FOCAL POINT

★ Veterinarians must understand the strengths and limitations of available vaccines and know the types of protection needed to defend animals against pathogenic organisms.

KEY FACTS

■ Commercial vaccines are not available for all important infectious agents.

■ The humoral (antibody) and cell-mediated systems can be stimulated by vaccination.

■ The immune response is further separated into systemic and mucosal compartments.

■ Passive immunity (maternal antibody) can interfere with active immunization.

■ Disease prevalence, housing stressors, population density, and exposure to other animals influence selection of a vaccination protocol.

Immunologic Principles and Immunization Strategy

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Controlling infectious disease is a primary concern for practicing veterinarians. Proper nutrition, environment enhancement, stress reduction, minimization of exposure to pathogens, and use of effective vaccines are all important for the control of infectious disease.

Numerous vaccines have been developed for controlling infectious agents that cause disease in animals. For the vaccines to produce an optimum immune response and decrease the risk of disease, they must be used in accordance with the principles of immunology. This article reviews basic immunologic principles that affect the development of protection against infectious agents and considers how to apply these principles when developing an immunization program.

BASIC IMMUNOLOGIC PRINCIPLES

Immunization implies that after vaccination, the animal develops a protective immune response against the invading pathogen. A proper vaccination protocol must select the correct antigen and deliver it in an optimum fashion and at the correct time to elicit a response that can protect the animal. In general, a successful immune response should produce the same humoral and cellular response that results from natural infection. To achieve this goal, the delivery system should place the vaccine in association with lymphoid cells in the target tissue.[1] Effectiveness of vaccination is influenced by the age, nutrition, immune status, and overall health of the recipient.

When selecting diseases to include in a vaccination program, immunization with every available vaccine may be wasteful and unjustifiable (see Some Questions to Consider). Some vaccines that have been manufactured for controlling certain infectious agents are not efficacious. Other diseases for which vaccines are available are not a primary concern in all geographic areas. The vaccines selected should be limited to those that produce protective immunity and for which there is a risk of disease.

Humoral Immune System

Two parts of an animal's defense mechanism can be stimulated by vaccination: the humoral (antibody) system and the cell-mediated system. The humoral system consists of B lymphocytes (non–thymus-derived lymphocytes); plasma cells; and immunoglobulins (antibodies)—that is, IgG, IgM, IgA, and IgE. For an antigen to stimulate immunoglobulin production, the antigen must be processed (primarily by macrophages) and presented to B lymphocytes. In the presence of antigen, specific lymphocytes are transformed into lymphoblasts that rapidly increase in number. These activated B cells either differentiate into immunoglobulin-producing plasma cells or remain as memory B cells. Each plasma cell produces only one class-specific immunoglobulin to a specific antigen.

Approximately 80% of immunoglobulin activity in serum is IgG. IgG binds to and opsonizes antigen. The resultant complex activates the complement cascade.[2] IgM, which is effective in complement fixation and opsonization, is the first immunoglobulin produced in an immune response.[2] Lymphoid tissue located near mucosal surfaces produces IgA. IgA does not activate complement or opsonize bacteria but acts to prevent antigens from binding to mucosal surfaces. IgE is associated with tissue mast cells. When IgE binds to an antigen, the mast cells release vasoactive agents such as histamine. The vasoactive agents cause a local inflammatory response that floods the tissue with immunoglobulins and phagocytic cells.[3] IgE also plays a primary role in the immune response to helminth parasites.

Immunoglobulin against surface antigens on bacteria may confer protection from disease. Examples include antigens from the flagellum, pilus, capsule, or cell wall. Conversely, a humoral immune response to antigens from inside living bacteria is unlikely to be protective because internal antigens are inaccessible to immunoglobulins. These internal antigens include cytoplasmic antigens as well as certain cell-wall and membrane antigens.

Similarly, immunoglobulins against external viral antigens (e.g., envelope and capsid antigens) may provide protective immunity from viruses. Immunoglobulins against internal viral antigens (e.g., core protein antigens) do not provide protective humoral immunity from viral pathogens.

Nonspecific Immune System

Assisting the humoral system is the nonspecific immune system, which includes phagocytic cells (granulocytes and macrophages), natural lymphocyte killer cells, and complement. The nonspecific immune system is not directly affected by either vaccination or previous exposure to an infectious agent. This system can respond to an infectious agent almost immediately and is crucial in the initial control of infection. The humoral and cell-mediated systems require 2 weeks to reach optimum function after the first exposure to an antigen. On second exposure to the same antigen, the response is rapid. This anamnestic response is the protective principle on which vaccination is based. The anamnestic response is the result of immunologic memory provided by special classes of lymphocytes (memory cells).

