with inactivated influenza vaccines do not predict protection after immunization with live attenuated influenza vaccines, which offer more protection than would be predicted based on the elicited serum HI or neutralization titers (Beyer et al., 2002). Whether cell-mediated effector mechanisms, mucosal antibody, or some other factor is primarily responsible for protection by live attenuated influenza vaccines or natural

infection remains controversial despite several decades of study.

A reliable correlate of protection is even more elusive for rotavirus, which causes diarrhea by infecting the small intestinal epithelium. Based on studies in animal models, homotypic neutralizing antibodies were thought to play a key role in protection, making viral serotype an important distinction. Yet, in humans, a monovalent live attenuated rotavirus vaccine provides substantial heterotypic protection (Leshem et al., 2014). There are a number of candidates for the mechanism of the greater breadth of protection, including heterotypic neutralizing antibody, heterotypic nonneutralizing antibodies that block intracellular replication, and cell-mediated effector mechanisms, with no mechanism widely accepted as responsible. Cell-mediated effector mechanisms are thought to play a significant role in vaccine-mediated protection from a few viruses, such as varicella zoster virus (in adults) and influenza virus (with live attenuated vaccines) (Forrest et al., 2008; Weinberg et al., 2009). Eliciting an effective T helper (Th) response is essential for mounting an affinity-matured, durable humoral response to vaccines. The control of more changeable viruses and the harnessing of cell-mediated effector mechanisms for prevention of viral diseases in humans are, therefore, two of the chief challenges in modern vaccinology.

### Immunological Assays for Viral Vaccines

Increasingly sophisticated tools of B cell repertoire analysis, transcriptome analysis, and advanced functional imaging are being used to understand vaccine mechanism of action (Tsang et al., 2014; Jiang et al., 2013; Karlsson et al., 2015). Yet, as is the case for the technologies used to manufacture most current human vaccines, the core immunoassays used to bring viral vaccines to licensure are generally simple, as exemplified by antibody-based viral neutralization assays, enzyme-linked immunosorbent assays, and multiplex binding assays. This restricted range of assay types largely reflects a need to standardize assays rigorously (Guidance for Industry. Bioanalytical Method Validation (Draft) 2013) and practical limitations on the sophistication of assays that can be used as primary endpoint in the large-scale clinical trials (often with tens of thousands of subjects) required to bring novel vaccines to licensure. More sophisticated measures of cell-mediated immunity have had a prominent role in vaccine research but a much more minor role in vaccine licensure. The difficulty of standardizing such assays and assigning a threshold assay value that indicates protection has limited their use as correlates of protection for vaccine trials being used for registration. Similarly, although mucosal immunity surely plays a major role in determining vaccine-mediated protection, challenges in sample collection and assay consistency have the consequence that serum antibody levels rather than measures of mucosal immunity are favored as correlates of protection for vaccine licensure. Nevertheless, measures of cell-mediated immunity, such as enzyme-linked immunosorbent spot assays and fluorescence-activated cell sorter-based T cell functional analyses, are routinely used as exploratory endpoints during vaccine development. Recently,

investigators have also added systems biology approaches, with transcriptional array, mass cytometry-based cell sorting, and luminex binding assays to their analytical tool box for investigating the early innate immune responses to vaccination (O’Gorman et al., 2014; Tsang et al., 2014). To date, none of these interesting approaches have proven sufficiently robust or predictive to be used as licensure criteria. Standardization of such assays could lead to a more prominent role for assays of cell-mediated, mucosal, and innate immunity in vaccine development.

### Challenges to Vaccine-Mediated Protection from Viral Diseases

Antigenic diversity and antigenic change remain key challenges for the development of effective vaccines for several important agents. Influenza vaccines must change composition on an almost annual basis to track antigenic drift or shift, and the vaccine varies considerably in effectiveness from year to year depending in part on the accuracy of the antigenic match between the vaccine and circulating strains. For pathogens that are more variable than influenza, such as HIV and HCV, attempts at immunization have thus far proven largely ineffective despite the identification of viral targets of broadly neutralizing antibodies (Burton and Mascola, 2015; Kong et al., 2012; Throsby et al., 2008; Whittle et al., 2011).

Evasion of vaccine-mediated immunity, for example, by replication in privileged reservoirs, poses another challenge for vaccine development. HIV evades vaccine-mediated immunity not only by selection of resistant variants in response to immune pressure, but also by replication in immune-privileged sites, such as cells of the central nervous system (Churchill and Nath, 2013). The latency of herpes viruses has posed a challenge for vaccine development, though not an insurmountable challenge, as evidence by the efficacy of varicella zoster vaccines against reactivation disease (shingles) (Lal et al., 2015; Oxman et al., 2005).

