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Chapter 4

Antiviral Immunity and Virus Vaccines

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As obligate intracellular organisms, viruses have co-evolved with their respective host species, which in turn have evolved diverse and sophisticated capabilities to protect themselves against viral infections and their associated diseases. Viruses have also evolved a remarkable variety of strategies to avoid or subvert these host defences. Antiviral immunity in higher animals is complex and reflects a combination of innate and acquired (adaptive) immune response mechanisms, although there is considerable interplay between these two broad categories. Innate immunity provides constant and relatively rapid protection against viral infections, and previous exposure to a particular virus is not required to activate these mechanisms. In contrast, adaptive immunity develops only after exposure to a virus and is specific to that particular pathogen and often its close relatives. Adaptive immunity involves cell and antibody (humoral)-mediated effector mechanisms, by T and B lymphocytes, respectively. Adaptive immune responses also exhibit memory, such that the response may be quickly reactivated after reexposure to the same virus. With many systemic viral infections, immunological memory after natural infection confers long-term, often lifelong, protection against the associated disease.

The development of efficacious vaccines has substantially reduced the deleterious impact of viral infections in humans and animals. The goal of vaccination is to stimulate the adaptive immune responses that protect animals from infection with specific viruses. An increasing variety of vaccine types are now commercially available for use in both companion and production animal species. These include conventional inactivated and live-attenuated virus vaccines, recombinant viruses that express protective proteins of heterologous viruses, virus-like particles (VLPs), and DNA vaccines. Vaccines are used extensively in regulatory programs for the control of individual viral diseases of livestock, often in combination with specific management procedures. Vaccines are also a critical component of the medical care of companion pets.

This chapter provides a comprehensive overview of immunity to viruses, including mechanisms that viruses utilize to avoid or “escape” these protective host responses, and gives an in-depth discussion of the large variety of vaccines available for animal vaccination. Also addressed are adverse effects of vaccines, vaccination policy and schedules, and the increasingly important fields of vaccination of poultry and fish.

IMMUNITY TO VIRUSES

Innate Immunity to Viral Infections

The cells mediating innate immunity do not respond to specific viral antigens as do their counterparts in the adaptive immune response. Rather, these cells are activated by the presence of the virus, using an array of different
sensors. In addition, cells of the innate system react to viral infections through production and recognition of cytokines, which are small proteins that affect the behavior of other cells. Cytokines made by lymphocytes are often termed interleukins. A key family of cytokines in the innate response to virus infection are the interferons.

**Interferon Responses**

In 1957, Isaacs and Lindenmann reported that influenza virus-infected cells produce a nonviral protein they termed “interferon” that can protect uninfected cells against the same (influenza virus) as well as unrelated viruses. It has since been determined that there are several types and subtypes of interferon and that these proteins are key elements of antiviral resistance at the cellular level. They also play a central role in both innate and adaptive immune responses to viral infections. A critical class of these proteins was collectively designated as type I interferon (IFN). These include IFN-α, which is encoded by several different genes in most species (e.g., 14 in cattle and 27 in swine). How many of the IFN-α genes are used by any species in response to any infection event is not clearly defined. There are also 7 IFN-β genes in cattle and one in swine. In addition, IFN-τ, IFN-δ, IFN-ε, IFN-κ, and IFN-ω are also type I interferons. All of these protein hormones bind a common receptor, the IFN-α receptor (IFNAR). This cell surface protein is a heterodimer of IFNAR 1 and 2, and functions to transduce a signaling cascade of enzymes including the tyrosine and Janus kinases that induce signal transducers and activators of transcription, and interferon regulatory factors. Activation of this signaling cascade ultimately results in induction of the interferon response genes in cells (Fig. 4.1). Humans and animals with deficits in signaling pathways triggered by interferon often die of common viral diseases that are not usually fatal.

Type II interferon, or IFN-γ, was originally reported as “immune interferon.” This cytokine is central to many aspects of both innate and adaptive immunity and defines multiple subtypes of T lymphocytes. Type III interferon is designated as IFN-λ. In humans, these protein hormones were originally described as members of the interleukin 10 (IL-10) cytokine family because they are bound by IFN-λ receptor 1 and IL-10 receptor 2. IFN-λ1, 2, and 3 were first described as IL-29, IL-28A, and IL-28B, respectively. As with type I and type II interferons, the IFN-λs have cytokine activities in addition to their inherent antiviral action. In cattle and swine, there are 2 IFN-λ genes reported to date, IFN-λ1 and IFN-λ3.

Induction of type I interferon in virus-infected cells involves activation via an array of cellular receptors.
called pattern recognition receptors, which detect pathogen-associated molecules that are broadly specific to different classes of viruses. The binding of pathogen-associated molecules to these cellular receptors stimulates the transcription of numerous genes encoding proteins that are involved in innate and adaptive immune responses, including the activation of interferon production and secretion. Importantly, these responses may be triggered by several redundant pathways, both cytoplasmic and extracytoplasmic. One class of pattern recognition receptors are the Toll-like receptors (TLRs), so named because of their homology to the Toll genes of *Drosophila*. Different Toll-like receptors detect different pathogen-associated molecular patterns (PAMPs). For instance, TLR7 and TLR8 bind single-stranded RNA (ssRNA), thus detecting RNA virus infections, which then induces production of type I interferon. This is an important response to influenza and human immunodeficiency virus infections for example. In contrast, TLR3 detects double-stranded RNA (dsRNA), a critical intermediate of RNA virus genome replication that is not present in normal cells. These Toll-like receptors are predominantly located in the endosome, where they can readily detect viruses internalized after endocytosis, including viruses or their nucleic acid released from adjacent apoptotic or lysed cells. Cytosolic pathways for pathogen sensing and type I interferon induction also can occur via TLR-independent signaling involving cytoplasmic RNA helicase proteins such as retinoic acid inducible gene (RIG-1) and melanoma differentiation-associated gene 5 (MDA5). Other intracellular pathways include mitochondrial antiviral signaling protein (MAVS; also referred to as IPS-1), which mediates activation of transcription factors that induce interferon production (Fig. 4.1).

Type I interferon released from virus-infected cells or activated innate response cells (see below) stimulates adjacent cells via interferon α receptor (IFNAR) binding (Fig. 4.1). This activates a signaling pathway leading to induction of the interferon response element. In mice, this results in the transcriptional activation of more than 300 interferon-stimulated genes (ISGs). In large mammals and humans it is clear that a similar group of interferon-stimulated genes is activated following binding of type I interferons to their specific receptors. Most of these genes encode proteins that regulate either signaling pathways or transcription factors that amplify interferon production, whereas others promote an antiviral state via cytoskeletal remodeling, apoptosis, posttranscriptional events (mRNA editing, splicing, degradation), or posttranslational modifications.

Proteins proven to be critical to the induction of the interferon-induced antiviral state include:

- ISG15, which is a ubiquitin homolog that is not constitutively expressed in cells. Addition of ubiquitin to cellular proteins is key to regulation of the innate immune response, and ISG15 apparently can exert a similar function with more than 150 target proteins in interferon-stimulated cells. Activities of ISG15 can regulate all aspects of the interferon pathway, including induction, signaling, and action.
- MxGTPase is a hydrolyzing enzyme that, like ISG15, is not constitutively expressed. The enzyme is located in the smooth endoplasmic reticulum, where it affects vesicle formation, specifically targeting the viral nucleocapsid in virus-infected cells to prevent virus maturation.
- The protein kinase R (PKR) pathway is constitutively expressed at only a very low level, but is quickly upregulated by IFNAR signaling. In the presence of dsRNA, the protein kinase phosphorylates elongation (translation) initiation factor eIF2α and prevents recycling of cyclic nucleotides (GDP), which in turn halts protein synthesis. This interferon-induced pathway is especially important for inhibiting replication of reoviruses, adenoviruses, vaccinia and influenza viruses, amongst many others.
- The 2′→5′ oligoadenylate synthetase (OAS) pathway, like the PKR pathway, is constitutively expressed only at a low level. After IFNAR stimulation and in the presence of dsRNA, this enzyme produces oligoadenylates with a distinctive 2′→5′ linkage, as contrasted with the normal 3′→5′ lineage. These 2′→5′ oligoadenylates in turn activate cellular RNAse that degrades RNA, which cleaves viral messenger and genomic RNA. Picornaviruses are especially susceptible to inhibition by this pathway, as is West Nile virus.

In summary, type I interferon is produced after virus infection of many different types of cells, and the interferon released from these cells then induces an antiviral state in adjacent cells. In addition, cells of the innate immune system can be activated to secrete interferon by virus infection, including nonproductive infections or by their ‘sensing’ of viral infection, which augments the level of antiviral signaling and the local antiviral state in tissue. In many instances, this response may control, or even eliminate, a viral infection before the development of systemic infection or the occurrence of overt disease. If the virus overwhelms the early innate immune response then systemic spread occurs and disease may be detected clinically.

**Natural Killer Cells**

Natural killer (NK) cells are specialized lymphocytes that lack an antigen-specific receptor, which can kill virus-infected cells, tumor cells and other cells they detect to be “in a state of stress”. This is accomplished via engagement of a
series of receptors for ligands expressed on the surface of potential target cells. As such, natural killer cells provide early and nonspecific resistance against viral infections. Natural killer cells express an extensive complex of receptors that recognize particular patterns of expression of their respective ligands on host cells. The receptors on natural killer cells are both activating and inhibitory, and the function of natural killer cells is stringently regulated by the balance of activating and inhibitory signals from these receptors. For example, one of the primary receptors on natural killer cells binds to class I major histocompatibility complex (MHC) proteins and this binding provides a negative (inhibitory) signal for natural killer cell activation. This allows natural killer cells to “scan” tissue without harming healthy cells, which are recognized as “self.” A common effect of virus infection is reduced expression of class I MHC protein on the surface of the infected cell. The lack of sufficient MHC ligand to bind the natural killer cell inhibitory receptor results in activation signals that reach the necessary threshold for cell activation. Virus-infected cells also express stress receptors that bind activating receptors on natural killer cells, and thus the balance of signal favors “antigen-independent” activation and the resulting killing response (Fig. 4.2).

The receptors mediating activation of natural killer cells to target cell killing, or the inhibition of that activation, are encoded in two large families of genes. The killer immunoglobulin-like receptors (KIR) are encoded by a cluster of genes in the leukocyte receptor complex. In humans and cattle, these receptors are highly polymorphic and individuals tend to have unique allelic patterns of expression. Mice lack KIR genes altogether, and KIR gene transcription products in horses have yet to be described. Only a single KIR gene transcript has been demonstrated in swine. The second receptor complex expressed by natural killer cells is the NK receptor complex. In this locus are the NK2G genes and the Ly49 genes. Mice express many Ly49 genes as do horses, whereas pigs, cattle, cats and dogs express a single gene product and humans lack a functional Ly49 gene. There are other receptor genes encoded in the MHC locus of these species including Nkp30 and Nkp44 or 46, depending on the species. Nkp46, also designated CD335, is the classic natural killer cell marker. However, cells of other lineages that are not natural killer cells can express this protein and kill other cells in a natural killer cell-like manner (i.e., antigen nonspecific). Most notable of these are γδ T cells (see below).

Natural killer cells kill virus-infected cells by the same pathway utilized by antigen specific, cytotoxic T lymphocytes (CTL), which is by inducing apoptosis (i.e., programmed cell death, or “cell suicide”—see Chapter 3: Pathogenesis of Viral Infections and Diseases). This cytocidal activity is central to the control of viral infections because it can eliminate infected cells (virus factories) before they can produce and release progeny virions. Like cytotoxic T lymphocytes, natural killer cells have cytosolic granules that contain the proteins perforin, granzyme A and granzyme B (Fig. 4.2). When activated by the stimulatory receptor-binding process, these granules are orientated toward the target cell and then released. Perforin creates pores in the target cell membrane through which the granzyme proteins enter and once inside, these proteins induce apoptosis of the target cell.

