Vaccines and Principles of Immunization

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Antiviral compounds for preventing virus entry into susceptible cells are experimental for the most part; therefore, viral diseases cannot be routinely controlled through chemotherapy. Vaccination must be utilized to prevent clinical disease or to lessen the effects of viral infection.

Immunoprophylaxis employs viral antigens to stimulate specific protective immune responses. Whereas the first veterinary viral vaccines were crude tissue extracts harvested from infected animals, the trend has been toward the manufacture of modified live virus vaccines comprised of highly specific inactivated antigens in a concentrated mass. The goals are to confer maximal specific immunity and to elicit minimal or no deleterious reactions in the vaccinate.

Factors to consider in viral vaccine production are (1) the portal of entry and the target organs of viral replication (exposed mucosae or deeper tissues); (2) the desired immune response (local IgA and cell-mediated immunity [CMI], systemic humoral/CMI); (3) the specific antigens required to elicit a protective response; (4) the method of culturing, attenuating, or inactivating the virus or synthesis of viral antigens; (5) the onset and duration of immunity; and (6) the cost of production. Modified live virus vaccines should not be shed by the animal, revert to virulence, or persist in the vaccinate after active immunity is established.

The three most common types of viral vaccines are (1) modified live virus (MLV) or attenuated, (2) killed virus (KV) or inactivated, and (3) subunit vaccines. Table 1 lists canine and feline viral diseases, the types of vaccines available, and the methods of administration.

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Table 1. *Diseases, Types of Vaccines, and Routes of Administration*

DISEASES	VACCINES		ROUTES OF ADMINISTRATION
Rabies	RV	MLV/KV	IM*
Canine distemper	CDV	MLV	SC,IM
	CDV-MV	MLV	IM
Infectious canine hepatitis	CAV-2 (or CAV-1)	MLV/KV	SC,IM
Canine parainfluenza	CPI	MLV	SC,IM
		MLV-IN	IN
	CDV-CAV-2-CPI + *Leptospira* bacterin	MLV	SC,IM
Canine parvovirus enteritis	CPV-2	MLV/KV	SC,IM
Canine coronavirus	CCV	KV	SC,IM
Feline panleukopenia	FPV	MLV/KV	SC,IM
		MLV-IN	IN
Feline rhinotracheitis	FRV	MLV/KV	SC,IM/IN
Feline calicivirus		MLV-IN	IN
	FRV-FCV	MLV/KV	SC,IM/IN
		MLV-IN	IN
	FRV-FCV-FPV	MLV	SC,IM
	FRV-FCV-FPV	MLV + KV	SC,IM
Feline leukemia	FeLV	subunit	IM
Mink virus enteritis (mink)	MEV	KV	IM
Canine distemper (mink and ferrets)	CDV CETCO*	MLV	IM
Canine herpesvirus	CHV	none	
Canine rotavirus	CRV	none	
Pseudorabies	PR	none	
Feline infectious peritonitis	FIP	none	

*Recently, some rabies vaccines have been approved for subcutaneous administration in cats and dogs.
Note: CETCO = chicken embryo tissue culture origin; MLV = modified live virus; KV = inactivated virus; IM = intramuscular; SC = subcutaneous; IN = intranasal.

CLASSES OF VIRAL VACCINES

Modified Live Virus Vaccines

Attenuated virus vaccines are comprised of living virions that are not pathogenic for the vaccinate but retain antigenicity and the ability to replicate in the host. A virus is attenuated by growing it for many generations in the cells of some other host and then selecting the nonvirulent mutants. Attenuated strains are also propagated in cell lines of the natural host. Reversion to virulence by vaccine virus under natural conditions has not been reported. When mutant virions are selected for their ability to replicate in the alternate host, they lose their ability to cause disease in the natural host. The first "attenuated" small animal vaccine was Green's distemperoid.[27,85] This highly virulent field isolate of canine distemper virus (CDV) was passed through ferrets more than 100 times and used as a vaccine. Unfortunately, it still

produced signs of distemper in a number of dogs. Obviously, the ferret was a poor choice as a host for viral attenuation.

The adaptation of canine distemper virus to the chicken embryo led to the first safe and effective MLV vaccine for small animals. When tissue culture techniques became available, many viruses were attenuated by serial passage in cells of the natural host (CDV in dog kidney cells)[15] or in the cells of an alternate host (rabies or CDV in chicken embryo cell culture[35,96] and infectious canine hepatitis [ICH] in pig kidney cell culture).[95]

Attenuation may be expedited by combining a cell culture procedure with the variation of incubation temperature. Temperature-sensitive mutants of feline rhinotracheitis virus (FVR) can be selected by propagation at 32°C.[113] Such mutants replicate in the upper respiratory tract rather than the lungs. In some instances, the pH of the medium can be adjusted to provide immunizing virions.

The dose of vaccine, whether tissue culture or egg infectious units, as in MLV vaccines, or antigenic mass, as in KV vaccines, is determined on the basis of seroconversion and response by vaccinates to challenge with a standard virulent virus. The requirements for each vaccine are designated by the Animal Health Inspection Service of the USDA.