Immunoglobulin molecules alone cannot destroy infectious agents but do activate the nonspecific defense mechanisms and improve their efficiency. Immunoglobulins may coat infectious agents, thereby minimizing the likelihood that the agents will attach to host cells. Immunoglobulins may agglutinate infectious agents (thus reducing their infectivity) and directly bind to and neutralize toxins. Immunoglobulins also act by working with other cells or effector molecules by binding to and marking an antigen so that phagocytic cells, cytotoxic cells, or complement destroys the infectious agent.

Cell-Mediated Immune System

The cell-mediated system consists of macrophages, lymphocytes, lymphokines, and monokines. The system is organized by thymus-matured lymphocytes (T lymphocytes) that recognize specific antigens.[3] When

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Some Questions to Consider

- Is it likely that sufficient immunity to protect the animal against a given disease is already present?
- Could immunity already be present and thus inactivate the vaccine?
- What is the nutritional status of the animal?
- What is the health status of the animal?
- Does the animal have possible immunosuppression attributable to physiologic stressors or pharmacologic intervention?
- What is the level of exposure that the animal is likely to encounter for the disease(s) being considered for vaccination?
- If cost is a factor for the client, which veterinary service (vaccinations or diagnostic screening procedures) is more appropriate?

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an animal is exposed to a disease or a vaccine that induces cell-mediated immunity, the T lymphocytes that recognize the antigen respond by replicating themselves through mitosis. With reexposure to the same antigen, a large number of T lymphocytes (T memory cells) recognize it and replicate. The T lymphocytes attempt to destroy the infectious agent directly and by secreting lymphokines. Lymphokines are proteins that direct and encourage other white blood cells to attack and destroy the agent.

Cell-mediated immunity is particularly important for protection against facultative intracellular viral and bacterial pathogens. The cell-mediated system can destroy infected cells, thereby releasing the invading organism so that it can be destroyed by phagocytic or killer cells. Internal antigens as well as external and secreted antigens can induce a protective cell-mediated response. This response differs from the humoral response because antigen processing and presentation of internal antigens by an antigen-presenting cell can induce a cell-mediated response that can destroy invaded host cells. The cell-mediated system may recognize antigens that remain on the surface of host cells after viral penetration, viral antigens synthesized by the host cells and expressed on the cell surface, or processed antigens associated with major histocompatibility complex (MHC) class I molecules.[4]

Cytotoxic T lymphocytes may destroy cells that express viral antigens associated with MHC class I molecules; such destruction is believed to be important in protective immunity against many viral diseases.[4] The T-helper lymphocytes responding to antigens associated with MHC class II molecules may mediate protection from viral disease through secretion of lymphokines. Lymphokines may make cells resistant to viral infection, damage viral infected cells, and enhance activity of cytotoxic cells (e.g., macrophages, neutrophils, and natural killer cells).[4]

Systemic and Mucosal Immune Systems

The immune response is further separated into two distinct compartments: systemic and mucosal. Systemic immunity derives from cells in the spleen or lymph nodes, whereas mucosal immune responses are generated by lymphoid tissue associated with mucosal surfaces or the associated draining lymph nodes.[5] It is especially difficult for the immune system to protect animals against infection on mucosal surfaces, such as the intestinal, reproductive, and respiratory tracts.[6] Most of the antibody classes responsible for humoral immunity as well as the white blood cells responsible for cell-mediated immunity are not found on mucosal surfaces.

Parenteral antigens usually result in a systemic immune response, whereas antigens transferred across a mucosal surface induce mucosal immunity.[5] Whether parenteral or local immunization is necessary for protection depends on the pathogenesis of the disease. Mucosal immunity is particularly beneficial when the route of exposure of a pathogen is the same as that of the target tissue. Examples of local immune stimulation are the intranasal use of attenuated infectious bovine rhinotracheitis (IBR) virus vaccine against respiratory disease in calves, intranasal use of *Bordetella* and *Parainfluenza* vaccines for control of infectious tracheobronchitis in dogs, and intraocular or intranasal use of vaccine to control calicivirus infection in cats. The intranasal route has the advantage of inducing systemic immunity as well as local antibody and cell-mediated immunity in the respiratory tract.