**FIGURE 4.2** Natural killer (NK) cell destruction of a virus-infected cell. Virus-infected cells express multiple stress indicators and virus infection inhibits expression of cell proteins. The NK cell’s multiple activating and inhibiting receptors are bound, and when activation stimuli overcome inhibition, cell killing is initiated. The cytotoxic granules orient to the cell junction and are released. The perforin creates access to the target cell cytosol delivering the granzymes, which are serine proteases that mediate target cell death by multiple pathways. Courtesy of J.R. Patch, W.T. Golde, Plum Island Animal Disease Center, USDA.
Natural killer cells also express CD16, a surface receptor for the Fc portion of immunoglobulin G molecules (FcRγIII). This receptor allows natural killer cells to bind and lyse antibody-coated target cells through the process of antibody-dependent cellular cytotoxicity. This results in a killing activity identical to the cell-killing mechanism just described, but bypassing all of the natural killer cell receptors. Finally, natural killer cells also can mediate functions in addition to direct killing. Notably, natural killer cells are very efficient at type II interferon (IFNγ) production and secretion following activation. IFNγ secretion by natural killer cells creates a strong inflammatory environment, activates other cells of the innate and the adaptive immune system, and induces an antiviral state in cells at the site of inflammation.

**T Cells in Innate Immunity**

The antigen-specific receptor on T cells is expressed as a heterodimer in a complex with subunits of the nonpolymorphic protein CD3. The receptor heterodimer is comprised of α and β chains requiring the CD3 complex for cell surface expression; these so-called αβ T cells are required for adaptive immune responses. There is also a unique subset of T cells that plays a prominent role in innate immunity, but with a different receptor comprised of γ and δ chain expressed as a heterodimer in association with CD3; thus, these are termed γδ T cells. These γδ T cells can express a series of scavenger receptors including those in the WC1 family. In mice, there are no circulating γδ cells but they constitute up to 5% of peripheral blood mononuclear cells in humans, especially in newborns. In pigs and calves, up to 50% of circulating lymphocytes can be γδ T cells, and 20–30% in adult swine and cattle. These cells function in adaptive immune responses via antigen-specific interactions with the T cell receptor, but they also can be activated in a nonspecific manner in response to cellular stress such as that associated with virus infection. Specifically, γδ T cells can respond to infection by expressing NKp46 and killing virus-infected cells in a natural killer cell-like manner. These cells while concurrently making strong cytokine responses, particularly production of IFNγ.

**Innate Responses of Dendritic Cells**

Dendritic cells (DCs) are critical to the initiation of the adaptive immune response but are also central to innate immunity. There are a number of dendritic cell subtypes, with overlapping (common) functions as well as capacities unique to each subtype. The classical dendritic cell is of bone marrow myeloid lineage, expresses a high density of class II MHC proteins, and is highly phagocytic in the naive state. These dendritic cells can also respond to stimulation by pathogen associated signatures (PAMPs) by secreting large amounts of type I interferon (notably IFNα and IFNβ). Other dendritic cells of the myeloid lineage populate the skin, both the dermis and epidermis. A substantial portion of dendritic cells in the skin are a specialized subset of cells called Langerhans cells, which have been described in many species of mammal. A unique dendritic cell population, first described in pigs and subsequently in mice, humans, cattle, and nonhuman primates, is termed the plasmacytoid dendritic cell. These differentiate from a lymphoid lineage and have a distinct morphology from myeloid dendritic cells. Plasmacytoid dendritic cells were first described as natural interferon producing cells as they are remarkably efficient in the production and secretion of type I interferon in response to virus infection.

**Adaptive Immunity to Viral Infections**

The adaptive immune response to viral infection requires recognition and binding of antigen by specific receptors on T and B lymphocytes. Induction of an adaptive immune response occurs in lymph nodes and is initiated by pathogen-stimulated dendritic cells that migrate through afferent lymphatics from the site of infection to the draining lymph node. A primary adaptive immune response takes several days to develop and involves clonal expansion of lymphocytes bearing identical antigen-specific receptors and the differentiation of these lymphocytes into effector cells. The adaptive immune response consists of two main arms: humoral immunity, mediated by antibodies secreted by terminally differentiated B lymphocytes called plasma cells, and cell-mediated immunity, driven by αβ T cell receptor expressing lymphocytes expressing lymphocytes (Fig. 4.3). Antibodies bind antigen directly in its native conformation on the pathogen surface and protect the host by clearing extracellular viruses, whereas T lymphocytes recognize processed antigen in the form of peptides bound to MHC molecules at the cell surface and so target virus-infected cells. Once a virus infection is cleared from the host, a proportion of the antigen-specific lymphocytes can develop into long-lived memory cells that can rapidly respond to the pathogen should it be encountered again; this establishment of immunologic memory is a hallmark of adaptive immunity and the basis of vaccination.

**Dendritic Cells Link Innate and Adaptive Immune Responses**

Classical dendritic cells are “professional” antigen-presenting cells (APCs), as they have a unique capacity to stimulate T cell responses to infectious agents, including viruses. Langerhans cells and other dendritic cells at epithelial surfaces exist as immature cells that are equipped to capture antigens and pathogens by phagocytosis. Many viruses directly infect dendritic cells. Pathogen infection or exposure results in engagement of Toll-like receptors or other pathogen recognition
receptors leading to interferon production, secretion, and signaling that induces a process known as maturation in which the dendritic cell transitions from innate immune responses to antigen-presenting cell function. A critical feature of dendritic cell maturation is the switch in chemokine (chemokines are cytokines that attract other cells via the process of chemotaxis) receptor expression from CCR5 to CCR7, thereby guiding dendritic cell migration. In addition, there is an accompanying change in the functional capacity of the migrating dendritic cell such that phagocytic capacity is reduced, interferon production is lost, and production of cytokines that activate naive T cells and B cells increases. Mature dendritic cells also have upregulated expression of MHC and costimulatory molecules that are particularly important in stimulating antigen-specific naive T cells resident in the lymph node paracortex. Dendritic cell and T cell engagement is facilitated by expression of adhesion molecules LFA-1 and CD2 on the T cell, and ICAM-1, ICAM-2, and CD58 on the dendritic cell. The mature dendritic cell provides three different kinds of signal to the naive T cell. Binding of the MHC/peptide complex to the T cell receptor/CD3 complex provides the first signal, and the second is mediated by binding of costimulatory molecules on the dendritic cell with CD28 on the T cell. These two signals promote activation and survival of the T cell. The third signal mediated by cytokines produced by the dendritic cell leads to T cell differentiation, as discussed below.

**Recognition and Killing of Virus-Infected Cells by Cytotoxic T lymphocytes (CTLs)**

Destruction of infected cells by cytotoxic T lymphocytes expressing αβ T cell receptors is the principal mechanism utilized by the adaptive immune system to control intracellular virus infections (Fig. 4.3). Cytosolic viral proteins within the infected cell are digested by a multicatalytic protease complex called the proteasome, which delivers short peptides to the endoplasmic reticulum through a pair of energy-dependent transporters known as TAP (transporters associated with antigen processing; TAP1 and TAP2). Within the endoplasmic reticulum, peptides are further trimmed to lengths of 8–11 amino acids and engage a series of chaperone molecules that allow peptides with the compatible sequence to bind nascent MHC class I molecules forming in the endoplasmic reticulum. The stable MHC class I/peptide complex is shuttled through the Golgi apparatus for presentation at the surface of the infected cell. T lymphocytes that bear antigen receptors that recognize the specific MHC class I/peptide complex presented by the infected cell bind the complex and become activated. T cells targeting infected cells in this manner also express the CD8 coreceptor which binds an invariant region of MHC class I protein and provides the signals that are essential for an effective T cell response (Fig. 4.4).
Antigens generated within a virus-infected cell are designated as endogenous antigens and their presentation by MHC class I molecules can occur in essentially any cell in the body, providing an effective means for CD8 T cell recognition and subsequent elimination of infected cells. Endogenous antigens are not the exclusive source of antigenic peptide for MHC class I loading, however, as peptides from extracellular sources, including phagocytized dead and dying cells that are infected with pathogens, can enter the MHC class I pathway through a process known as cross-presentation. This pathway is important in allowing dendritic cells not directly infected with a pathogen to engage and stimulate naïve virus-specific CD8 T cells in the lymph node paracortex during the initial establishment of a primary adaptive response.

CD4 Helper T Cells in Immunity to Virus Infection

A second population of αβ T cells bears the CD4 coreceptor and, once activated, these cells can differentiate into several subsets of functionally distinct effector cells based on the type of cytokines they produce (Fig. 4.3). Although CD4 T cells can participate directly in the killing of virus-infected cells (ie, as cytotoxic T lymphocytes), that function is more characteristic of CD8 T cells and is mediated by only a minor population of virus-reactive CD4 T cells. Rather, CD4 T cells play an especially important role in antiviral immunity by facilitating both cell-mediated and humoral immune responses, hence the term T helper cell. There are at least five different subsets of CD4 T cells that are specialized in providing help to immune responses to infections with different classes of pathogens. T-helper 1 (TH1) cells produce IFN-γ and activate macrophages, which facilitates killing of intracellular pathogens phagocytized by macrophages, creating the antiviral state in IFN-γ receptor expressing cells, and inducing differentiation of discrete aspects of B lymphocyte function. T-helper 2 (TH2) cells produce interleukins (IL-4, IL-5, and IL-6) that recruit eosinophils, mast cells and basophils, providing protection at mucosal surfaces. Both TH2 cells and T follicular helper cells (TFH) cells that reside in B cell follicles of lymph nodes, engage B cells and promote antibody production via secretion of IL-4 and IL-13. TH17 cells produce IL-17 and IL-21 that induce fibroblasts and epithelial cells to recruit neutrophils to sites of microbial infection during the early stages of an adaptive immune response. A final class of CD4 T cell is the regulatory T cell, a heterogeneous population of cells that suppress T cell activity and limit autoimmunity. These cells are characterized by production of antiinflammatory cytokines such as IL-10 and transforming growth factor.

CD4 T cells recognize antigenic peptides presented by MHC class II proteins, which are limited in expression to antigen presenting cells (Dendritic cells, macrophages and B cells). In many species of large mammal, including primates, cattle, and swine, T cells can be activated to MHC
class II gene expression and contribute to antigen presentation thereby expanding secondary adaptive immune responses. Peptides presented by MHC class II molecules derive largely from exogenous antigens, those antigens that are made outside of the cell, such as endocytosed virus particles and particulate antigens derived from dead and dying cells. Antigen taken up by cells from the extracellular space is internalized into endosomes which become acidified, activating proteases that degrade antigen into peptide fragments and individual amino acids that are then available for new protein synthesis. Critical for adaptive immunity, these peptides are also available for binding to MHC class II molecules (Fig. 4.4).

Newly formed MHC class II molecules in the endoplasmic reticulum are protected from binding peptides within that compartment by a chaperone protein called invariant chain, which blocks the peptide-binding groove of the molecule and targets delivery of MHC class II to a low-pH compartment. Proteases process the invariant chain leaving a truncated form of protein termed class II-associated invariant chain peptide (or CLIP) that continues to protect the peptide-binding groove. Vesicles containing endocytosed exogenous proteins (eg, viral proteins) fuse with vesicles containing MHC class II molecules and antigenic peptides displace the CLIP chaperone protein, facilitated by the MHC class II-like molecule HLA-DM. The fully formed MHC class II/peptide complex is transported to the cell surface where a T cell receptor with specificity for the particular peptide/MHC combination can bind to form a trimolecular complex of T cell receptor, MHC class II and peptide. This interaction is further facilitated by the CD4 coreceptor expressed by these T cells, which binds to an invariant region of MHC class II and promotes an effective T cell response (Fig. 4.4).

A key function of effector CD4 T cells is to provide help to CD8 T cells, an essential step in the activation of cytotoxic T lymphocytes in the majority of viral infections. Within lymph nodes, CD4 T cells engage virus-derived peptides bound to MHC class II presented by antigen presenting cells such as dendritic cells, which also engage naïve CD8 T cells through presentation of different viral peptides in the context of MHC class I. The effector CD4 T cell expresses CD40 ligand that binds CD40 on the dendritic cell, thus activating the dendritic cell and inducing the upregulation of essential costimulatory molecules such as CD80 and CD86 that are required for activation of the CD8 T cell. Effector CD4 T cells also secrete abundant IL-2 that drives CD8 T cell proliferation. Further, CD4 T cells are essential in the effective activation and differentiation of B cells in most humoral immune responses, as detailed below.