The duration of immunity elicited by viral vaccines also varies. In the best case, a single immunization with the live attenuated yellow fever vaccine appears to confer lifelong protection (although current recommendations are for a booster every 10 years for those at risk) (Gotuzzo et al., 2013). At the other extreme, the need for annual immunization against influenza does not appear to be solely due to antigenic changes in circulating viruses, as the duration of immunity elicited by nonadjuvanted, inactivated influenza vaccines appears to be limited, with vaccine strain-specific effectiveness decreasing over the course of a season more than can be accounted for by the antigenic drift of circulating viruses (Kissling et al., 2013; Pebody et al., 2013).

Special populations pose a particular challenge for viral vaccines. The neonatal response to immunization differs from responses later in life, with preferential elicitation of memory B cells rather than plasma cells and a more Th2-biased T cell response (Wood and Siegrist, 2011). Passively transferred maternal antibodies can interfere with vaccine immunogenicity, particularly in infants below 6 months of age (Crowe, 2001). Safety is a critical issue for neonatal immunization. For example, findings of excess wheezing and hospitalization

prevent a live attenuated influenza vaccine from being used in infants under 2 years of age despite its utility in older children (Prutsky et al., 2014). To overcome these factors and to take advantage of prime-boost effects, infant immunization is generally carried out with multiple doses, often including remote boosters.

For RSV, a major cause of the infant disease for which there is currently no licensed vaccine, there is insufficient time to elicit an active immune response before the peak incidence of severe disease at approximately 2 months of age. This early peak of disease is a chief rationale underlying proposals for maternal immunization against RSV (Anderson et al., 2013). Immunizing pregnant women can increase the levels of pathogen-specific antibodies transferred transplacentally or in breast milk to newborns. Immunization of pregnant women is gaining increasing acceptance, in part due to the experience of immunizing pregnant women during the 2009 influenza pandemic (which caused disproportionately severe illness in pregnant women) (Fell et al., 2012), evidence of improved birth outcomes with influenza immunization during pregnancy (Steinhoff et al., 2012), and the success of maternal immunization to prevent neonatal tetanus (Blencowe et al., 2010).

At the other end of the age spectrum, immune senescence creates challenges for immunizing the elderly. High-dose formulations of inactivated influenza vaccine and varicella zoster vaccine and an adjuvanted formulation of inactivated influenza vaccine have been used to increase vaccine effectiveness in the elderly (DiazGranados et al., 2014; Mannino et al., 2012; Oxman et al., 2005). Immunodeficiencies also pose a challenge for immunization. For example, renal failure patients on hemodialysis are at particular risk for the acquisition of hepatitis B. Yet, the HBV vaccine is less likely to elicit protective antibody titers in this population. This has led to the licensure of a hepatitis B vaccine adjuvanted with an MPL-analog adsorbed on aluminum phosphate for those with renal insufficiency in the European Union (Kundi, 2007). There are also safety concerns around the use of live attenuated vaccines in the immunodeficient, with some live attenuated vaccines (such as those targeting smallpox virus, yellow fever virus, or rotavirus) causing clinical illness due to infection with the attenuated vaccine virus in those with immunodeficiency (Bakare et al., 2010; Reed et al., 2012; Seligman, 2014).

The potential for vaccine-mediated disease enhancement has substantially hindered the development of an RSV vaccine (Anderson et al., 2013). In the 1960s, a formalin-inactivated RSV vaccine candidate primed immunized infants for more severe RSV disease upon natural RSV challenge (Kapikian et al., 1969). A preponderance of evidence suggests that an immunopathologic Th2-biased cellular response coupled with a minimal neutralizing antibody response to the vaccine was responsible for the enhanced disease, although the mechanism of the vaccine-mediated disease enhancement is still not completely understood (Graham, 2011). Observations of vaccine-enhanced disease have also been made in humans after immunization with an inactivated measles vaccine (Fulginiti et al., 1967) and in a ferret model with experimental immunization against the severe acute respiratory syndrome coronavirus (Weingartl et al., 2004).

## Prospects for Applying Modern Immunology for Improved Vaccines

As is evident from the preceding comments, there is ample opportunity to apply state-of-the-art immunology as well as more modern manufacturing practices to vaccines. There remains a large gap between the large diversity of immunization approaches in research and early development and the limited application of new science and technology to vaccines that have been licensed or that are in late-stage development. In part, this reflects the challenges of replacing sufficiently effective, old vaccines with new ones. The old vaccines have a long track record of safe administration; for the new vaccines, knowledge of safety, efficacy, and effectiveness is necessarily more limited, no matter what theoretical advantages new technologies may offer. In addition, many of the old, established vaccines still in use are remarkably inexpensive, undermining the economics that would permit replacement with much more expensive new vaccines, burdened by new development costs. Nevertheless, key viral pathogens, such as HIV, RSV, CMV, herpes simplex virus, dengue virus, and HCV have resisted successful vaccine development using conventional techniques and are prime targets for innovative approaches.