Protective Responses

The type of immune response that protects an animal against infectious disease varies between pathogens and depends on the route of introduction and site of replication of particular organisms. Protection may result from the presence of circulating immunoglobulins (humoral immunity), sensitized T lymphocytes (cell-mediated immunity), immunoglobulins on mucosal surfaces, or a combination of these factors.[4]

Humoral immunity is important for protection against extracellular phases of systemic viral and bacterial infection and for protection against endotoxin- and exotoxin-induced diseases.[4] Cell-mediated immunity is important in combating facultative intracellular bacterial pathogens (e.g., *Brucella*), intracellular viral infection (e.g., herpesvirus), fungal disease, and protozoal disease.[4]

ANIMAL FACTORS OF IMMUNOLOGY

Numerous factors can influence an animal's defense mechanisms and thus affect the immune response to vaccination. Factors important to an effective vaccination program include the blocking effect of colostral antibody, the age and nutritional condition of the animal, and the effect of concurrent infection. All of these factors affect an individual's immune status.

One of the most common problems associated with vaccination is interference of passive immunity (maternal antibody) with active immunization.[7] For example, immunization of puppies against canine parvovirus 2 is commonly prevented or delayed because of maternal interference. The interference probably occurs when passively derived maternal antibody binds to the vaccine antigen, thus resulting in clearance of the vaccine antigen from the body before it stimulates an immune response.[3] The level of maternal antibody that can in-

Viral Vaccines

Modified Live Viral Vaccines

- Provide longer-lasting and more complete immunity than noninfectious vaccines

- Stimulate cellular and secretory immunity

- Do not require multiple vaccinations for immunologic memory

- Often do not require revaccination or require fewer revaccinations during the life of the animal

- Rarely cause the hypersensitivities but may be virulent for certain individual animals or revert to virulence

Noninfectious Viral Vaccines

- Provide short-lived systemic immunity

- Offer poor cellular and secretory immunity

- Require multiple vaccinations for immunity

- Often require revaccination to ensure immunologic memory

- Often cause hypersensitivity reactions

- Cannot cause disease even in immunologically compromised animals

terfere with parvovirus vaccination is less than that needed to protect the puppy from infection. A gap in immune protection therefore occurs when the puppy is susceptible to parvovirus infection yet maternal antibody interferes with immunization. Such susceptibility is the reason for frequent revaccination between 6 and 16 weeks of age for kittens and between 6 and 22 weeks of age for puppies. By vaccinating young animals every 3 weeks, the period of susceptibility to disease is shortened but not eliminated.[8] Studies have shown that, at least in the case of canine parvovirus[9] and probably canine distemper,[10] vaccines with high antigen titers are likely to be more effective in the presence of maternal-derived antibodies.[9]

The general health and nutritional state of the animal is important for successful immunization because an optimum immune response requires extensive cell division and synthesis of proteins. Inadequate nutrition, including deficiencies of protein and certain micronutrients (e.g., copper and zinc), is known to restrict immune responses.[11] Animals under stress as a result of transportation, climate, or other environmental stressors are poor

subjects for immunization because the immune response may be diminished by various mechanisms, including immunosuppression by endogenous corticosteroids.[12–14] Glucocorticoid administration may interfere with the ability of animals to produce a primary response after vaccination, although proof is lacking.[15,16] It seems prudent, however, to reduce or eliminate glucocorticoid therapy for a week before and 2 weeks after primary vaccination if possible.[15]

Vaccination will fail to immunize an animal adequately if it is already incubating the organism at the time of administration. Clinical illness or stress from the presence of disease unrelated to the vaccine antigen may also be associated with immunosuppression and may interfere with successful immunization.[17]

ENVIRONMENTAL FACTORS

Disease prevalence, housing stressors, population density, and exposure to other animals are important factors in the selection of a vaccination protocol. As veterinarians perform risk-to-benefit assessments in different geographic areas, they should develop different vaccination protocols. For example, risk of Lyme disease is much lower in Kansas than in Connecticut; that difference should be taken into consideration. Stressful housing situations (e.g., kennel or cattery environments) decrease immune response to vaccination, increase susceptibility, and can activate latent viral infection. Animals in these types of housing situations have very different vaccination requirements for proper immunization than would an indoor cat in a single-pet household. Vaccination protocols also should take into account the increased exposure to potential carriers for animals that roam, share common exercise areas, attend exhibitions or training classes, or are frequently kennelled.