**T Cell Memory**

Memory is a critical aspect of adaptive immune responses, in contrast to innate immunity where there is
no recall on reexposure to specific antigens. During the period of antigenic stimulation, a portion of the reactive helper (TH) and cytotoxic (CTL) T cells differentiate into memory T cells. These cells return to quiescence and reside primarily in the local (ie, “draining”) lymph nodes and to a lesser extent, other lymphoid organs like the spleen. When there is a subsequent exposure to the virus, these cells mediate the recall response. The induction and maintenance of T cell memory is a critical aspect of vaccination. In swine and cattle, memory T cells express both CD4 and CD8. Humans and nonhuman primates also have a small percentage of peripheral T cells that express both CD4 and CD8 whereas this phenomenon is very rare in mature T cells of mice.

**Humoral Immunity to Virus Infection**

Humoral immunity is mediated by antibodies (syn. immunoglobulins (Ig)), which are the effector molecules of B lymphocytes (Fig. 4.3). Immunoglobulins consist of a combination of proteins called heavy and light chains, which each have variable (V) and constant (C) regions. The antigen-binding region is unique to each antibody and is formed by the combined V regions of both heavy and light chains at one end of the molecule. The C region of the heavy chain is at the other end of the molecule, called the Fc region, and determines both the class of antibody and its functional specialization. There are four different classes of secreted antibody. IgM antibodies are found primarily in blood and are the first antibodies produced during a developing immune response. IgG is the principal class of antibody in blood and extracellular fluid, and exists as several different subtypes. IgA is the main antibody in secretions of the respiratory, genital and gastrointestinal tracts. IgE is present at very low concentrations in blood and extracellular fluid and mediates allergic reactions. A fifth class of antibody, IgD, is expressed almost exclusively as a cell surface molecule by naive B cells.

To generate antibody a B cell must first encounter and bind epitopes on accessible proteins of the virus through engagement of its B cell receptor, the cell surface version of immunoglobulin. IgM and IgD are expressed by naive B cells of many species of mammal, whereas only IgM is expressed in other species. The binding of antigen to its specific receptor initiates internalization of the virus particle and its subsequent degradation in acidified vesicles. Within these vesicles, viral peptides, including those derived from internal proteins that are not accessible to the B cell receptor, are loaded onto MHC class II molecules for presentation at the cell surface. Virus-specific CD4 T cells engage the MHC class II/peptide complex and deliver activating and survival signals to the B cell in the form of CD40 ligand (which engages CD40 on the B cell) and cytokines, inducing proliferation and differentiation of B cells into antibody-secreting cells. Engagement of CD4 T cells also promotes the formation of a germinal center within the lymph node cortex, the site of intense B cell proliferation and death.

During an ongoing humoral immune response, B cell expansion/proliferation is characterized by somatic hypermutation and isotype class switching within the immunoglobulin genes. Both processes are critical to generation of effective antiviral immunity. Somatic hypermutation in the V-region of the immunoglobulin gene locus of B cells occurs spontaneously during B cell activation and leads to *affinity maturation*. This process ensures antibodies are generated with increasing affinity for antigen as the immune response evolves. Thus, while the first antibodies made following a virus infection are low-affinity IgM antibodies, as the immune response matures there is a switch to high-affinity IgG and IgA antibodies produced by the concurrent events of somatic hypermutation and affinity maturation. Germinal center B cells that produce immunoglobulins with increasing affinity for antigen as a result of somatic hypermutation will preferentially survive, as the process of antigen binding, degradation, and presentation on MHC class II molecules to CD4 T cells is sustained even as antigen diminishes.

Isotype class switching involves genetic rearrangement of the C region of the immunoglobulin heavy chain gene and results in replacement of the original C\_\text{\textmu } heavy chain, encoding IgM, with an alternative C region. Switching to a C\_\gamma heavy chain results in production of IgG molecules, whereas expression of C\_\epsilon or C\_\alpha results in production of IgE or IgA molecules, respectively. Further, in mammalian species, these B cells maintain expression of the membrane form of the antibody as the antigen receptor, or B cell receptor, while also secreting antibody. The two forms of the antibody, membrane and secreted, are coexpressed by alternate mRNA splicing of the membrane or secretory sequences to the end of the rearranged/spliced antibody mRNA. B cells that terminally differentiate into plasma cells are solely dedicated to synthesis and secretion of antibody and no longer express surface antibody nor undergo somatic mutation or further class switching. These cells have a finite life span. As antigen diminishes, for instance when a virus infection is controlled, some B cells, especially those in the lymphoid tissues, return to a resting state and can remain surface immunoglobulin-expressing until a new encounter with the same antigen. Under the influence of cytokines produced by T cells, these cells can become memory B cells with a very long life span, just as with T cells. Together, these are the cells that mediate the recall response.

Which antibody class is selected during isotype class switching is a function of the cytokines the B cell is exposed to, with IL-4 inducing IgG1 and IgE expression
and IFN-γ inducing IgG3 and IgG2a production in mice. However, individual animal species exhibit a variety of different immunoglobulin isotypes as the duplications leading to large immunoglobulin superfamiliy families occurred after speciation. For instance, Bos taurus cattle have three IgG isotypes; IgG1, IgG2, and IgG3, although the IgG3 constant region gene is not used. Because the IgG1 isotype is secreted in mucosal fluids, older reports assume there is no IgA in cattle. Bovine IgA was discovered only later and has unique expression profiles relative to bovine IgG1, but is a minor antibody in mucosal secretions of cattle. Likewise, IgG2 can be expressed in lower concentrations than IgG1 in serum, but can dominate the antibody response in some instances. In swine there are six IgG isotypes. IgG 2, 4, and 6 differ by only a few amino acids and their functions are identical and redundant, but they are distinct genes and are all expressed in individual animals. Porcine IgA is the predominant antibody isotype in mucosal secretions of swine, as is the case for humans. For species other than mouse and human, data are limited relating certain cytokines with induction of class switch to particular immunoglobulin isotypes in activated B cells.

Species-specific differences also occur in the expression of the light chain of antibody molecules. For example, humans express both κ and λ light chains, but mice express only κ and horses only λ. Cattle, swine, canines, and felines express a mixture, like humans. The combinatorial interaction between heavy and light chains determines the properties of the antigen binding cleft, and mutations that are under selection pressure by antigen driven somatic mutation, are focused in this region. Specifically, mutations that yield higher affinity for antigen are selected and propagated.

**Antiviral Functions of Antibodies**

Neutralizing antibodies can be important both in mediating virus clearance during primary viral infections and in preventing reinfection with viruses to which the animal previously has been exposed. Virus neutralization occurs *in vivo* when antibody binds to its complementary epitope on the virus surface, preventing virus from binding to and/or productively infecting target cells. All other functions of antibodies are dependent upon the class of immunoglobulin and are mediated by the Fc region at the end of the antibody molecule distant to the binding portion. One of these functions is activation of complement, a system of plasma proteins that are activated through sequential proteolytic cleavage reactions resulting in production of a number of immunologically active proteases. IgM is the most effective antibody class at activating complement as it exists in a pentameric form, providing multiple Fc regions for the binding of C1q, the first protein in the classical pathway of complement activation. In viral infections, complement activation leads to more efficient activation of B cells through binding to complement receptor 2 (CD21), a component of the B cell coreceptor complex. Another major function of antibody in viral infections is opsonization, which facilitates binding of the Fc portion of antibody to various Fc receptors on effector cells. Different cell types express different sets of Fc receptors, and the antibody class thus determines which type of cell will be engaged in an immune response. Many Fc receptors are expressed by phagocytes and facilitate phagocytosis of antibody-coated particles. In addition, natural killer cells express FcγRIII (CD16) that can bind to the Fc portion of IgG after it has attached to viral proteins expressed at the surface of infected cells. This binding results in activation of the natural killer cell and killing of the virus-infected cell through the process of antibody-dependent cellular cytotoxicity that was described earlier.

**Passive Immunity**

A critical aspect of adaptive immunity in veterinary species involves maternal immunity that is “passively” transferred to neonatal animals. For most mammalian species, neonates are born with a naïve immune system. The final stages of immunological development occur after birth, following separation from the maternal blood and population by the microbiome. During pregnancy, placental structure influences immunoglobulin transfer and only in a few species, notably humans and to a lesser extent carnivores, does antibody, usually of the IgG isotype, cross the placenta to circulate in the fetus. In most mammals, including all farm animal species, passive transfer of antibodies occurs through the neonate’s ingestion of colostrum immediately after birth. Colostrum contains immunoglobulin at 10–100-fold its concentration in milk. Colostrum is also a source of maternally-derived leukocytes (> 1 million/mL in cattle), which are absorbed and enter the neonate’s circulation. In addition, colostrum contains bioactive compounds that may influence gut mucosal development and provides a source of bacteria that colonize the neonatal gastrointestinal tract, which is increasingly recognized to play a central role in normal development of the immune system.

Vaccination of pregnant animals can influence the specificity of antibodies present in colostrum and can be used to provide pathogen-specific passive protection of the neonate. When the newborn ingests colostrum from its mother, the transfer of immunoglobulins and leukocytes provide passive protection until it is able to generate its own adaptive immune responses. As such, vaccination schedules are arranged with knowledge of when the species being treated develops the autonomous capacity for mounting the immune response. Vaccination before the newborn’s immune system is fully functional may result in a weak or ineffectual response, potentially
compromising vaccine effectiveness. Furthermore, the presence of maternal antibodies can clear viral antigens in the vaccine and prevent induction of an effective immune response. Therefore, vaccination against common viral diseases of livestock and companion pets often starts when the animal is a few weeks or months of age, when maternal antibodies have waned and the individual is capable of developing a strong immune response.

**Viral Mechanisms of Avoidance and Escape**

Viruses have developed remarkably sophisticated mechanisms to avoid the various host protective immune responses. In addition to the many different strategies utilized by viruses to facilitate persistent infection, including growth in immune cells and/or in immunologically privileged sites, latency, integration, and antigenic drift, individual viruses have also developed diverse and complex mechanisms of avoiding protective host innate and adaptive immune responses. Examples of these mechanisms will be discussed in this section but the reader also should consult the chapters on individual virus families for specific examples.

**Shutdown of Host Macromolecule Synthesis**

Many viruses initiate infection within the cell by inhibiting normal transcription and/or translation of cellular proteins, and rapidly subvert the machinery of the infected cell for production of progeny virions. This rapid shutdown of the host cell quickly impairs the innate immune response to the infecting virus, including the production of critical proteins such as class I MHC and antiviral cytokines such as Type I interferon. The result is that, without effective innate immune responses, the infecting virus can quickly replicate and disseminate before the host can develop an adaptive immune response. This strategy is widely used by RNA viruses, many of which have rapid replication cycles.

**Avoidance of Cytotoxic T lymphocyte (CTL)-Mediated Killing**

Cytotoxic T lymphocyte-mediated killing of virus-infected cells requires the presentation of viral antigens on the surface of the infected cell in the context of the appropriate class I MHC molecule (Fig. 4.4). Thus, viruses have developed different strategies to suppress the normal expression of class I MHC proteins, which prevents cytotoxic T lymphocyte-mediated lysis of virus-infected cells by removing the ligand for the CTL receptor. These strategies include: (1) suppression of cellular production of class I MHC molecules by shutdown of host protein synthesis; (2) production of virus-encoded proteins that disrupt normal production of class I MHC proteins, or their transport from the endoplasmic reticulum to the Golgi apparatus or to the cell surface; (3) production of virus-encoded proteins that disrupt the function or viability of class I MHC molecules; and (4) production of virus-encoded homologs of class I MHC molecules that can bind β2 microglobulin and viral peptides, but are otherwise dysfunctional as ligands for the CTL response.

**Prevention of Natural Killer (NK)-Cell-Mediated Lysis of Virus-Infected Cells**

In contrast to cytotoxic T lymphocyte-mediated lysis, which requires the presence of appropriate concentrations of class I MHC antigen on the surface of virus-infected cells, natural killer-cell-mediated lysis of virus-infected cells is promoted by reduced levels of class I MHC antigen on the cell surface (Fig. 4.2). Also important to natural killer cell activity is the balance of inhibitory molecules (such as class I MHC) and stimulatory molecules (such as heat-shock proteins) on the cell surface. Some viruses evade the natural killer cell response by selectively inhibiting cellular production and expression of molecules that provide stimulatory signals for natural killer cell activity.