Advantages. Modified live virus vaccines have several advantages over the KV vaccines. The onset and duration of immunity they stimulate is similar to that induced by a natural infection and, therefore, is usually more rapid and durable. Because the vaccine viruses are living, a single small dose is amplified by replication in the host. Viral replication promotes persistent antigenic stimulation of antibody protection and CMI. Modified live virus vaccines may be administered by the natural route of infection, thereby inducing local immunity such as secretory IgA.[3,24,77] They are also more efficient stimulants of both local and systemic CMI. Examples are oral rabies vaccine for foxes,[8,9,116] an intranasal FVR-FCV product for cats,[29] and an aerosol vaccine for mink distemper.[48] Some attenuated vaccines such as canine adenovirus-2 apparently can produce local immunity when administered parenterally.[3]

Because the onset of immunity is rapid with MLV vaccines, they have a distinct advantage in controlling outbreaks where a number of animals are assembled. Examples include distemper on mink farms, parvovirus in kennels, and respiratory viruses in catteries.

Disadvantages. Modified live virus vaccines can cause mild to severe reactions that are discussed below. Modified live viruses, by their replication in the host, cause an infection. The ability of a live virus to mutate allows the possibility of reversion to virulence.

Certain MLV vaccines may lead to persistent infections.[76,107] In addition, some vaccine viruses are shed for a period of time following vaccination. The carrier state could serve as a reservoir of vaccine virus. Prier[104] states that eradication of certain diseases may be difficult due to the use of MLV vaccines.

In general, attenuated vaccines should not be administered to pregnant animals because of their potential teratogenic activity. Virulent and attenuated FPV vaccines have caused cerebellar hypoplasia in late gestation kitten feti and in both neonatal kittens and ferret kits.[33,67] Similarly, administration

of MLV products to immunosuppressed animals may result in vaccine-induced clinical disease.

Even with the rigorous tests for contaminants in vaccine lots, an adventitious organism will occasionally remain undetected. These contaminants are typically other viruses, *Mycoplasma* spp., or other bacteria. When dog kidney cells were used as primary tissue cultures, there were numerous instances in which the cultures were infected with a wild infectious hepatitis virus causing "blue eyes" and occasionally death.

Finally, modified live viruses are susceptible to disinfectants, and the imprudent use of these agents prior to vaccination may inactivate the vaccine. Attenuated vaccines are always lyophilized because they are not stable after reconstitution and must be promptly used.

Inactivated Virus Vaccines

Killed vaccines are comprised of suspensions of viruses, cultivated similarly to live vaccine viruses, that have been treated by chemical or physical means to prevent them from replicating in the vaccinate. Adjuvants are used to increase the retention and activity of the killed antigen virus within the host.

In the production of inactivated vaccines, a high titer of virus is essential. Killed virus vaccines are often prepared by using virulent or wild-type virus as the antigenic material and must be carefully tested. It is imperative to inactivate the viral nucleic acid completely to prevent replication without affecting viral capsid or envelope proteins in order to retain antigenicity. The physical methods of inactivating virus include heat, X-irradiation, gamma irradiation, ultrasonication, and ultraviolet light; however, these procedures are not currently used because they cannot completely destroy infectivity or preserve antigenicity.

Formalin is the most common chemical agent employed to inactivate viruses, although it can denature protein and cause irritation at the vaccination site. Other agents used to inactivate nucleic acids are beta-propiolactone, acetylethyleneimine, ethyleneimine, and ethylene oxide.[107] Perchloroethylene also gives results comparable with or better than those of formalin.[107] Beta-propiolactone (BPL) and ethyleneimine do not cause irritation at the vaccination site or a decrease in immunogenicity, but BPL may be a carcinogen. Killed vaccines must be evaluated quantitatively for their immunogenicity and qualitatively for residual infectivity.[76]

Advantages. The most important advantage of KV vaccines is safety. Killed viruses do not replicate in the host and, therefore, eliminate the reversion to virulence or the inclusion of pathogenic virions in the inoculum. Any contaminants are inactivated with the production virus. Recent studies have shown that adjuvants may potentiate the cell-mediated immune response[84] and enhance immunogenicity by retention of the killed antigen within the host. Killed vaccines are indicated for pregnant animals, colostrum-deprived neonates, or stressed, immunosuppressed, or otherwise debilitated animals.[50]

Disadvantages. The most frequently cited disadvantage of killed vaccines is the type and duration of immune response that they evoke. The protection is generally of shorter duration, and multiple inoculations are

usually required. If the animal is "incompletely" immunized, subsequent exposure to virulent virus may result in a mild or inapparent infection followed by an anamnestic response.[19] Killed vaccines require a large antigenic mass and, with the inevitable inclusion of nonviral material, repeated inoculation may lead to hypersensitivity reactions.[50] Occasionally, allergic responses occur with subsequent infection by virulent virus.

Most workers would agree that neither adequate CMI nor sufficient levels of local secretory IgA are induced consistently by inactivated vaccines if these types of immunity are necessary for protection. The antigenicity of inactivated vaccines may be reduced or altered during the inactivation process, and the inactivating agent may cause pain at the injection site. Serious complications arose when the parvovirus causing Aleutian disease contaminated formalized mink virus enteritis vaccine and mink distemper vaccine.