More sophisticated application of immunology to vaccine development could address some of the challenges in the clinical development of vaccines. For example, increased use of immunological markers of vaccine safety, immunogenicity, and efficacy could limit clinical trial sizes and durations. More intensive study of smaller numbers of subjects in early clinical trials could lead to the testing of more vaccine candidates and better informed decisions on whether to progress vaccine candidates to larger-scale clinical testing or to switch to more promising alternative vaccine candidates (Rappuoli and Aderem, 2011). For example, CD4<sup>+</sup> T cell responses observed 3 weeks after immunization with a H5N1 influenza vaccine predict neutralizing antibody responses 6 months after immunization, potentially enabling more rapid answers about immunogenicity from clinical trials of such vaccines (Galli et al., 2009).

Although analysis of biomarkers and more sophisticated immunological analyses may enhance our ability to assess some aspects of vaccine safety and immunogenicity in smaller trials, it is less likely that such approaches will enable the detection of the unanticipated, idiosyncratic adverse events that large phase III trials and postlicensure surveillance are designed to detect. Examples of such idiosyncratic events include the Guillain-Barré syndrome observed after influenza immunization following the 1977 swine flu outbreak (Schonberger et al., 1979), the narcolepsy associated with AS03-adjuvanted 2009 pandemic influenza vaccine in northern European adolescents (Ahmed et al., 2014), or the intestinal intussusception associated to greater or lesser degrees with different live attenuated rotavirus vaccines (Rosillon et al., 2015). However, if a core set of safety and immunogenicity parameters could be assessed in smaller trials through more intensive study of immunological and other biomarkers, more of the burden of detecting rare events could be shifted from large prelicensure trials to postlicensure surveillance systems, decreasing the barriers to advancing vaccines through licensure to meet the unmet medical needs.

B cell repertoire analysis has provided a wealth of new information on the diversity of antibodies that collectively make up a serum antibody response to a vaccine. A key finding has been that, among the neutralizing antibodies elicited in response to influenza virus, HIV, or RSV infection or immunization, some have remarkably broad specificity (Burton and Mascola 2015; Corti et al., 2013, 2011). To date, B cell repertoire analysis coupled with the functional analysis of antibodies and the structural analysis of antigen–antibody complexes has defined the antibodies we want our vaccines to elicit and the target structures on viruses recognized by such antibodies. Progress has also been made in our ability to design epitope-focused antigens that elicit such antibodies (Correia et al., 2014; Schickli et al., 2015). However, to date, such engineered antigens have not proven more effective at eliciting high titers of broadly neutralizing antibodies than the intact glycoprotein domains that contain the epitopes of interest. Iterative improvement of such rationally designed antigens does, however, hold promise for improved vaccines that target patches of conservation on variable, multiepitope antigens.

Vaccines based on conserved T cell epitopes or internal protein antigens that are the targets of T cell–based effector mechanisms are an area of active research. Theoretically, vaccines that target the conserved nonsurface proteins of viruses that are expressed in infected cells could overcome the antigenic diversity of surface neutralization determinants. For example, in a small human live viral challenge study, the vectored expression of the influenza internal matrix protein and nucleoprotein together significantly reduced the number of days of virus shedding during laboratory-confirmed influenza infection (Lillie et al., 2012). It remains to be determined whether such mechanisms of protection will prove effective and durable enough to be the primary basis for vaccine-mediated protection, however. Even absent the ability to suffice as the primary basis for influenza vaccines, conserved targets of T cell effectors may prove useful supplements to increase the breadth of coverage by vaccines that also include targets of more potently protective, strain-specific humoral immunity (Antrobus et al., 2014). As T cell effector mechanisms involve the killing of host cells, priming for increased T effector responses does carry some risk of exacerbating disease even as it accelerates viral clearance.