**Interference With Apoptosis**

In addition to apoptosis induced by natural killer cell or cytotoxic T lymphocyte-mediated cell lysis, virus infection alone can initiate apoptosis via either the extrinsic (death receptor) or intrinsic (mitochondrial) pathways (see Chapter 3: Pathogenesis of Viral Infections and Diseases). Apoptosis is the process of programmed cell death, essentially a mechanism of cell suicide that can be activated to eliminate viral factories before virus replication is complete. Apoptosis is especially deleterious to the relatively slow-growing DNA viruses (eg, poxviruses, herpesviruses, and adenoviruses), thus, these DNA viruses in particular have developed a remarkable variety of strategies to optimize their replication by inhibiting the various pathways that normally lead to apoptosis. The need for these viruses to prevent apoptosis to promote their own survival is reflected by the fact that individual viruses may use a combination of strategies, including: (1) inhibition of the activity of executioner caspases that mediate cell death—notably by the serpins, which are protease inhibitors produced by poxviruses that bind to and block the proteolytic activity of caspases; (2) inhibition of the expression, activation, and signaling of death receptors, such as by production of viral receptor homologs that bind tissue necrosis factor (TNF) so that it cannot initiate the extrinsic (death receptor) pathway, or molecules that specifically block the signaling cascade initiated by death receptor activation; (3) production of virus-encoded homologs of antiapoptotic proteins such as Bcl-2; (4) production of proteins that sequester p53, which is a pro-apoptotic molecule that accumulates in cells infected with certain viruses; (5) other as yet poorly defined mechanisms of inhibition of apoptosis that are apparently used by a myriad of viral proteins.
**Vaccines and Vaccination Against Viral Diseases**

Vaccination is the most effective way of preventing viral diseases. Although deliberate exposure to virulent viruses such as smallpox (syn. variolation) was long recognized as an effective, albeit dangerous, method of prophylaxis. The concept of vaccination is considered to have been widely introduced by Edward Jenner in 1798 to protect humans against smallpox. Nearly a century later, the concept was shown by Louis Pasteur to have wider applications and, most notably, could be used to prevent rabies. With the advent of cell culture techniques in the 1950s, a second era of vaccination was introduced and many live-attenuated virus and inactivated virus vaccines were developed. More recently, the field of vaccinology has witnessed the introduction of a number of novel “new generation” vaccines produced through various forms of recombinant DNA and related technologies. While live-attenuated and inactivated virus vaccines of the second era are still the “work horses” of veterinary practice, new generation vaccines are now complementing and, increasingly, replacing them (Table 4.1).

There are some important differences between vaccination practices in humans and animals. Economic constraints are generally of less importance in human medicine than in veterinary medicine. There is also greater agreement about the safety and efficacy of vaccines in use in human medicine than there is with animal vaccines, and better mechanisms for reporting potential adverse consequences associated with the use of specific products. At the international level, the World Health Organization exerts persuasive leadership for human vaccine usage, and maintains a number of programs that have no equivalents for animal vaccine usage by its sister agencies, the Food and Agriculture Organization and the Office International des Epizooties (syn. the World Organization for Animal Health). Furthermore, within countries, greater latitude is allowed in the manufacture and use of vaccines for veterinary diseases than is allowed by national regulatory authorities for human vaccines.

Before the recent advent of the new generation vaccines based on recombinant DNA technology, there were just two major strategies for the production of virus vaccines: one employing live-attenuated (syn. modified-live) virus strains and the other employing chemically inactivated (syn. killed) virus preparations. Live-attenuated virus vaccines replicate in the vaccine recipient and, in so doing, amplify the amount of antigen presented to the
### TABLE 4.1 Examples of Commercially Available Veterinary Virus Vaccines by Type

| Animal | Target Pathogen/Disease | Vaccine Characteristic |
|--------|-------------------------|-----------------------|
| **Live-Attenuated Virus Vaccines** | | |
| Carp   | Cyprinid herpesvirus 3  | Cyprinid herpesvirus 3 modified by serial passage in cultured cells and UV irradiation |
| Cattle | Infectious bovine rhinotracheitis | Bovine herpesvirus-1 modified by serial passage in cultured cells |
| Horse  | Equine influenza         | Cold-adapted equine influenza virus |
| Poultry| Marek’s disease          | Turkey herpesvirus |
| Rabbit | Myxomatosis              | Shope fibroma virus |
| **Nonreplicating (Killed) Virus Vaccines** | | |
| Cat    | Feline leukemia virus    | Feline leukemia virus |
| Cow    | Bovine respiratory syncytial virus | Bovine respiratory syncytial virus |
| Horse  | Equine rhinopneumonitis  | Equid herpesvirus-1 and 4 |
| Poultry| Viral arthritis/tenosynovitis | Avian reovirus |
| **Gene-Deletion and Chimeric Viruses** | | |
| Cattle | Infectious bovine rhinotracheitis | Bovine herpesvirus-1 with glycoprotein E deletion |
| Pig    | Pseudorabies             | Pseudorabies virus with thymidine kinase and glycoprotein E deletion |
| Pig    | Porcine circovirus       | Chimeric porcine circovirus type 1 expressing porcine circovirus type 2 capsid |
| **Viral Vector-Based Vaccines** | | |
| Cat    | Rabies                   | Canarypox virus expressing rabies virus glycoprotein |
| Dog    | Canine distemper         | Canarypox virus expressing canine distemper virus fusion and hemagglutinin |
| Horse  | Equine influenza         | Canarypox virus expressing equine influenza virus hemagglutinin |
| Pig    | Porcine epidemic diarrhea virus | Venezuelan equine encephalitis virus replicon expressing porcine epidemic diarrhea virus spike |
| Poultry| Infectious laryngotracheitis | Turkey herpesvirus expressing infectious laryngotracheitis virus glycoproteins |
| Poultry| Newcastle disease and influenza | Newcastle disease virus expressing avian influenza virus hemagglutinin |
| Rabbit | Myxomatosis and rabbit hemorrhagic disease | Myxoma virus expressing rabbit hemorrhagic disease virus VP60 |
| **Subunit Vaccines** | | |
| Poultry| Newcastle disease        | Newcastle disease virus hemagglutinin-neuraminidase expressed in plant cells |
| Pig    | Classical swine fever    | Classical swine fever virus E2 glycoprotein expressed by baculovirus |
| Pig    | Porcine circovirus       | Virus-like particle of porcine circovirus type 2 capsid expressed by baculovirus |
| Salmon | Infectious pancreatic necrosis | Infectious pancreatic necrosis virus VP2 expressed by *Escherichia coli* |
| **DNA Vaccines** | | |
| Salmon | Infectious hematopoietic necrosis virus | Naked DNA encoding infectious hematopoietic necrosis virus surface glycoprotein |
host’s immune system. There are important benefits in this approach, because the replication of vaccine virus mimics infection to the extent that the host immune response is more similar to that occurring after natural infection than is the case with inactivated or some subunit vaccines. When inactivated virus vaccines are produced, the chemical or physical treatment used to eliminate infectivity may be damaging enough to diminish the immunogenicity of the vaccine virus, especially the induction of virus-specific cell-mediated immune responses. As a result, inactivated vaccines often induce an immune response that is shorter in duration, narrower in antigenic spectrum, weaker in cell-mediated and mucosal immune responses, and possibly less effective in inducing sterilizing immunity. Nonetheless, very serviceable and safe inactivated vaccines are available and widely used.

The majority of vaccines in large-scale production for use in animals continue to include either live-attenuated or inactivated virus; however, new generation vaccines developed through recombinant DNA technologies offer significant improvements and potential advantages in terms of both their safety and their efficacy. A remarkable variety of such vaccines have recently been developed, an increasing number of which are now in commercial production.

**Live-Attenuated Virus Vaccines**

Live-attenuated virus vaccines, when they have been proven to be safe, have historically been the best of all vaccines. Several of them have been dramatically successful in reducing the incidence of important diseases of animals and humans. Most live-attenuated virus vaccines are injected intradermally, subcutaneously, or intramuscularly, but some are delivered orally, and a few by aerosol or to poultry in their drinking water. For these vaccines to be successful, the vaccine virus must replicate in the recipient, thereby eliciting a lasting immune response while causing little or no disease. In effect, a live-attenuated virus vaccine mimics a subclinical infection. The individual virus strain incorporated in a live-attenuated virus vaccine may be derived from any one of several sources.

**Avirulent Viruses in Heterologous Species**

The original vaccine (vacca meaning cow) introduced by Edward Jenner in 1798 for the control of human smallpox, utilized cowpox virus, a zoonotic pathogen (see Chapter 7: Poxviridae). This virus produced only a mild infection and lesions in humans, but, because it is antigenically related to smallpox virus, it conferred protection against the human disease. The same principle has been applied to other diseases—for example, the protection of chickens against Marek’s disease using a vaccine derived from a related herpesvirus of turkeys, and the protection of piglets against porcine rotavirus infection using a vaccine derived from a bovine rotavirus. Similarly, rabbits can be effectively protected against the poxvirus disease, myxomatosis, with the naturally avirulent Shope rabbit fibroma virus.

**Attenuation of Viruses by Serial Passage in Cultured Cells**

Most of the live-attenuated virus vaccines in common use today were derived empirically by serial passage of virulent “field” virus (syn. “wild-type” virus) in cultured cells. The cells may be of homologous or, more commonly, heterologous host origin. Typically, adaptation of virus to more vigorous growth in cultured cells is accompanied by progressive loss of virulence for the natural host. Loss of virulence may be demonstrated initially in a convenient laboratory model such as a mouse, before being confirmed by clinical trials in the species of interest. Because of the practical requirement that the vaccine must not be so attenuated that it fails to replicate satisfactorily in its natural host, it is sometimes necessary to compromise by using a virus strain that replicates sufficiently well that it may induce mild clinical signs in a few of the recipient (vaccinated) animals.

During repeated passage in cultured cells, viruses typically accumulate nucleotide substitutions in their genome, which in turn leads to attenuation. With the recent advent of next generation genome sequencing procedures, the genetic basis of virulence and attenuation has been established with some viruses, human poliovirus for example, which allows better prediction of vaccine efficacy and safety. Furthermore, it is increasingly clear that several genes can contribute to virulence and tropism of individual viruses, and do so in different ways. For example, in contrast to the severe, systemic infections that result from infections with some wild-type or “field” viruses, live-attenuated vaccine strains of these same viruses administered by the respiratory route may replicate, for instance, only in the upper respiratory tract, or undergo only limited replication in the intestinal epithelium after oral administration.

Despite the outstanding success of empirically derived live-attenuated virus vaccines, there is a strong perceived need to replace what some veterinary scientists consider to be “genetic roulette” with rationally designed, specifically engineered vaccines. In these engineered live-attenuated vaccines, the mutations associated with attenuation of the parental virus are defined and predictable, as is the potential for reversion to virulence. However, the regulatory
approval process for commercial use of genetically engineered vaccines in animals can be more complicated than it is for traditional live-attenuated virus vaccines.

Attenuation of Viruses by Serial Passage in Heterologous Hosts

Serial passage in a heterologous host was an historically important means of empirically attenuating viruses for use as vaccines. For example, rinderpest and classical swine fever (hog cholera) viruses were each adapted to grow in rabbits and, after serial passage, became sufficiently attenuated to be used as vaccines. Other viruses were passaged in embryonated hens’ eggs in similar fashion, although some such passaged viruses acquired novel and very undesirable properties. For example, live-attenuated bluetongue vaccine viruses propagated in embryonated eggs can cross the placenta of ruminants vaccinated during pregnancy, with resultant fetal infection and associated developmental defects or loss. Similarly, embryonated-egg-propagated African horse sickness virus, which is not naturally zoonotic, caused devastating consequences in humans infected after aerosol exposure to this vaccine virus.