Practitioners have the responsibility to tailor vaccination protocols so that each animal has the greatest opportunity to develop protection from the infectious diseases that it will come in contact with while minimizing risk of vaccine-induced complications.

VACCINE TYPES

Animals require different classes of immunity for protection from various pathogens. The type of vac-

cine, route of administration, and particular adjuvant characteristics affect the type of immune response produced. Two types of vaccine are currently used in veterinary medicine: attenuated (modified live) and noninfectious (see Viral Vaccines). Noninfectious vaccines include both killed (inactivated) and subunit products. Attenuated vaccines use infective agents that are altered so that they are no longer virulent yet retain the antigenic properties that induce a protective immune response.

Attenuated Vaccines

Attenuated vaccines, which can be given locally or parenterally, do not contain enough antigen to immunize an animal unless the organism can infect and replicate in the host.[3] An effective local immune response requires a replicating organism and cannot be produced by noninfectious (killed) vaccines.[3] Because attenuated vaccine organisms replicate in the host, they more closely resemble virulent infection and generally produce a stronger and more durable protective immune response than do the noninfectious vaccines.[3] Attenuated vaccines may also induce interferon production during the first few days after immunization, thus providing additional early protection against some virulent viral infections.[3]

Attenuated vaccines also have disadvantages. Some attenuated infectious agents can induce immunosuppression, may be shed into the environment, may be contaminated with unintended viruses, or may revert to virulence or cause vaccine-induced disease.[18–20]

Noninfectious Vaccines
Inactivated Vaccines

Inactivated vaccines cannot replicate and are unable to cause infectious disease. To induce a protective immune response, inactivated vaccines require a large antigenic dose, multiple immunizations, and the use of adjuvants that can cause tissue irritation at the injection site. These factors substantially increase the cost of inactivated vaccines and the probability of local and systemic vaccine reactions.[3] Several studies have shown that the use of feline leukemia and rabies vaccines in cats are associated with an increased frequency of sarcoma. The incidence of vaccine-associated fibrosarcoma is low and does not outweigh the risk of feline leukemia in at-risk cats.[21,22]

Inactivated vaccines generally produce weaker immune responses with a shorter duration than the immune responses produced by attenuated vaccines. To lessen this shortfall, adjuvants are added to increase vaccine efficacy in stimulating immune protection. Modified Freund's adjuvant, *Bordetella pertussis*, and *Propi-*

onibacterium acnes stimulate the activity of macrophages and enhance their ability to present antigen.[23] Another method of enhancing the immune response is to ensure that the antigen remains in the body for a prolonged period, which can be done by incorporating antigen in such insoluble adjuvants as alum or oil.[23] Several new adjuvants that apparently facilitate cell-mediated responses are under investigation.[1]

Subunit Vaccines

Subunit vaccines being introduced to veterinary medicine have many of the same advantages and disadvantages as killed vaccines because the subunit vaccines also are noninfectious. Subunit vaccines differ from attenuated and inactivated vaccines in that subunit vaccines contain a portion of an organism rather than the entire organism. For a subunit vaccine to be effective, the portion of the organism in the vaccine must include one or more antigens that induce a protective response. The advantage of subunit vaccines is the reduction in risk of an allergic reaction to nonessential elements.[3]

Bacterins

Bacterins (killed bacterial vaccines) are generally less effective than viral vaccines and provide short-lived, partial immunity.[1] The immune response required for protective immunity to bacteria is much more complex than that required to protect against viruses. With regard to structure and antigens, viruses are simpler than bacteria. Most viruses have only a few critical immunodeterminants that stimulate a protective immune response. In contrast, bacteria are more complex structurally and have many immunodeterminants.[3] Because of these differences, vaccines for some bacterial diseases, such as that caused by *Bordetella bronchiseptica* in dogs, may require more frequent (annual) revaccination than do vaccines for many viral diseases.

Revaccination

Viral diseases for which long-term, protective immune responses are developed after vaccination do not require annual revaccinations once the animal reaches adulthood. Controlled studies indicate that immunization with attenuated canine distemper vaccine maintains titers for at least 5 years,[a] and attenuated canine parvovirus vaccine maintains titers for at least 3 years.[a]

For these two diseases, the correlation between the presence of immunoglobulin titers and protection from disease is good, although exceptions do occur.[a] Serum neutralization titers greater than 1:10 for canine distemper and hemagglutination inhibition titers greater

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[a]Schultz RD: Personal communication, School of Veterinary Medicine, University of Wisconsin, Madison, WI, 1996.