Killed vaccines are effective only if administered parenterally. Adjuvants are frequently used to prolong antigenic stimulation and they, too, may cause irritation when injected.

Subunit Vaccines

Subunit vaccines differ from MLV or killed vaccines in that they are composed of viral capsid or envelope proteins instead of intact virions.[45,76,78] Multiple subunit vaccines may contain more than one type of specific antigen that will induce protective immunity. The feline leukemia vaccine is a multiple subunit vaccine consisting of a viral envelope protein, gp70, and FOCMA (feline oncornavirus-associated cell membrane antigen), a tumor-specific antigen. The gp70 and other proteins induce formation of antibodies to neutralize FeLV in the blood, and FOCMA induces immunity to FeLV-related neoplasms. Olsen[78] found that FeLV-infected cell cultures grown in serum-free medium synthesize these proteins and release them into the culture fluid. The vaccine is derived from this supernatant cultural fluid.

Other methods used to produce subunit vaccines are recombinant DNA technology,[7,53,54] chemical peptide synthesis,[36,70,75,127] and the splitting of virus into its component parts by sucrose gradient centrifugation.[26] Recombinant DNA techniques involve insertion of the viral DNA that codes for the relevant antigen(s) into plasmids, which are then expressed in bacteria, yeasts, or even other viruses such as vaccinia virus. Immunogenic peptides may be synthesized chemically only after their complete amino acid sequences are determined. Procedural advances are essential before synthetic vaccines become cost-effective.

Advantages. The most obvious advantage of subunit vaccines is their safety. Only the specific immunogenic viral surface proteins are used and, therefore, infection of the host does not occur. Post-vaccinal replication is not a problem. Specificity is a distinct advantage, because there is a minimal quantity of foreign protein present in the vaccine. Moreover, subunit vaccines have been shown to be efficacious in the protection against rabies,[7,26] canine parvovirus enteritis,[7] and feline leukemia.[71] Subunit vaccines may be used safely in pregnant, immunosuppressed, or debilitated animals.

Disadvantages. Presently, the cost of producing subunit vaccines may be prohibitive, although the presence of commercially available feline leukemia vaccine would tend to refute this claim. Current technical difficulties

may prove to be insurmountable in some instances. The efficacy of the FeLV vaccine has recently been questioned.[91] Currently, adjuvants are necessary, and their associated problems must be considered. At this writing, subunit vaccines will behave like KV vaccines with regard to the onset and duration of immunity.

Criteria for the Selection of Vaccines

There are occasions when the selection of one type of vaccine product is clearly indicated. Although MLV vaccines are used in routine immunization regimens, they may be contraindicated in certain circumstances. Colostrum-deprived neonates or pregnant, debilitated, or immunosuppressed animals should be given killed or subunit vaccines if available. Although modified live feline rhinotracheitis virus vaccine has been administered intranasally to pregnant cats without ill effect,[29] this practice is not recommended. If there are several animals in a household, kennel, or cattery that vary with regard to age, immunity, or breeding status, the killed or subunit vaccines may be preferred if the modified live vaccine virus is known to be shed in the feces, urine, or saliva, or if there is a possibility of reversion to virulence. Vaccination against a zoonotic disease such as rabies dictates the use of killed or subunit vaccines. Certain MLV vaccines may cause mild disease (intranasal FRV), and some killed vaccines may produce swelling at the injection site.

The type of immune response required to confer protection varies with the disease. Killed vaccines induce low levels of local immunity. On the other hand, attenuated vaccines elicit powerful local and systemic humoral and cell-mediated immunity. Mucosal immunity stimulated by some MLV vaccines resembles that achieved through natural infection. Properly prepared inactivated and subunit vaccines are adequate stimulants of systemic humoral immunity and perhaps low level cell-mediated immunity.

ADJUVANTS

Adjuvants are used to increase the potency and efficacy of KV vaccines toward those of MLV vaccines.[107] They allow for aggregation of antigens, and by making the emulsion insoluble in tissues, require a smaller antigenic mass.[84] The antigens remain in tissues longer, thereby prolonging the stimulus for an immune response.[84,107] Adjuvants enhance macrophage response to the antigen by facilitating presentation of the antigen to B lymphocytes and helper T lymphocytes.[1,60,107,122]

Freund's complete or incomplete adjuvants (water-in-mineral oil substances) have been employed but are no longer used, because they produce granulomatous reactions and they are potential carcinogens. In the case of complete Freund's, it causes the development of cutaneous hypersensitivity to tuberculin. The newer mineral gels, such as aluminum hydroxide, aluminum phosphate, and alum, are commonly used in veterinary vaccines.[78,107,118,131]

Other adjuvants discussed by Osebold[84] are muramyl dipeptide (MDP), a synthetic glycopeptide; polyadenylic-polyuridylic acid complexes

(PolyA:U), a double-stranded homoribopolymer; lipopolysaccharide from *Escherichia coli* (LPS), *Bordetella pertussis, and Corynebacterium parvum;* saponin, a glycoside of plant origin; colchicine; bestatin, an enzyme inhibitor; liposomes; or combinations of the above. Occasionally, adjuvants cause irritation at the injection site and are associated with abscess formation.[107]