Attenuation of Viruses by Selection of Mutants and Reassortants

The observation that temperature-sensitive mutants (viruses that are unable to replicate satisfactorily at certain temperatures, usually including normal body temperature) generally display reduced virulence suggested that they might make satisfactory live-attenuated vaccines, although some viruses with temperature-sensitive mutations have displayed a disturbing tendency to revert toward virulence during replication in vaccinated animals. Attention accordingly moved to cold-adapted mutants, derived by adaptation of virus to grow at suboptimal temperatures. The rationale is that such mutant viruses would be safer vaccines for intranasal administration, in that they would replicate well at the lower temperature of the nasal cavity (about 33°C in most mammalian species), but not at the temperature of the more vulnerable lower respiratory tract and pulmonary airspaces. Cold-adapted influenza vaccines that contain mutations in most viral genes do not revert to virulence, and influenza vaccines based on such mutations are now licensed for human use; vaccines against equine influenza have been developed utilizing the same principle.

Nonreplicating Virus Vaccines

Inactivated (Killed) Whole Virions

Inactivated (syn. killed) virus vaccines are usually made from virulent virus; chemical or physical agents are used to destroy infectivity while maintaining immunogenicity. When prepared properly, such vaccines are remarkably safe, but they need to contain relatively large amounts of antigen to elicit an antibody response commensurate with that induced by a much smaller dose of live-attenuated virus vaccine. Normally, the primary vaccination course comprises two or three injections, and further (“booster”) doses may be required at regular intervals thereafter to maintain immunity. Killed vaccines usually must be formulated with chemical adjuvants to enhance the immune response, but these also can result in more adverse reactions to vaccination.

The most commonly used inactivating agents are formaldehyde, β-propiolactone, and ethylenimine. One of the advantages of β-propiolactone, which is used in the manufacture of some rabies virus vaccines, and ethylenimine, which is used in the manufacture of some foot-and-mouth disease vaccines, is that they are completely hydrolyzed, within hours, to nontoxic products. Because virions in the center of aggregates may be shielded from inactivation, it is important that aggregates be broken up before inactivation. In the past, failure to do this occasionally resulted in vaccine-associated disease outbreaks—for example, several foot-and-mouth disease outbreaks have been traced to this problem. Furthermore, production of inactivated virus vaccines requires the initial production of large quantities of virulent virus prior to its inactivation, which itself can pose a considerable threat if this virus escapes from the production facility into the environment.

Purified Native Viral Proteins

Lipid solvents such as sodium deoxycholate are used in the case of enveloped viruses, to solubilize the virion and release the components, including the glycoprotein spikes of the viral envelope. Differential centrifugation is used to semipurify these glycoproteins, which are then formulated for use as so-called split vaccines for influenza. Examples include vaccines against herpesviruses, influenza viruses, and coronaviruses.

Vaccines Produced Using Recombinant DNA and Related Technologies

Molecular biology and its many associated technologies have facilitated the development of new vaccine strategies, each with inherent potential advantages and, in some instances, disadvantages as compared with those of the
traditional vaccines. Such novel technologies have been used in the creation of new vaccines that already are in use and, given their substantial inherent potential advantages, it is anticipated that the availability and types of such products will only increase in the future.

**Attenuation of Viruses by Gene Deletion or Site-Directed Mutagenesis**

The problem of the reversion to virulence of live-attenuated virus vaccines (ie, a mutation by which the vaccine virus regains virulence) may be largely avoided by deliberate insertion of several attenuating mutations into key viral genes, or by completely deleting nonessential genes that contribute to virulence. Gene deletion is especially feasible with the large DNA viruses that carry a significant number of genes that are not essential for replication, at least for replication in cultured cells. “Genetic surgery” is used to construct deletion mutants that are stable over many passages. Several herpesvirus vaccines have been constructed using this strategy, including a thymidine kinase (TK) deletion pseudorabies vaccine for swine that also includes a deletion of one of the glycoprotein genes (gE). The deleted glycoprotein may be used as capture antigen in an ELISA so that vaccinated, uninfected pigs, which would test negative, can be distinguished from naturally infected pigs (the differentiation/discrimination of infected from vaccinated animals (DIVA) strategy), enabling eradication programs to be conducted in parallel with continued vaccination. A gE-deleted marker vaccine for infectious bovine rhinotracheitis virus (bovine herpesvirus-1) has also been developed.

Site-directed mutagenesis facilitates the introduction of defined nucleotide substitutions into viral genes at will. As the particular genes that are influential in virulence and immunogenicity of individual viruses are increasingly defined, it is anticipated that existing empirically derived live-attenuated virus vaccines will be replaced by those engineered for attenuation through “customized” alteration of critical genes. The production of live-attenuated virus vaccines from molecular clones facilitates both the deliberate introduction of defined attenuating nucleotide substitutions into the vaccine virus, and consistent production of vaccine virus from a genetically defined “seed” virus. This strategy also potentially enables the use of differential serological tests to DIVA.

**Subunit Vaccines Produced by Expression of Viral Proteins**

Eukaryotic expression vectors offer the potential for large-scale production of individual viral proteins that can be purified readily and formulated into vaccines. Once the critical viral protein conferring protection has been identified, its gene (or, in the case of an RNA virus, a complementary DNA (cDNA) copy of the gene) may be cloned into one of a wide choice of expression plasmids and expressed in any of several cell systems. Mammalian cells offer the advantage over cells from lower eukaryotes in that they are more likely to possess the machinery for correct posttranslational processing and authentic maturation of complex viral proteins.

Useful eukaryotic expression systems include plant and yeast cells (*Saccharomyces cerevisiae*), insect cells (*Spodoptera frugiperda*), and various mammalian cells. Yeast offers the advantage that there is extensive experience with scale-up for industrial production; the first vaccine produced by expression of a cloned gene, human hepatitis B vaccine, was produced in yeast. Insect cells offer the advantage of simple technology derived from the silk industry: moth cell cultures (or caterpillars) may be made to express very large amounts of viral proteins through infection with recombinant baculoviruses carrying the gene(s) of the virus of interest. The promoter for the gene encoding the baculovirus polyhedrin protein is so strong that the product of a viral gene of interest inserted within the baculovirus polyhedrin gene may comprise up to half of all the protein the infected cells make. Baculovirus-expressed E2 protein is a highly effective subunit vaccine against classical swine fever virus, as is the capsid protein of porcine circovirus 2.

Expression of protective viral antigens in plant cells can theoretically provide a cost-effective and efficient method of vaccinating production animals. For example, plant cell lines have been developed that express the hemagglutinin and neuraminidase proteins of Newcastle disease virus for protective immunization of birds. Similarly, bacterial expression systems based on *Escherichia coli* are very effective and efficient at generating large quantities of vaccine antigen, and such a system is used for the production of VP2 protein of infectious pancreatic necrosis virus used as a vaccine for salmon.

**Viral Proteins that Self-Assemble Into Virus-like Particles (VLPs)**

The expression of genes encoding the capsid proteins of viruses within certain families of nonenveloped icosahedral viruses leads to the self-assembly of the individual capsid proteins into VLPs that can be used as a vaccine. This strategy has been developed for various picornaviruses, caliciviruses, rotaviruses, and orbiviruses, and an effective VLP-based vaccine has been developed recently against human genital papillomaviruses. Baculovirus-expressed capsid protein of porcine circovirus 2 self-assembles into VLPs and this vaccine confers protective immunity against porcine-circovirus-associated diseases such as multisystemic wasting disease. The advantage of recombinant VLPs over traditional inactivated vaccines is
that they are devoid of viral nucleic acid, and therefore completely safe. They may also be equated to an inactivated whole-virus vaccine, but without the potentially damaging loss of immunogenicity that can accompany chemical inactivation. However, the potential limitations of the strategy include production costs and low yields with some constructs, stability of the VLP after production, and less effective immunity as compared with some existing vaccines.

**Viruses as Vectors for Expression of Heterologous Viral Antigens**

Recombinant DNA techniques allow foreign genes to be introduced into specific regions of the genome of either RNA or DNA viruses, and the product of the foreign gene is then carried into and expressed in the target cell. Specifically, the gene(s) encoding key protective antigens (those against which protective responses are generated in the host) of the virus causing a disease of interest are inserted into the genome of an avirulent virus (the recombinant vector). This modified avirulent virus is then administered either as a live-attenuated virus vector or as a nonrepli- 
cating (“suicide”) expression vector. Infected cells within the immunized host express the foreign protein, to which the animal will in turn mount an adaptive immune response (humoral and/or cellular). The approach is safe, because only one or two genes of the disease-causing virus typically are inserted into the expression vector, and because well-characterized viruses (such as existing live-attenuated vaccine viruses) can be used as the expression vector. Furthermore, animals vaccinated with such recombinant vaccines can be distinguished readily from infected animals (or those vaccinated with live-attenuated virus vaccines) using serological tests that detect antibodies to viral proteins that are not included in the vaccine construct (the DIVA strategy).

**DNA Viruses as Vectors**

Individual genes encoding antigens from a variety of viruses have been incorporated into the genome of DNA viruses, especially vaccinia and several other poxviruses, adenoviruses, herpesviruses, and adeno-associated viruses (which are parvoviruses). Vaccination of animals with a significant number of different recombinant poxvirus-vectored vaccine constructs has effectively generated antibody and/or cell-mediated immune responses that confer strong protective immunity in the recipient animals against challenge infection with virulent strains of the heterologous viruses from which the genes were derived. For example, recombinant vaccinia virus vectored rabies vaccines incorporated into baits administered orally protect both foxes and raccoons against this zoonotic disease; this vaccine contains only the gene encoding the surface glycoprotein (G) of rabies virus. Similarly, the avian poxviruses have been increasingly used as expression vectors of heterologous genes in recombinant vaccine constructs. Fowlpox virus is a logical choice as a vector for avian vaccines but, perhaps surprisingly, fowlpox virus has also been shown to be a very useful expression vector in mammals: even though this virus, and the closely related canarypox virus, do not complete their replication cycle in mammalian cells, the inserted genes are expressed and induce strong cellular and humoral immune responses in inoculated animals. Because the large genome of poxviruses can accommodate at least a dozen foreign genes and still be packaged satisfactorily within the virion, it is theoretically possible to construct, as a vector, a single recombinant virus capable of protecting against several different viral diseases.

Recombinant poxvirus-vectored vaccines that have been widely used to immunize mammals include vaccinia—rabies constructs used for the vaccination of foxes in Europe and raccoons and coyotes in the United States, and canarypox virus vectored vaccines to prevent influenza and West Nile disease in horses, distemper in dogs, ferrets and certain zoo animals/wildlife species, and feline leukemia and rabies in cats. Amongst many others, experimental recombinant canarypox virus vectored vaccines also have been successfully developed to prevent African horse sickness, bluetongue, Japanese encephalitis, and Nipah, and extensive trials have been carried out in humans with an experimental HIV—recombinant canarypox virus vaccine. Raccoonpox, capripox, and other poxviruses have also been successfully developed as recombinant expression vectors for potential use as vaccines in mammals. Rabbits can be effectively immunized against both myxomatosis (pox virus) and rabbit hemorrhagic disease (calicivirus) with a recombinant live-attenuated myxoma virus that expresses the VP60 gene of rabbit hemorrhagic disease virus. This combined vaccination strategy has the considerable advantage that rabbit hemorrhagic disease virus cannot be grown in cell culture, so that vaccination against rabbit hemorrhagic disease alone currently requires inactivation of virus collected from the livers of virus-infected rabbits. Similarly, recombinant adenoviruses successfully have been developed for immunization of animals against diseases such as rabies (in wildlife) and foot-and-mouth disease (in livestock).

A number of DNA virus vectored vaccines have also been developed for use in poultry, including recombinant turkey herpesvirus-vectored vaccines against Newcastle disease virus, infectious laryngotracheitis virus, and infectious bursal disease virus; these vaccines include only genes encoding the protective antigens of the heterologous viruses, but they generate protective immunity in chickens against both Marek’s disease (which is caused by another herpesvirus) and the other diseases represented.
in the construct (Newcastle disease, infectious laryngotracheitis, and infectious bursal disease). Fowlpox virus vectored vaccines against Newcastle disease and H5 influenza viruses have also been developed, and the latter has been widely used in Mexico and Central America.

Chimeric DNA viruses also have been developed as vaccines in which the genes of a virulent virus are inserted into the genetic backbone of a related avirulent virus. For example, a chimeric circovirus vaccine used in swine includes a genetic backbone of porcine circovirus 1, which is avirulent (nonpathogenic) in swine, with the gene encoding the immunogenic capsid protein of pathogenic porcine circovirus 2. Antibodies to the capsid protein of porcine circovirus 2 confer immunity in vaccinated pigs. Like porcine circovirus 1, the chimeric virus replicates to high titer in cell culture, which makes vaccine production more efficient and cost-effective.