Guidelines for Proper Handling of Vaccines

- All vaccines should be maintained at the recommended temperature from the time they leave the manufacturer until they are administered to the animal.
- All vaccines should be protected against exposure to ultraviolet light.
- The diluent provided by the manufacturer has been pH-adjusted for a particular organism. Therefore, practitioners should never use a diluent intended for a different vaccine to rehydrate a lyophilized product— even a diluent from the same manufacturer.
- Because different vaccines require different pH values and may have various activating agents or adjuvants that can affect the pH, practitioners should never mix vaccine products unless it is recommended by the manufacturer.
- Attenuated vaccines should be used soon after they have been reconstituted. Therefore, practitioners should ensure that the vaccines are not stored for use at a later date.
- Chemically sterilized syringes should not be used because traces of disinfectant can inactivate attenuated vaccines.
- Before administering any vaccine, practitioners should properly restrain the animal and ensure that the injection site has been cleansed.

HANDLING OF VACCINES

Proper handling of vaccines is essential for the development of immunity (see Guidelines for Proper Handling of Vaccines). Attenuated vaccines are particularly sensitive to improper storage; but exposing either attenuated or inactivated vaccines to freezing temperatures, excessive heat, or ultraviolet light also may result in reduced immunogenicity.[3] All vaccines should be stored according to manufacturer recommendations.

Lyophilized products should be used immediately after reconstitution and should not be exposed to ultraviolet light or temperature extremes. Different vaccines (even from the same manufacturer) should not be mixed in the same syringe unless directed by manufacturer instructions. Syringes used to deliver attenuated products should not be chemically sterilized because traces of disinfectant can inactivate infective agents.

than 1:100 for parvovirus are indications that revaccination is unwarranted.[a] These titers may not always be able to protect against pathogen exposure; but they can result in vaccine clearance, making revaccination ineffective.[a] Immunization with attenuated feline panleukopenia vaccine results in lifelong protection. Immunoglobulin titers for panleukopenia as well as feline calicivirus have good correlation with protection.[a] The correlation between immunoglobulin titer and protection for feline rhinotracheitis is not as strong.[a]

Potential Vaccine Developments

Immunoglobulin A is produced in lymphoid tissue located on mucosal surfaces (i.e., Peyer's patches and related structures) and prevents antigen from binding to surfaces.[2] There is evidence for migration of reactive lymphocytes between various mucosal sites, including the gut, respiratory tract, urogenital tract, and mammary gland.[3] In the future, oral vaccination to stimulate mucosal immunity through IgA secretion may be possible by using microsphere polymers that can carry antigens through the stomach and into the intestine, where they are absorbed by Peyer's patches.[24] These developments could lead to oral vaccines that stimulate mucosal and humoral immunity on any mucosal surface.

IMMUNE RESPONSE FAILURE

Even when a vaccine is handled properly and induces an immune response, that immune response can fail to protect the animal. Such failure can occur when:[25–27]

- The animal produces a humoral response to the vaccination, but a cell-mediated response is necessary for protection.
- The antigen for which the animal builds an immune response is not important for control of the disease.
- The infectious agent the animal is exposed to differs from that the animal was immunized against.
- The protection induced by the vaccine wanes after a period of time so that a low to moderate exposure to the pathogen can overcome the animal's defense mechanisms.
- An immune response is overwhelmed by extreme exposure to a particular pathogen.

OBSTACLES TO PLANNING A VACCINATION PROGRAM

To be certain that a relevant immune response is stimulated, the veterinarian must know which specific antigens confer protective immunity and what aspects of the immune response are responsible for protection against a given disease. Such information is

not readily available for many diseases. Lack of information is a major impediment to veterinarians who are trying to develop scientifically sound immunization programs.

SUMMARY

Vaccines against organisms that cause serious animal disease are available. To be beneficial, however, the products must be used after the veterinarian has gained a good understanding of the principles of immunology and has combined such understanding with sound management practices that control risk. Vaccines are available for only a few diseases. Furthermore, even the available vaccines cannot provide optimum immunity in the presence of nutritional deficiencies, immunosuppression, concurrent disease, or management shortfalls.

Because of the limitations, vaccination programs should be considered part of a sound wellness program rather than the cornerstone of veterinary care. By understanding the strengths and limitations of the various types of vaccines and by learning the types of protection needed to defend the host from specific pathogens, veterinarians can determine how to incorporate the available products into a program that can reduce the risk of infectious disease.

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