ROUTES OF VACCINE ADMINISTRATION AND THEIR EFFICACY

The goal of vaccination is to produce complete immunity against viral diseases with minimum complications or adverse reactions. According to the premise that natural infection induces the most prolonged and complete protection, it is logical to assume that the administration of MLV vaccines via their route of entry (aerosol exposure of mink and ferrets to CDV)[48] would be more efficacious in promoting local humoral and cell-mediated immunity at mucosal epithelium than would parenteral administration. Systemic immunity is more important against viruses such as CDV that spread to target tissues via the hemo-lymphatic systems.[60] Parenteral vaccination is efficacious for canine distemper. Mucosal immunity has no advantage over systemic immunity unless it prevents subsequent infection, as in the case of the intranasally administered CPI vaccine.[3,24] Local cell-mediated immunity and IgA are important in preventing those diseases in which reinfection may occur (FRV).[110]

Rabies

Modified live rabies virus vaccine, given intramuscularly (IM), is 100 times more effective than by subcutaneous (SC) administration,[111,120] although recent vaccines are purported to be as efficacious when given subcutaneously. Much research has been performed with oral vaccination of foxes with MLV rabies vaccines.[8,9,13,32,133] Virus-neutralizing (VN) antibodies in serum were recorded, and the foxes resisted challenge exposure. Oral vaccination with a KV vaccine did not induce immunity.[13] Introduction of a MLV vaccine with a stomach tube bypassing the oral mucosa also failed to immunize foxes.[9,13] Black and Lawson[13] and Baer and colleagues[9] concluded that the virus replicated in the oral mucosa and was inactivated by gastric acids. These results were used by Steck and others[117] in arresting two different rabies epizootics in wild fox reservoirs in the Rhone Valley in Switzerland. This was accomplished by spreading chicken heads impregnated with MLV vaccine over a large area.

Canine Distemper

Although distemper is manifested clinically as a respiratory infection, and the virus later localizes in the brain, CDV replicates initially in lymphoid tissue. Intramuscular, subcutaneous, and intranasal inoculation of modified live CDV vaccine induces protective immunity.[110,112] The related measles virus, however, is more effective if administered IM rather than SC.[50,110] Gorham and colleagues[48] successfully immunized mink and ferrets by aerosol exposure to a modified live CDV vaccine. This procedure is now used worldwide.

Canine Parainfluenza (CPI) and Canine Adenovirus Type-2 (CAV-2)

Both parenteral and intranasal (IN) MLV vaccines have been tested for efficacy against CPI and CAV-2 with differing results.[3,24] Parenteral modified live CPI vaccines induce systemic immune responses but not local immunity. Disease is prevented, but subsequent infection of the respiratory mucosa by virulent virus may still occur. The IN modified live CPI vaccine stimulates local immunity, which prevents infection.[3,24] The IN vaccine has also been successful in immunizing 3-week-old puppies possessing maternal antibodies.[3]

The parenteral adminstration of modified live CAV-2 induces both local and systemic immunity, thereby preventing both infection and disease upon challenge.[3] The IN modified live CAV-2 vaccine stimulates local immunity and may also be used to protect puppies with circulating maternal CAV-2 antibodies.

Canine Coronavirus (CCV)

Oronasal vaccination with a modified live CCV vaccine protected dogs against challenge. Dogs vaccinated subcutaneously produced low titers of VN antibodies but mounted an anamnestic response after oral challenge.[6,19] The modified live CCV vaccine was replaced by a commercially available killed CCV vaccine discussed below.

Feline Viral Rhinotracheitis (FVR) and Feline Calicivirus (FCV)

The modified live FVR-FCV vaccines may be administered parenterally or intranasally.[61,62] Parenterally administered vaccines induce local and systemic humoral immunity as well as cell-mediated immunity[60,124] and are preferred over the IN vaccines because they do not evoke sneezing and other postvaccinal signs. The IM route is slightly more efficacious than the SC route.[50,111] The IN FVR-FCV vaccine is preferred for controlling epizootics because cats are protected against challenge within 24 to 48 hours after vaccination.[30]

Feline Panleukopenia (FPV)

Feline panleukopenia virus first replicates in lymphoid tissue prior to spread to the gastrointestinal tract; therefore, parenteral vaccination is protective. Both IM and SC routes are effective.[110] Intranasal vaccination has some effect, but oral vaccination alone is ineffective.[109]

COMBINATION VACCINES

Vaccines comprised of several live and/or killed viral (and bacterial) agents are used routinely in veterinary medicine. The small dose of modified live vaccine virus necessary to stimulate immunity allows the combination of several MLVs into one product without appreciably increasing the volume of inoculum. Polyvalent vaccines are convenient in that they are easy to handle and save time. The two main components of combination vaccines are typically (1) lyophilized MLV with (2) KV such as CPV or FPV and

bacterins such as *Leptospira canicola* and *L. icterohemorrhagiae* in a liquid diluent.

In addition to the usual stability, potency, and safety testing, polyvalent vaccines are evaluated for freedom from interference or synergism between the individual agents, so that their efficacy equals that of separately administered monovalent vaccines.