It is anticipated that commercially available veterinary vaccines increasingly will utilize DNA viruses as expression vectors in the future, because of their inherent advantages in terms of safety and efficacy, and the ability in control programs to distinguish vaccinated animals from those exposed to infectious virus.

**RNA Viruses as Vectors**

As with DNA virus vectored vaccines, RNA viruses, especially virus strains of proven safety, can be used as “genetic backbones” for insertion of critical immunogenic genes from other (heterologous) viruses. Chimeric RNA viruses utilize the replicative machinery of one virus for expression of the protective antigens of the heterologous virus. For example, chimeric vaccines have been developed in which the genes encoding the envelope proteins of the traditional live-attenuated vaccine strain of yellow fever virus are replaced with corresponding genes of other flaviviruses such as Japanese encephalitis virus, West Nile virus, or dengue virus, or even with genes encoding critical immunogenic proteins of distinct viruses such as influenza. A chimeric vaccine based on yellow fever virus that includes the premembrane (preM) and envelope (E) proteins of West Nile virus was used briefly for protective immunization of horses.

Positive-sense RNA viruses are especially convenient for use as molecular clones for the insertion of foreign genes because the genomic RNA of these viruses is itself infectious. Infectious clones also have been developed for negative-sense RNA viruses by including the replicase proteins at transfection. In poultry, a recombinant Newcastle disease virus vaccine that expresses the H5 gene of influenza virus has been developed and widely used in China for protective immunization of birds against both Newcastle disease and H5 avian influenza. Additional negative-sense RNA viruses such as rhabdoviruses are also being evaluated as potential gene vectors (eg, vesicular stomatitis virus), as have other positive-sense RNA viruses such as the nidoviruses (coronaviruses, arteriviruses).

Recombinant replicon particles offer a similar but slightly different strategy that has been developed with certain RNA viruses, including flaviviruses and alphaviruses such as Venezuelan equine encephalitis, Semliki Forest, and Sindbis viruses. Recombinant alphavirus replicon particles are created exclusively from the structural proteins of the donor alphavirus, but the genomic RNA contained in these particles is chimeric, in that the genes encoding the structural proteins of the replicon alphavirus are replaced by those from the heterologous virus. As an example, replicon particles derived from the vaccine strain of Venezuelan equine encephalitis virus that coexpress the GP5 and M envelope proteins of equine arteritis virus induce virus-neutralizing antibody and protective immunity in immunized horses; neither infectious Venezuelan equine encephalitis virus nor equine arteritis virus is produced in immunized horses, as the replicon genome includes only the nonstructural proteins of Venezuelan equine encephalitis virus and the structural protein genes of equine arteritis virus. A similar strategy has been used to make a porcine epidemic diarrhea virus (a coronavirus) vaccine for pigs using Venezuelan equine encephalitis virus replicons expressing the porcine epidemic diarrhea virus spike gene.

For influenza viruses and other viruses with segmented genomes, the principle of chimeric viruses was well established before the advent of recombinant DNA technology. Reassortant viruses were produced by homologous reassortment (segment swapping) by cocultivation of an existing vaccine strain virus with the new isolate. Viruses with the desirable growth properties of the vaccine virus but with the immunogenic properties of the recent isolate were selected, cloned, and used as vaccine.

**DNA Vaccines**

The discovery, in the early 1990s, that viral DNA itself can be used for protective immunization offered a potentially revolutionary new approach to vaccination. Specifically, a plasmid construct that included the β-galactosidase gene expressed the enzyme for up to 60 days after it was inoculated into mouse skeletal muscle. From this early observation, there has been an explosion of interest in the development of DNA vaccines and this methodology has been utilized experimentally for a wide range of potential applications. The first commercially available “naked” DNA vaccine was developed to protect salmon against infectious hematopoietic necrosis virus, and a DNA-based vaccine to prevent West Nile disease in horses was approved for use in 2005 but has since been
discontinued. Indeed, commercial utilization of this strategy in veterinary vaccines has been slow, and a DNA vaccine is yet to be approved for use in humans.

With hindsight, the discovery that DNA itself could confer protective immunity was perhaps not that surprising. In 1960, it was shown that cutaneous inoculation of DNA from Shope papillomavirus induced papillomas at the site of inoculation in rabbit skin. Subsequently, it was shown for many viruses that genomic viral DNA, RNA, or cDNA of viral RNA, could complete the full replicative cycle following transfection into cells. The strategy of DNA vaccines is to construct recombinant plasmids that contain genes encoding key viral antigens. The DNA insert in the plasmid, on injection, transfects cells and the expressed protein elicits an immune response that in turn simulates a response to the respective viral infection. DNA vaccines usually consist of an Enterobacteriaceae plasmid with a strong promoter with broad cell specificity, such as the human cytomegalovirus immediate early promoter. The plasmid is amplified, commonly in Enterobacteriaceae, purified, and then simply injected into the host. Intramuscular immunization is most effective. Significant improvement in response to vaccination has been achieved by coating the plasmid DNA onto microparticles—commonly gold particles 1–3 μm in diameter—and injecting them by “bombardment,” using a helium-gas-driven gun-like apparatus (the “gene gun”).

Theoretical advantages of DNA vaccines include purity, physiochemical stability, simplicity, a relatively low cost of production, distribution, and delivery, potential for inclusion of several antigens in a single plasmid, and expression of antigens in their native form (thereby facilitating processing and presentation to the immune system). Repeated injection may be given without interference, and DNA immunization can induce immunity in the presence of maternal antibodies. However, DNA vaccination is yet to be widely used, because the practical application of the technology is considerably more challenging in humans and animals than it is in laboratory animals. Unsubstantiated concerns have also been raised regarding the fate and potential side-effects of the foreign, genetically engineered DNA and, for animals that will enter the human food chain, the costs of proving safety are likely to be significant.

Other Potential Vaccine Strategies

Bacteria as Vectors for Expression of Viral Antigens

Viral proteins (or immunogenic regions thereof) can be expressed on the surface of engineered bacteria that infect the host directly. The general approach is to insert the DNA encoding a protective viral antigen into a region of the genome of a bacterium, or one of its plasmids, which encodes a prominent surface protein. Provided that the added viral protein does not seriously interfere with the transport, stability, or function of the bacterial protein, the bacterium can multiply and present the viral epitope to the immune system of the host. Enteric bacteria that multiply naturally in the gut are the ideal expression vectors for presenting protective epitopes of virulent enteric viruses to the gut-associated lymphoid tissue, and attenuated strains of Enterobacteriaceae spp., and Mycobacterium spp. are being evaluated for immunization against enteric pathogens, including viruses, and/or for the preferential stimulation of mucosal immunity. A commercial subunit vaccine based on infectious pancreatic necrosis virus VP2 gene expressed by Enterobacteriaceae is effective in controlling this disease in salmonids.

Synthetic Peptide Vaccines

With the increased ability to locate and define critical epitopes on viral proteins, it is also possible to synthesize peptides chemically that correspond to these antigenic determinants. Appropriately designed synthetic peptides can elicit neutralizing antibodies against many viruses, including foot-and-mouth disease virus and rabies virus, but in general this approach has been disappointing, probably because of the conformational nature of many critical epitopes included in the authentic protein. Specifically, conformational epitopes are not composed of linear arrays of contiguous amino acids, but rather are assembled from amino acids that, while separated in the primary sequence, are brought into close apposition by the folding of the polypeptide chain(s). An effective antigenic stimulus requires that the three-dimensional shape that an epitope has in the native protein molecule or virus particle be maintained in a vaccine. Because short synthetic peptides lack any tertiary or quaternary structure, most antibodies raised against them are incapable of binding to virions, hence neutralizing antibody titers may be orders of magnitude lower than those induced by inactivated whole-virus vaccines or purified intact proteins. In contrast, the epitopes recognized by T lymphocytes are short linear peptides (bound to MHC protein). Some of these T cell epitopes are conserved between different strains of a particular virus and, therefore, may elicit a cross-reactive T cell response in some hosts. However, the MHC proteins that bind these peptides are highly polymorphic within any species and even more so between species. That makes the identification of common peptide epitopes across strains of the virus and all of the genotypes of animals responding to the virus very challenging. Today’s sophisticated bioinformatics capabilities make this approach more viable.
Vaccine Adjuvants

The immunogenicity of inactivated vaccines, especially that of purified protein vaccines and synthetic peptides, usually needs to be enhanced to optimize their utility. This may be achieved by mixing the antigen with an adjuvant, incorporation of the antigen in liposomes, or incorporation of the antigen in an immunostimulating complex. Similar approaches are also used to enhance the immunogenicity of recombinant vaccines, and the immunogenicity of these vaccines can be potentially even further enhanced through incorporation of immunopotentiating agents into or along with the expression vector.

Adjuvants are formulations that, when mixed with vaccines, potentiate the immune response, humoral and/or cellular, so that a lesser quantity of antigen and/or fewer doses will suffice. Adjuvants differ greatly in their chemistry and in their modes of action, but they typically can prolong the process of antigen degradation and release and/or enhance the immunogenicity of the vaccine by recruiting and activating key immune cells (macrophages, lymphocytes, and dendritic cells) to the site of antigen deposition. Alum and mineral oils have been used extensively in veterinary vaccines, but many others have been developed or are currently under investigation, some of which remain proprietary. Among many examples, synthetic biodegradable polymers such as polyphosphazene can serve as potent adjuvants, especially when used with microfabricated needles for intradermal inoculation of antigen. Immunomodulatory approaches to enhance the immunogenicity of vaccines also continue to be investigated—specifically, molecules that can enhance critical innate and adaptive immune responses or inhibit suppressors thereof.

Liposomes consist of artificial lipid membrane spheres into which viral proteins can be incorporated. When purified viral envelope proteins are used, the resulting “viroosomes” (or “immunosomes”) somewhat resemble the original envelope of the virion. This not only enables a reconstitution of viral envelope-like structures lacking nucleic acid and other viral components, but also allows the incorporation of nonpyrogenic lipids with adjuvant activity. When viral envelope glycoproteins or synthetic peptides are incorporated with cholesterol plus a glycoside known as Quil A, spherical cage-like structures 40 nm in diameter are formed. Several veterinary vaccines include this “immunostimulating complex adjuvant (ISCOM)” technology.

As discussed earlier in this chapter, viruses contain characteristic signatures designated as PAMPs that efficiently stimulate pathogen recognition receptors in dendritic cells and other innate cells that are critical in induction of adaptive immune responses. These PAMPs include ssRNA, dsRNA, and certain viral proteins. Whole-virus vaccines, both live-attenuated and killed, often retain these microbial signatures because the vaccines include intact virions, promoting vaccine-induced responses. TLR9 recognizes DNA molecules with methylation patterns not usually found in eukaryotic cells, and cytosine guanine oligonucleotides (CpG ODNs) have been developed in an effort to activate the TLR9 pathway in conjunction with various antigens and DNA vaccines. Other vaccine adjuvants in development or under evaluation include polynosinic-polycytidylic acid, which resembles viral dsRNA and stimulates TLR3, and saponin, an amphipathic glycoside derived from tree bark. Enhanced production of cytokines induced by the innate immune response can be achieved by expressing relevant cytokines in a viral expression vector along with the antigen of interest. Alternatively, a DNA vaccine expressing a viral antigen can be given along with a DNA molecule encoding a given cytokine. Numerous studies have shown enhanced immune responses when cytokines are used to augment the response naturally induced by an immunization process.

Given the recent development and increasing commercial production of new vaccine types and adjuvants, it anticipated that vaccine formulations and their methods of delivery will change quickly in the coming years.

Factors Affecting Vaccine Efficacy and Safety

In much of the world, vaccines are made under a broad set of guidelines, termed Good Manufacturing Practices. Correctly prepared and tested, all vaccines should be safe in immunocompetent animals. As a minimum standard, licensing authorities insist on rigorous safety tests for residual infectious virus in inactivated virus vaccines. There are other safety problems that are inherent to live-attenuated virus vaccines and, potentially, new generation recombinant virus vaccines.