Increased insights into innate immune mechanisms have informed the search for new generations of rationally designed adjuvants. The adjuvants in currently licensed vaccines, aluminum salts, oil-in-water emulsions, and MPL-analogs have been the products of largely empirical development efforts (O’Hagan and Fox, 2015). The elucidation of Toll-like receptors as key sentry molecules that detect potential pathogens and recruit antigen-presenting cells for a subsequent antigen-specific response has enabled the rational design of a new generation of potential vaccine adjuvants (Wu et al., 2014). Using well-established principles of small-molecule screening and optimization for ligand binding, new Toll-like receptor agonists are now reaching clinical testing. Similarly, the ability to target vaccine-primed responses to mucosal tissues through the use of retinoic acid as an adjuvant, thus far demonstrated in preclinical testing, provides an additional mode of action for adjuvants to shape the character of vaccine-mediated immune responses

(Tan et al., 2011). It is important to note, however, that demonstration of safety, especially as it applies to rare idiosyncratic events, remains a critical hurdle to the licensure of vaccines containing novel adjuvants.

## Conclusion

Largely empirical approaches to vaccine development have provided tremendous public health benefits. The understanding of vaccine immunology has advanced greatly since many vaccines still in use were originally developed. Applying this new knowledge could improve current vaccines and lead to the development of vaccines for viral diseases that have resisted vaccine-mediated protection to date.

**See also:** **B Cell Activation:** Antigen Acquisition *In Vivo* and its Role in B Cell Activation; Cytokine Regulation of B Cell Activation and Differentiation; Extrafollicular Antibody Responses; Germinal Centers; Long-Lived Plasma Cells; Marginal Zone B Cell Responses to Antigens; Memory B Cells; Regulation of B Cell Migration and Location in Response to Antigens; Somatic Hypermutation and Class Switch Recombination; T Cell–Dependent B Cell Activation; The Humoral Immune Response to T Cell–Independent Antigens; Vaccines to Program Protective Humoral Immunity. **Cells of the Innate Immune System:** Dendritic Cells and Dendritic Cell Subsets. **Cytokines and Their Receptors:** Inflammasomes; Viral Anticytokine Strategies. **Immunity to Bacterial, Parasitic and Fungal Infections:** Immune-Driven Pathogen Evolution; Immunology of Bacterial and Parasitic Diseases: An Overview; Immunology of Central Nervous System Pathogens. **Immunity to Viral Infections:** Activation of CD8 T Lymphocytes during Viral Infections; Antibody Responses and Functions in Defense against Viral Infection; CD4 T Cell Immunity to Viral Infection; CD8 T Cell Memory to Pathogens; Chemokines and Viral Infections; Dendritic Cells in Viral Infection; Ebola Virus; Human Genetic Defects Resulting in Increased Susceptibility to Viral Infections; Immune Responses to Viruses in the CNS; Immunity to HIV; Immunity to Oncogenic Viruses; Innate Cytokine Responses and Their Functions during Viral Infections; Intrinsic Cellular Defenses (TRIMS) in Modulating Viral Infection and Immunity; NK Cells in Antiviral Defense; NK Cells in HIV-1 Infection; NK Cells in T Cell Regulation; Protective and Pathogenic T Cell Responses to Virus Infections; Sensors of Viral Infection; T Cell Responses during Acute Respiratory Virus Infection. **MHC Structure and Function:** Immune Epitope Database and Analysis Resource; Ligand Selection and Trafficking for MHC I; Ligand Selection and Trafficking for MHC II; Origin and Processing of MHC Class II Ligands; Origin and Processing of MHC-I Ligands; Structure of Classical Class I MHC Molecules; Structure of Classical MHCII (HLA-DP, DQ, DR, and Orthologs). **Physiology of the Immune System:** Immunology of the Female Reproductive Mucosa; Neonatal Immunity; The Intestinal Immune System. **Signal Transduction:** Immunological Synapses. **Structure and Function of Diversifying Receptors:** AID in Somatic Hypermutation and Class Switch Recombination; Error-Prone Mismatch and Base Excision DNA Repair in Somatic

Hypermutation; Genomic Organization of Murine and Human Immunoglobulin Light Chain Loci; Genomic Organization of the Murine and Human IGH Locus; IgG Structure and Function; Organization and Rearrangement of TCR Loci; Structure and Function of IgA; Structure and Function of TCR $\alpha\beta$  Receptors; Structure, Function, and Production of Immunoglobulin M (IgM); Structure, Function, and Spatial Organization of the B Cell Receptor. **T Cell Activation:** Conventional Dendritic Cells: Identification, Subsets, Development, and Functions; Recirculating and Resident Memory CD8<sup>+</sup> T Cells; Th1 Cells; The Differentiation and Function of Th2 and Th9 Cells; Transition of T Cells from Effector to Memory Phase. **Tumor Immunology:** Therapeutic and Prophylactic Cancer Vaccines; Vaccines and Their Role in CD8 T Cell-Mediated Antitumor Immunity.

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