Common feline vaccines are FVR-FCV and FVR-FCV-FPV combinations. *Chlamydia*, pneumonitis, may also be included. Typical canine vaccinations are CDV-CAV-2 (or CDV-MV and CDV-CAV-2-MV combinations for use in puppies with maternal CDV antibodies) or CDV-CAV-2-CPI combinations. *Leptospira* bacterins and CPV-2 are often added to polyvalent vaccines.

The same difficulties encountered with the monovalent forms of the vaccines will occur with polyvalent products. Synergism and interference, however, should not be problems in vaccines that have been adequately evaluated and administered to clinically healthy animals. One should never combine biologicals that are not specifically designated by the manufacturer as compatible, because diluents containing killed agents may inactivate the MLV components.

HETEROLOGOUS VIRAL VACCINES

Jenner noticed that cowpox infection in humans could protect against smallpox. He was the first to apply an antigenically related, moderately virulent virus as a vaccine to protect the host against a highly virulent pathogen. There are several examples in veterinary medicine of this cross protection.

Feline Panleukopenia Virus Used to Immunize Against Canine Parvovirus-2

Before the availability of CPV-2 vaccines, killed FPV vaccines were used to protect dogs against canine parvovirus. It was determined that 1000 times more killed FPV was necessary to protect dogs than was required to protect cats.[99] The killed FPV vaccine was safe, but the resultant immunity was short-lived.[120] The killed CPV-2 and modified live FPV were more effective in preventing CPV infections.[57,120]

There are no recorded cases of post-vaccinal disease in dogs administered modified live FPV vaccine.[19,46] Carmichael and Olin[19] reported that killed FPV and killed CPV-2 are equally efficacious in immunizing dogs for 6 to 7 months if the antigenic mass is similar.[6,98,101,130,132] The modified live homotypic CPV-2 vaccine may be preferred over inactivated CPV-2 vaccines because the MLV produces a more rapid onset of immunity.[57,105,120] Inactivated CPV-2 vaccines should be used for the immunization of pregnant bitches.[57] The close antigenic relationship of CPV-2 and FPV precludes the administration of FPV vaccines to young puppies, because the FPV vaccine virus is rapidly neutralized by maternal antibodies.[19]

Measles Virus (MV) Used to Protect Against Canine Distemper

Measles virus vaccines may temporarily protect puppies with maternal CDV antibodies or puppies of unknown immune status against canine dis-

temper after the maternal CDV antibodies decline and until they can be vaccinated with modified live CDV.[5,19,54,107,110,120] The measles vaccine prevents disease, but not infection with CDV, when administered to 6- to 8-week-old puppies. The measles vaccine is not efficacious in pups if maternal CDV antibodies are extremely high.[19,109,110] Pups with low maternal CDV antibodies may be immunized successfully against CDV with combination MV-CDV vaccines.[110] Vaccination with modified live MV is usually contraindicated in puppies selected as breeding bitches when they are 12 weeks of age and older, because antibodies secreted later in the bitch's colostrum may preclude the use of measles vaccine to protect her puppies as described above.[40,110]

Canine Adenovirus-2 Used to Protect Against Infectious Canine Hepatitis (CAV-1)

Vaccination of dogs with modified live CAV-2 has been shown by Appel and colleagues[4] and others[10,109] to provide protection against challenge with highly virulent infectious canine hepatitis. This vaccine virus is recommended because it does not evoke the renal or ocular lesions discussed below that may occur with CAV-1 vaccination.[4,10] Moreover, CAV-2 is not shed from vaccinates, in that contact dogs are not immunized.[46] Bass and others[10] demonstrated tht CAV-2 retained genetic stability through five serial back passages by IN administration. Parenteral administration of modified live CAV-2 does not elicit respiratory signs.[40] The CAV-2 vaccinates develop VN antibodies against both CAV-2 and CAV-1.[10] It has been suggested that CAV-2 induces both systemic (humoral and CMI) and local immunity, whereas CAV-1 only stimulates systemic immunity.[46]

VACCINATION FAILURE DUE TO MISHANDLING

In addition to host factors, vaccines may also fail to immunize if they are improperly maintained or administered. Greene[50] cites mistreatments that may result in failure of vaccines to immunize hosts. Improper storage (excessive heat or freezing)[58] may inactivate MLV vaccines. Both MLV and KV vaccines should be refrigerated at 5°C. and, in the case of freeze-dried products, their vacuum should be preserved.[58] Improper product mixing, delay in use after reconstitution of MLV vaccines, or use of a product after the expiration date[58] frequently results in vaccination failures. Reused syringes should be autoclaved, not treated with disinfectants or detergents.[58] Disposable plastic syringes are preferred. The most appropriate vaccine strain, the correct vaccination schedule, and the recommended effective dose (no dose-splitting)[58] should be used. Concurrent administration of antisera is always contraindicated.

ADVERSE REACTIONS TO VACCINATION

Immunologic Reactions

There are recorded cases of deleterious immune responses to vaccination. Type I immediate hypersensitivity may be caused by stimulation

with nonviral antigens, such as culture-medium ingredients or cellular antigens. Serum may be added during the maturation and release of virus[55] but is usually present in negligible amounts. Walton[128] reported penicillin sensitivity in a cat that had intermittent skin lesions and a long history of drinking discarded milk containing penicillin. The penicillin-streptomycin combination may be used during cell culture-based vaccine production.[55] Stabilizers containing milk products and deactivators such as formaldehyde that are added during the final processing could conceivably induce immediate hypersensitivity reactions. The amount of cellular antigens in a vaccine varies with the type of virus cultivated, though culture cells lose their surface antigenic specificity rapidly in vitro. Children known to be allergic to egg protein were given chicken embryo fibroblast-based vaccines without ill effects,[63,64] although this practice is not recommended. Dogs and cats might be subject to the same type of protein hypersensitivity.