The objective of vaccination is to protect against disease and, ideally, to prevent infection and virus transmission within the population at risk. If infection with wild-type virus occurs as immunity wanes after vaccination, the infection is likely to be subclinical, but it will boost immunity. For enzootic viruses, this is a frequent occurrence in farm animals, cats and dogs in shelters, and birds in crowded pens.

In many species, IgA is the most important class of immunoglobulin relevant to the prevention of infection of mucosal surfaces, such as those of the intestinal, respiratory, genitourinary, and ocular epithelia. One of the inherent advantages of orally administered live-attenuated virus vaccines is that they often induce prolonged synthesis of local IgA antibody, which confers relatively transient immunity to those respiratory and enteric viruses the pathogenic effects of which are manifested mainly at the site of entry. In contrast, IgG mediates long-term, often lifelong, immunity to re-infection against most viruses that reach their target organ(s) via systemic (viremic) spread.
Thus, the principal objective of vaccination is to mimic natural infection—that is, to elicit a high titer of neutralizing antibodies of the appropriate class, IgG and/or IgA, directed against the relevant epitopes on the virion in the hope of preventing infection.

The efficacy of live-attenuated virus vaccines delivered by either the mouth or nose is critically dependent on subsequent replication of the inoculated virus in the intestinal or respiratory tract, respectively. Interference can occur between the vaccine virus and enteric or respiratory viruses, incidentally infecting the animal at the time of vaccination. It is also proposed that interference can occur between different attenuated viruses contained in certain vaccine formulations. Special difficulties also complicate vaccination against viruses known to establish persistent infections, such as herpesviruses and retroviruses. These vaccines must be remarkably effective if it is to prevent, not only the primary disease, but also the establishment of lifelong latency. Live-attenuated virus vaccines are generally more effective in eliciting cell-mediated immunity than inactivated ones, however, they also carry some risk of themselves establishing persistent infections in the immunized host.

Potential Adverse Effects of Vaccines

Under-Attenuation

Some live-attenuated virus vaccines cause clinical signs in some vaccinated animals—in effect, a mild, or even severe case of the disease. For example, some early canine parvovirus vaccines that had undergone relatively few cell culture passages produced an unacceptably high incidence of disease. However, attempts to attenuate virulence further by additional passages in cultured cells may lead to a decline in the ability of the virus to replicate in the vaccinated animal, with a corresponding loss of immunogenicity.

Such side-effects are typically minimal with appropriately evaluated animal virus vaccines, and do not constitute a significant disincentive to vaccination. However, it is important that live-attenuated virus vaccines are used only in the species for which they were produced; for example, canine distemper vaccines cause fatalities in some members of the family Mustelidae, such as the black footed ferret, so that recombinant or inactivated whole-virus vaccines must be used. An additional unintended consequence of live-attenuated virus vaccines is the potential for transmission of viable vaccine virus from one animal to another, as has been reported among unvaccinated livestock adjacent to animals that were vaccinated with live-attenuated bluetongue virus vaccine. The unintended, natural transmission of live-attenuated vaccine viruses provides an opportunity for them to revert to virulence through genetic instability or recombination with “field” viruses.

Genetic Instability and Recombination

Some vaccine virus strains may revert toward virulence during replication in the recipient or in contact animals to which the vaccine virus has spread. Ideally, live-attenuated vaccine viruses are incapable of such spread, but in those that do there may be an accumulation of mutations (reversions) that gradually can result in restoration of virulence. The principal example of this phenomenon is the very rare reversion to virulence of Sabin poliovirus type 3 oral vaccine in humans, which eventually led to its replacement by the safer, although not necessarily more efficacious, nonreplicating vaccine. Temperature-sensitive mutants of bovine viral diarrhea virus have also proven to be genetically unstable. A more recent and ominous concern regarding genetic alteration of vaccine viruses comes from Australia, where the concurrent use of different infectious laryngotracheitis virus vaccines in poultry led to the emergence and spread of a novel recombinant virulent virus derived from distinct live-attenuated vaccine strains. Similarly, it is abundantly clear that live-attenuated vaccine strains of segmented RNA viruses such as bluetongue virus and African horse sickness virus (both orbiviruses) can re assort their genes with either field viruses or other vaccine viruses, in both the insect vector and animal host, to create novel progeny with potentially undesirable properties.

Heat Lability

Live-attenuated virus vaccines are vulnerable to inactivation by high ambient temperatures, a particular problem in the tropics, where maintenance of the “cold chain” from manufacturer to the point of administration to animals in remote, hot, rural areas can be challenging. To some extent the problem has been alleviated by the addition of stabilizing agents to the vaccines, selection of vaccine strains that are inherently more heat stable, and by packaging them in freeze-dried form for reconstitution immediately before administration. Simple portable refrigerators for use in vehicles and temporary field laboratories are also invaluable.

Presence of Contaminating Viruses

Because vaccine viruses are grown in animals or in cells derived from them, there is always a possibility that a vaccine will be contaminated with another virus from that animal or from the medium used for culturing its cells. An early example, which led to restrictions on international trade in vaccines and sera that are still in effect, was the introduction into the United States in 1908 of foot-and-mouth disease virus as a contaminant of smallpox vaccine produced in calves. Similarly, the use of embryonated
eggs to produce vaccines for use in chickens may pose problems (eg, the contamination of Marek’s disease vaccine with reticuloendotheliosis virus). Another important source of virus contaminants is fetal bovine serum, used universally in cell cultures; all batches of fetal bovine serum must be screened for contamination with bovine viral diarrhea virus in particular. Likewise, porcine parvovirus is a common contaminant of crude preparations of trypsin prepared from pig pancreases, which is used commonly in the preparation of animal cell cultures. The risk of contaminating viruses is greatest with live-attenuated virus vaccines, but may also occur with inactivated whole-virus vaccines, as some viruses are more resistant to inactivation than others; the prion agents are notoriously resistant to traditional methods of sterilization, for example. In some instances serious adverse effects relating to use of attenuated virus vaccines have an unknown origin; for example the chimeric West Nile vaccine based on yellow fever virus was highly effective at preventing West Nile disease in horses but was recalled after multiple reports of acute anaphylaxis, colic, respiratory distress and death following vaccination of horses.

**Adverse Effects in Pregnant Animals**

Live-attenuated virus vaccines are not generally recommended for use in pregnant animals, because they may be abortigenic or teratogenic. For example, live-attenuated infectious bovine rhinotracheitis vaccines can be abortigenic, and the live-attenuated feline panleukopenia, classical swine fever, bovine viral diarrhea, Rift Valley fever, and bluetongue vaccines are all teratogenic if they cross the placenta to infect the fetus at critical stages of gestation. These adverse effects are usually the result of primary immunization of a nonimmune pregnant animal at a susceptible stage of gestation, so that it may be preferable to immunize pregnant animals with inactivated vaccines, or to immunize the dam with a live-attenuated vaccine before mating. Contaminating viruses in vaccines sometimes go unnoticed until used in pregnant animals; for example, the discovery that bluetongue virus contamination of canine vaccines caused abortion and death in pregnant bitches was most unexpected.

**Adverse Effects From Nonreplicating Vaccines**

Some inactivated whole-virus vaccines have been found to potentiate disease. The earliest observations were made with inactivated vaccines for measles and human respiratory syncytial virus, in which immunized individuals developed more severe disease than did those that remained unvaccinated before infection. Similar events have occurred in veterinary medicine, including the enhanced occurrence of feline infectious peritonitis in cats immunized with a recombinant vaccinia virus that expressed the feline coronavirus E2 protein before challenge infection. Despite the production of neutralizing antibodies after immunization, the kittens were not protected and died quickly of feline infectious peritonitis after challenge. There are numerous instances of disease induced by incomplete inactivation of nonreplicating vaccines, and others wherein contaminating viruses survived the inactivation process.

**Vaccination Policy and Schedules**

Beyond the schedule of primary vaccination, there is little agreement and much current debate as to how often animals need to be revaccinated. For most vaccines, there is comparatively little definitive information available on the duration of immunity. For example, it is well recognized that immunity after vaccination with live-attenuated canine distemper vaccine is of long duration, perhaps lifelong. However, the duration of immunity to other viruses or components in a combined vaccine may not be of such long duration. In companion-animal practice, the cost of vaccination, relative to other costs, is typically modest when clients visit their veterinarian, so it has been argued that, if revaccination does no harm, it may be considered a justified component of the routine annual “check-up” in which a wide spectrum of healthcare needs may be addressed. In many countries, annual revaccination has become a cornerstone of broad-based companion-animal preventive healthcare programs, although the rationale for this approach is conjectural at best.

This concept of annual vaccination was further disturbed in the mid-1990s by reports of highly aggressive subcutaneous fibrosarcomas in cats at sites of vaccination (often behind the shoulder). All the factors responsible for these vaccine-associated cancers remain to be thoroughly proven; however, a contaminating virus within the vaccines themselves is not responsible, and the prevailing suspicion is that irritation induced by the vaccine constituents is responsible. Regardless, this phenomenon rekindled the debate of frequency of revaccination in companion animals, leading to new recommendations on the preferred vaccination site, vaccination interval (extended from 1 to 3 years for some vaccines), and systems for reporting adverse responses.

The available range of vaccines, often in multivalent formulations and with somewhat different recommendations from each manufacturer regarding vaccination schedules, means that the practicing veterinarian needs to educate her/himself constantly about vaccine choice and usage. Multivalent vaccine formulations confer major practical advantages by reducing the number of inoculations an individual animal must receive. Also, multivalent vaccines allow more extensive use of vaccines against agents of secondary importance. Unlike the situation in
human medicine, however, where there is general agreement on vaccine formulations and schedules for vaccination against all the common viral diseases of childhood, there is no such consensus in veterinary medicine. Furthermore, unlike the situation in human medicine in which there are few vaccine manufacturers, there are many veterinary vaccine manufacturers, each promoting their own products. The reader is referred to the specific resources on vaccination schedules specific for each animal species provided at the end of this section, but some general considerations for vaccination are described here.

**Optimal Age for Vaccination**

The risk of many viral diseases is greatest in young animals. Most vaccines are therefore given during the first 6 months of life. Maternal antibody, whether transferred transplacentally in primates or, as in domestic animals and birds, in the colostrum or via the yolk sac, inhibits the immune response of the newborn or newly hatched to vaccines. Optimally, vaccination should be delayed until the titer of maternal antibody in the young animal has declined to near zero. However, any delay in vaccine administration may leave the animal vulnerable during the resulting “window of susceptibility.” This is potentially life-threatening in crowded, highly contaminated environments or where there is intense activity of arthropod vectors. There are a number of approaches to handling this problem in different animal species, but none is fully satisfactory. The problem is complicated further because young animals do not necessarily respond to vaccines in the same way as older animals do. In horses, for example, antibody responses to inactivated influenza vaccines are poor until recipients become yearlings.

Because the titer of passively acquired antibody in the circulation of newborn animals after receiving colostrum is proportional to that in the dam’s blood, and because the rate of its subsequent clearance in different animal species is known, it is possible to estimate, for any given maternal antibody titer, the age at which no measurable antibody remains in the offspring. This can be plotted as a nomograph, from which the optimal age of vaccination against any particular disease can be read. The method is seldom used, but might be considered for exceptionally valuable animals in a “high-risk” environment.

In practice, relatively few vaccine failures are encountered if one simply follows the instructions from the vaccine manufacturers, who have used averaged data on maternal antibody levels and rate of IgG decay in that animal species to estimate an optimal age for vaccination. It is recommended commonly, even in the case of live-attenuated virus vaccines, that a number of doses of vaccine be administered, say at monthly intervals, to cover the window of susceptibility in animals with particularly high maternal antibody titers. This precaution is even more relevant to multivalent vaccine formulations, because of the differences in levels of maternal antibody against each virus.

**Dam Vaccination**

The aim of vaccination is generally thought of as the protection of the vaccinee. This is usually so, but in the case of certain vaccines (eg, those for equine herpes (abortion) virus-1, rotavirus infection in cattle, parvovirus infection in swine, infectious bursal disease of chickens) the objective is to protect the vaccinee’s offspring either in utero (eg, equine abortion) or as a neonate/hatchling. This is achieved by vaccination of the dam. For neonates/hatchlings, the level of maternal antibody transferred in the colostrum and milk or in the egg ensures that the offspring have a protective level of antibody during the critical early days. Because many live-attenuated virus vaccines are abortigenic or teratogenic, inactivated vaccines are generally recommended for vaccination of pregnant animals.