The transient thrombocytopenia reported following MLV canine distemper vaccination is thought to be the result of a Type II cytotoxic autoimmune phenomenon. There is a decrease in the number of circulating platelets several days following vaccination.[59,74,97,119] Straw[119] reported a mild thrombocytopenia, never below 100,000 platelets/mm^3, whereas McAnulty and Rudd[74] reported that it could be more severe, as low as 10,000/mm^3. Minimal[74] or no[59,119] external signs of bleeding were observed in otherwise normal dogs. Jones[59] noted no significant changes in platelet aggregability. The possibility of a hemorrhagic diathesis, however, must be considered in dogs with coagulation factor deficits.[74] The thrombocytopenia may persist for 3 weeks but usually lasts only a few days before the platelet count increases.[59,74,119] The condition is ameliorated by the administration of levamisole HCl.[97] The mechanism by which levamisole works is unknown.

Vaccination with canine adenovirus (CAV-1) can lead to renal and ocular lesions[4,12,16,17,20,109,135] characterized as Type III immune-complex hypersensitivity.[17] The anterior uveitis and corneal opacity observed clinically 1 to 3 weeks after vaccination are similar to that seen in dogs convalescing from naturally occurring ICH infection. The sight hounds, such as the Greyhound and Afghan Hound, seem especially susceptible.[114] The pathogenesis of the lesions was described by Carmichael.[16,17] He injected immune serum intraocularly in dogs given attenuated CAV-1 vaccine. The virus-antibody-complement complexes attract macrophages that release enzymes and damage corneal endothelium, resulting in corneal edema and keratitis ("blue eye"). Although the tendency to induce ocular lesions may vary among CAV-1 strains, attenuated CAV-2 viruses do not produce similar lesions.[10,109] To demonstrate the safety of modified live CAV-2 for use as an alternate vaccine for ICH immunization, Curtis and Barnett[28] inoculated dogs intravenously with large doses (greater than 10^8 TCID$_{50}$) of either modified live CAV-1 or modified live CAV-2. Four of 10 dogs inoculated with modified live CAV-1 had gross ocular lesions, whereas dogs inoculated with CAV-2 had neither gross nor histopathologic ocular changes. Although the ocular lesions are usually transient, they may result in permanent damage or loss of vision. Other adverse reactions to the use of modified live CAV-1 vaccines are glomerulonephritis and prolonged shedding of vaccine virus in the urine.[4,12,20,135]

Early rabies vaccines made from nervous tissue of adult mammals led to Type IV (delayed) cell-mediated autoimmunity against the vaccinate's own brain tissues. Postvaccinal encephalomyelitis is especially severe in the cat.[47] More recently, vaccines have been produced from tissues of immature animals (suckling mouse brain, hamster embryo, and duck embryo), which reduced the incidence of aberrant antigen-induced encephalomyelitis. Other rabies vaccines and their adverse reactions will be discussed below. For additional information, the reader is directed to Greene's[50] excellent discussion of the immune-mediated complications of vaccination.

Nonimmunologic Post-vaccinal Problems

Local tissue injury following parenteral vaccination may result in inflammation or abscessation, especially with adjuvanted products.

Rabies.   Approved attenuated rabies vaccine viruses have induced disease in cats,[11,37,38] dogs (LEP Flury chicken embryo origin),[93] and pet skunks (HEP Flury canine origin vaccine).[23,31] When the SAD[37] and LEP Flury[123] strains produced rabies in cats, they were no longer approved for use in this species. More recently, the ERA[38] and HEP Flury[11] strains have been implicated in paralytic rabies in cats. Paralysis typical of lower motor neuron involvement begins about 2 weeks after vaccination in the hindlimbs. The paralysis usually involves the injected hindlimb initially and spreads craniad to the forelimbs and cranial nerves (as manifested by unresponsive dilated pupils). Occasionally, a cat was found to be FeLV-positive by the ELISA test.[11,38] The nature of the presenting signs and their progression are well described by Esh and colleagues[38] and Bellinger and others.[11] Fluorescent antibody of brain tissue and/or mouse inoculation tests confirmed rabies infection. Vaccine strains were identified by the use of specific fluorescent[38] or monoclonal[11] antibodies.

Because of these lethal reactions to MLV rabies vaccines, several authors[11,22,38,44] recommend the use of KV vaccines in dogs and cats. Killed virus vaccines are safer than and are administered according to the same vaccination schedules as the MLV rabies vaccines. The risk of the potential public health danger is difficult to assess. The Flury (LEP) strain of vaccine virus was isolated from feline salivary glands.[123] This vaccine is not approved for cats.