**Availability and Recommendation of Vaccines**

The types of vaccines available for each viral disease (or the lack of any satisfactory vaccine) are discussed in each chapter of Part II of this book. There is clearly enormous geographic variation in the requirements for individual vaccines, particularly for highly regulated viral diseases such as foot-and-mouth disease. There are also different requirements appropriate to various types of livestock husbandry (eg, for intensively raised dairy cattle as compared to free-ranging beef cattle and their calves, or cattle in feedlots; also in poultry for breeders, commercial egg layers, and broilers). Similarly, vaccination schedules for dogs, cats, horses, pet birds, and other species such as rabbits should reflect science-based criteria in addition to individual risk. Thus, the reader is referred to specialty organizations that publish guidelines for the vaccination of, for example: horses (American Association of Equine Practitioners (http://www.aaepl.org/vaccination_guidelines.htm)), cats (American Association of Feline Practitioners (http://www.catvets.com/professionals/guidelines/publications/?Id5176)), and dogs (American Animal Hospital Association (http://secure.aahanet.org/eweb/dynamic-page.aspx?site5resource&webcode5CanineVaccineGuidelines)). Relatively few vaccines are widely available for use in pet birds, but those that are include vaccines for polyoma virus, Pacheco’s disease virus, canarypox and, in enzootic areas, West Nile virus.

For some species, including production animals, protection against viral infections and diseases is by exclusion. Laboratory rodents, for example, are maintained in
Various types of microbial barrier environments. Rarely, laboratory mice at high risk for ectromelia virus infection during outbreaks in highly valuable mouse populations may be individually vaccinated with the IHD-T strain of vaccinia virus.

Commercially raised rabbits, as well as pet rabbits, are often vaccinated against myxoma virus and rabbit hemorrhagic disease virus, where these agents are highly prevalent, as in Europe. These rabbit diseases also illustrate the political context of veterinary vaccination: vaccines may not be available in some countries, such as the United States, because vaccination may obscure surveillance for natural outbreaks of disease.

**Vaccination of Poultry and Fish**

Poultry production is economically important worldwide, an estimated >$20 billion per year industry in the United States for example. To help protect this industry, all commercially produced birds are vaccinated against several different viral diseases, although there is variation in the types of vaccines used in different countries. The strategy for vaccination of poultry against viral diseases is no different than that for mammals, but the cost of each vaccine dose is tiny; much of this economy of scale is linked to low-cost delivery systems (aerosol and drinking water). Further economies have been achieved by the introduction of in-ovo immunization of 18-day-old embryonated eggs; an instrument (called an Inovoject), capable of immunizing 40,000 eggs per hour, is used. The most frequently used vaccines are against Marek’s disease; formerly inoculated individually into 1-day-old chicks, these are now delivered in this way. By 2009, more than 95% of meat chickens (broilers) in the United States were vaccinated by this method.

In commercial aquaculture, vaccination is used to prevent infectious hematopoietic necrosis and infectious pancreatic necrosis in salmonids. Vaccines to these diseases include DNA and subunit protein vaccines that are administered either by injection or orally. A live-attenuated virus vaccine against cyprinid herpes virus 3 infection of koi carp (*Cyprinus carpio haematopterus*) was recently approved for use in Israel; this vaccine has a genetic deletion that allows differentiation between vaccinated and infected fish. The objective of vaccination in fish is the same as in mammals; indeed, the phylogenetic origins of the vertebrate immune system can be traced to the first jawed vertebrates, including bony fish (teleosts). Antiviral immunity, although less understood in fish as compared to mammals or birds, involves both innate and acquired response mechanisms. Specifically, cellular and humoral innate responses involve equivalent cell types, signaling molecules, and soluble factors as are found in mammals. These include phagocytes equipped with pattern recognition receptor such as the TLRs that lead to pro-inflammatory responses and interferon induction; induction of type 1-like interferons is essential for antiviral innate immune responses in fish, and their production is stimulated by dsRNA and signaling pathway in a manner analogous to that in mammals. Similarly, it appears that the innate immune response induces an antiviral state in addition to priming adaptive immunity in fish as it does in mammals.

Adaptive responses involving T and B lymphocytes and specific immunoglobulin production are also critical for antiviral immunity in fish. The structure of the T cell receptor complex (αβ or γδ) has remained virtually constant throughout the evolution of jawed vertebrates, including teleosts, whereas the organization and usage of the B cell receptors in fish varies from that of other vertebrates, as fish possess two distinct B cell lineages (sIgM+ or slgγ/δ+)—both of which are important for antiviral immunity and affinity maturation of immunoglobulins—and a less pronounced memory response is typical of the adaptive response in fish as compared with mammals or birds. As fish are poikilotherms, the magnitude of the immune response in most fish is profoundly influenced by water temperature, which may play a causal role in seasonal viral disease patterns in both captive and wild fish populations.

**OTHER STRATEGIES FOR ANTIVIRAL PROPHYLAXIS AND TREATMENT**

**Passive Immunization**

It is possible to confer short-term protection against specific viral disease by the subcutaneous administration of an appropriate antibody, such as immune serum, immunoglobulin, or a monoclonal antibody. Indeed, original vaccination strategies such as those employed by Arnold Theiler in an effort to prevent African horse sickness, employed the simultaneous inoculation of virulent virus and immune sera to susceptible horses. Although not commonly used, homologous immunoglobulin is now preferred, because heterologous protein may provoke a hypersensitivity response, as well as being more rapidly cleared by the recipient. Pooled normal immunoglobulin contains sufficiently high concentrations of antibody against all the common viruses that cause systemic disease in the respective species. Higher titers occur in convalescent serum from donor animals that have recovered from infection or have been hyperimmunized by repeated vaccinations; such hyperimmune globulin is the preferred product if available commercially.
Chemotherapy of Viral Diseases

If this had been a book about bacterial diseases of domestic animals, there would have been a large section on antimicrobial chemotherapy. However, the antibiotics that have been so effective against bacterial diseases have few counterparts in our armamentarium against viral diseases. The reason is that viruses are intimately dependent on the metabolic pathways of their host cell for their replication, hence most agents that interfere with virus replication are toxic to the cell. In recent years, however, and spurred in large part by investigation of devastating human viral diseases such as acquired immunodeficiency syndrome (HIV-AIDS), influenza, and B- and C-hepatitis, increased knowledge of the biochemistry of virus replication has led to a more rational approach in the search for antiviral chemotherapeutic agents, and a number of such compounds have now become a standard part of the armamentarium against particular human viruses. Antiviral chemotherapeutic agents are not in common use in veterinary practice, partly because of their very high cost, but some of the antiviral drugs used in human medicine have already also been utilized in veterinary medicine. Accordingly, it is appropriate to outline briefly some potential developments in this field.

Several steps in the virus replication cycle represent potential targets for selective antiviral drug attack. Theoretically, all virus-encoded enzymes are vulnerable, as are all processes (enzymatic or nonenzymatic) that are more essential to the replication of the virus than to the survival of the cell. A logical approach to the development of new antiviral drugs is to isolate or synthesize substances that might be predicted to serve as inhibitors of a known virus-encoded enzyme such as a transcriptase, replicase, or protease. Analogs of this prototype drug are then synthesized with a view to enhancing activity and/or selectivity. A further refinement of this approach is well illustrated by the nucleoside analog, acycloguanosine (aciclovir)—an inhibitor of herpesvirus DNA polymerase. Aciclovir is in fact an inactive prodrug that requires another herpesvirus-coded enzyme, thymidine kinase, to phosphorylate it to its active form. Because this viral enzyme occurs only in infected cells, aciclovir is nontoxic for uninfected cells, but very effective in herpesvirus-infected cells. Aciclovir and related analogs (eg, valacyclovir, ganciclovir) are now available for treatment of herpesvirus infections in humans, and they have also been used on a limited scale in veterinary medicine, such as for treatment of feline herpesvirus-1 induced corneal ulcers and equine herpesvirus-1 induced encephalomyelitis. They have also been used in humans exposed to the zoonotic herpes virus of macaques, herpes simiae (B virus) that may have catastrophic consequences in infected humans.

Drugs also have been developed to treat influenza virus infections in people and, potentially, animals. For example, oseltamivir phosphate (Tamiflu) is a prodrug that, after its metabolism in the liver, releases an activate metabolite that inhibits neuraminidase, the virus-encoded enzyme that releases budding virions from the surface of infected cells and cleaves the virus receptor so that released virions do not bind to already infected cells. Inhibition of neuraminidase, therefore, slows virus spread, giving the immune system the opportunity to “catch up” and mediate virus clearance.

Ribavirin is also a prodrug that is metabolized to purine RNA metabolites that interfere with the RNA metabolism that is required for virus replication. This drug has been used in the treatment of human respiratory syncytial virus and hepatitis C virus infections.

X-ray crystallography has opened a major new approach in the search for antiviral drugs. Now that the three-dimensional structure of many viruses is known, it has been possible to characterize receptor-binding sites on capsid proteins at the atomic level of resolution. Complexes of viral proteins with bound cellular receptors can be crystallized and examined directly. For example, for some rhinoviruses, receptor-binding sites on virions are in “canyons”—that is, clefts in the capsid surface. Drugs have been found that fit into these clefts, thereby preventing virus attachment to the host cell. Further information is provided by mapping the position of the particular amino acid residues that form these clefts, thereby allowing the design of drugs that better fit and better interfere with the viral infection process. This approach also lends itself to the development of drugs that block virus penetration of the host cell or uncoating of virus once inside the cell. If any of these strategies are successful in human medicine, adaptation to veterinary usage may follow.

VIRUSES AS VECTORS FOR GENE THERAPY

In addition to their central role as pathogens, viruses also have contributed much to the current understanding of both cellular and molecular biology. Individual viruses, or components thereof, have been exploited as molecular tools, and viruses also offer a novel and useful system for the expression of heterologous genes. Specifically, with the advent of cloning and genetic manipulation, foreign genes can readily be inserted into the genome of many viruses so that they can be used as expression vectors. These viral gene vectors include those that deliver the gene of interest without replicating in the host (“suicide” vectors) and those that do replicate in the host, with or without integration into the genome.

The use of both DNA and RNA viruses as recombinant vaccine vectors was described earlier in this chapter,
but this same strategy also can potentially be exploited for therapeutic use. Viral-vector gene therapy strategies offer a novel and especially attractive approach to the correction of specific genetic disorders, particularly those with a defined missing or dysfunctional gene. Correction of such disorders requires the long-term expression of the specific protein that is absent or dysfunctional; thus viruses with the capability of safely and stably inserting the target gene into the genome of the affected individual are a logical choice as vectors for this purpose. To this end, a variety of viruses have been evaluated as potential gene vectors, including retroviruses because of their inherent ability to integrate into the host genome, poxviruses, adenoviruses, adeno-associated viruses (which are paroviruses), herpesviruses, and various positive- and negative-sense RNA viruses.

Adeno-associated viruses have received much recent attention as potential vectors for gene therapy. They are small DNA viruses (family Paroviridae, subfamily Parovirinae, genus Dependoparvovirus) that can infect both dividing and nondividing cells, and they can insert their genome into that of the host cell. Furthermore, integration of the viral genome of adeno-associated viruses occurs at specific sites within the host genome, as opposed to that of retroviruses, insertion of which is typically random and potentially mutagenic. Adeno-associated viruses are considered to be avirulent (non-pathogenic), and the capacity for integration is readily abolished by genetic manipulation. Recombinant adeno-associated viruses that express appropriate proteins have been evaluated for the correction of a variety of human genetic disorders, including hemophilia and muscular dystrophy. Adeno-associated viruses have also gained favor as expression vectors of broadly neutralizing antibodies against HIV that may provide preexposure prophylaxis and protection against infection in “vaccinated” individuals.

The strategy of targeted gene delivery is also potentially applicable for therapeutic intervention by the delivery of molecules with the capacity to modulate disease processes, especially chronic diseases with an immune-mediated pathogenesis that might be susceptible to regional expression of immunomodulatory molecules.

Another potential application of targeted gene delivery using recombinant viruses is to control the reproduction of wildlife and feral species, including those species considered to be pests, by targeted delivery of immunogenic proteins critical for reproductive activity.