Canine Distemper.   Post-vaccinal canine distemper encephalitis has been reported under varying circumstances.[2,68,109] It has been observed in very young or immunosuppressed dogs[109] and in dogs with CPV-2 infections.[68] Reversion to virulence by the canine distemper vaccine virus has been reported by Goto and colleagues[49] and by Appel.[2] At this writing, the question of whether modified live CDV vaccines can immunosuppress and interfere with the successful immunization of modified live CPV-2 (or the converse) has not been resolved.

Canine parvovirus infection was enhanced by the administration of a modified live CDV-CAV-1 vaccine several days before experimental infection with CPV-2.[100] On the other hand, distemper encephalitis was demonstrated in gnotobiotic puppies vaccinated with a CDV-CAV-2-CPI-*Leptospira* bacterin product several days before oronasal inoculation with virulent CPV-2.[68] A modified live CCV vaccine was available commercially in 1983 for a

short period of time. When administered with a monovalent modified live CPV vaccine, no complications arose. However, when administered simultaneously with a modified live CDV, an encephalitis ensued. At this time, there is no published information demonstrating whether the encephalitis was caused by CCV alone or induced by immunosuppression by CDV with subsequent CCV encephalitis. The killed CCV vaccine does not replicate within the host, but Evermann[39] recommends conservative use of the new product until its efficacy is determined through field use. He suggests vaccination of high-risk dogs in research facilities, kennels, and shelters as well as vaccination of pregnant bitches to promote colostral CCV antibody production to protect puppies. Swango[120] claims that the highly attenuated chicken embryo tissue culture origin (CETCO) CDV vaccine is safer than and as efficacious as CDV vaccines of canine origin.

Canine Parvovirus-2. Although modified live CPV-2 has not been shown to cross the placental barrier, it is contraindicated in pregnant bitches.[57] Puppies vaccinated at less than 4 to 5 weeks old with modified live CPV-2 may develop myocarditis.[52,56,66,106,121] The modified live CPV-2 vaccine is contraindicated in immunosuppressed dogs. Greene[50] offers guidelines for immunizing young puppies and preventing their exposure to virulent CPV-2. Wallace and McMillen[125] and Wallace and colleagues[126] demonstrated that the new killed CPV-2 vaccine of high antigenic mass can effectively immunize dogs and puppies for 1 year if given two initial doses 21 days apart. Wallace and colleagues[126] successfully combined the killed CPV-2 with *Leptospira canicola* and *L. icterohaemorrhagiae* bacterin and used them in a diluent to reconstitute a lyophilized CDV-CAV-2-CPI vaccine. They recommend use of the killed CPV-2 product in order to avoid viral replication in the intestine and shedding of virus in the feces.

Canine Parainfluenza. An attenuated CPI virus is incorporated in most combined parenteral canine vaccines. Although it is more efficacious if administered intranasally, it may cause mild signs in the upper respiratory tract.

Feline Panleukopenia. The most common complication of vaccination with modified live FPV is the well-known effect of cerebellar hypoplasia.[33,67] This condition occurs in fetuses when modified live FPV is administered to pregnant queens and in kittens vaccinated before 5 weeks of age.[40] Killed FPV vaccine is recommended for pregnant queens and immunosuppressed or diseased cats. It is usually efficacious without the use of adjuvants. In addition to this, Povey[102] recommends routine vaccination with killed FPV in order to prevent shedding of vaccine virus.

Feline Viral Rhinotracheitis and Feline Calicivirus. The modified live FVR-FCV vaccines may cause problems whether they are administered parenterally or intranasally. Problems seen with IN products are transient sneezing with serous nasal and ocular discharge 4 to 9 days after vaccination.[25,111] Occasionally the tongue becomes ulcerated.[25,111] Vaccine virus is shed up to 13 days (FVR)[83] or from 3 to 83 days (FCV).[83] The advantages of the modified live IN FVR-FCV vaccine may outweigh the disadvantages. There is a rapid onset of immunity, within 24 to 48 hours after vaccination.[30] The IN vaccine provides local and systemic immunity, and it is safe in pregnant queens.[29] There is a rapid anamnestic response observed with

virulent challenge,[110] and the IN vaccine may protect against persistent infections.[83]

Complications have been reported with the use of parenteral (modified live or killed) FVR-FCV vaccines.[42] Disadvantages include the delay of onset of protection,[86] and the mild disease experienced by vaccinates after virulent virus challenge.[34,86] There is a possibility that parenteral vaccines promote the asymptomatic carrier state.[42,43,82,83] Accidental oronasal exposure at the time of vaccination may result in signs of respiratory infection.[103] Modified live viruses do not induce disease if administered parenterally. Although KV FVR-FCV vaccines are safe for use in pregnant queens and colostrum-deprived kittens less than 4 weeks of age,[50] they are not efficacious if administered intranasally.[86]

Feline Leukemia.   The first modified live and killed FeLV vaccines[79,80] not only failed to immunize cats, but the KV vaccinates became more susceptible to FeLV-induced disease. When challenged with virulent virus, more KV vaccinates developed viremia and malignant neoplasms and had a poorer FOCMA antibody response than the nonvaccinates.[78] Mathes and colleagues[73] found that the FeLV virion contains a protein, FeLVp15E, that depresses lymphocyte blastogenesis and other functions of the feline immune system. Additional research determined methods for producing the envelope glycoprotein, FeLVgp70,[71,81] and FOCMA[69,71,81,134] for protection against viremia and FeLV-associated neoplasias, respectively, without incorporating the immunosuppressive FeLVp15E protein. The long-term efficacy of the commercial FeLV subunit vaccine will be determined through further use. Its efficacy was initially tested by Olsen and colleagues in 1979[79] and 1980,[81] and recently by Pedersen and colleagues[91] in 1985.

VACCINATION OF EXOTIC SPECIES

Rabies vaccines are not licensed in the United States for use in wild or exotic animals. There is evidence that MLV rabies vaccines have induced disease in pet skunks.[23,31]

Canids

Foxes are vaccinated against distemper and ICH ("fox encephalitis"). There is a licensed killed ICH product for farm-raised blue and silver foxes. Attenuated distemper vaccines may be used, but only modified live CDV of chicken embryo tissue culture (CETC) origin should be used in gray foxes as they are highly susceptible (especially the "samson" foxes, having an absence of guard hairs) to vaccine-induced distemper.[51]

Felids

Wild cats should be vaccinated with killed FPV vaccine.[115] Oral vaccination of wild cats has been performed in some zoological parks but has been shown to be ineffective.[109,110]

Mustelids

Skunks should be vaccinated for distemper with only the modified live CDV of CETC origin and vaccinated against feline panleukopenia. Infectious canine hepatitis has been documented in skunks.[41]

Mink require vaccination against mink enteritis virus and canine distemper. A killed MEV product and an attenuated CDV of CETC origin are used.

Ferrets must be protected against distemper by vaccination with modified live CDV of CETC origin.[108] The first dose of vaccine should be administered at 6 to 8 weeks of age (4 to 6 weeks in kits from unvaccinated dams) and a second injection given 2 weeks later. Booster injections should be given every 3 years thereafter. Killed CDV vaccine provides only a short-term immunity that is slow to develop.[87] Distemper vaccines prepared from ferret cell culture should never be used in ferrets, because the vaccine virus will cause distemper.

Ferrets are not susceptible to feline panleukopenia, mink enteritis virus, canine infectious hepatitis, feline rhinotracheitis, or feline calicivirus. There is no definitive evidence that ferrets are susceptible to disease produced by canine parvovirus; therefore, vaccination is not warranted. Because clinical trials have never been conducted in ferrets on the safety or efficacy of the commonly used four- and five-way canine vaccines, the use of these products cannot be recommended. Only killed CDV vaccine should be administered to black-footed ferrets, *Mustela nigripes*, because modified live CDV of CETC origin may induce distemper.[21] The killed CDV vaccine should be administered at 6-month intervals for the life of this species of ferret.

Procyonids

Raccoons, lesser pandas, and kinkajous are susceptible to both panleukopenia and canine distemper. Raccoons should be vaccinated with chick embryo-passaged modified live CDV vaccine and killed FPV or killed CPV-2 to protect them against FPV or raccoon parvovirus strains. Care should be exercised in an immunization program, as modified live canine cell origin CDV-induced distemper has been reported in the lesser panda[14] and the kinkajou.[65] Fowler[41] discusses vaccination in wild species.

VACCINES FOR FUTURE CONSIDERATION

It will be difficult to improve substantially some of the current small animal vaccines. As more effective adjuvants come into use and techniques for purifying virus and antigen are refined, the use of killed, subunit, and polyvalent KV vaccines will probably escalate. Some diseases for which vaccines could have potential benefit will be discussed in the following section.

Pseudorabies

Pseudorabies is a rare disease, and there is currently no vaccine available for companion animals. The MLV swine biologics are lethal when administered to dogs, and inactivated vaccines are not protective.[94]

Canine Rotavirus

Canine rotavirus has been isolated from feces of dogs experiencing mild to fatal enteritis, but the disease cannot be reproduced experimentally and, therefore, the clinical significance is not established.

Canine Herpesvirus

Some authors feel that there is no need for a vaccine for canine herpesvirus because of the sporadic occurrences of the disease.[40,50] Although adult dogs exposed to virulent CHV become protected during the inapparent infection, neonates frequently succumb. Immune bitches secrete antibodies in their colostrum that protect their puppies. Perhaps it would be prudent to immunize breeding bitches routinely prior to conception. Carmichael and Medic[18] isolated a modified live CHV that has potential as a vaccine virus.

Feline Infectious Peritonitis

Research conducted for the development of a FIP vaccine has been frustrating. Pedersen and Black[88] demonstrated the formation of virus-neutralizing and indirect fluorescent antibodies in cats oronasally exposed to an avirulent strain of FIP. Gaskell[43] and Pedersen and Black[88] observed that the presence of antibody exacerbated the disease upon challenge with virulent FIP and that serum from cats that resisted FIP failed to immunize susceptible cats.[88] They felt that the resultant immunity may be cell-mediated. Some protection was achieved by the administration of sublethal amounts of virulent virus, but the results were unpredictable.[88] In this and other work, cats possessing antibodies against a cross-reacting non-FIP-coronavirus experienced a more severe disease than cats without antibody.[81,89,90,92] Effusive FIP appears to be the result of an Arthus-type response.[89,129] Many questions need to be answered before practical immunoprophylaxis is achieved.

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