# Adverse Vaccinal Events in Dogs and Cats

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**KEYWORDS**

• Vaccinal events • Causality • Reactions
• Cytokines • Immunogenicity

Vaccines are the most successful application of immunologic principles to animal and human health, dramatically reducing the mortality and morbidity of infectious diseases. This disease reduction has also decreased public awareness of infectious disease risk and, perhaps paradoxically, shifted current public focus to the safety of vaccines. The immunologic stimulation from vaccines that provides protection sometimes produces undesired side effects, decreasing public confidence in and compliance with vaccination recommendations.

Undesired biologic events can occur for a myriad of reasons, and cause and effect may be difficult to determine in events following vaccination. Bradford-Hill in 1965 proposed a set of criteria as supporting evidence that an association is cause and effect.[1] Of these criteria, temporality (the cause precedes the effect) and/or biologic plausibility often provide strong support for cause and effect, particularly when the adverse events occur within a few minutes or a few hours after vaccination. Because of the uncommon or rare occurrence of some adverse events, however, causal support may be quite weak for other important criteria such as strength (large relative risk), consistency (repeatedly observed), or specificity (one cause leads to one effect). In general, the association of vaccination with development of disease is based upon a close temporal relationship *and* additional supportive epidemiological evidence. Defining an association as causal is further complicated by the occurrence of similar immune-mediated diseases in unvaccinated individuals or individuals without a history of recent vaccination.

Assessment of suspected adverse events is markedly hindered by current reporting systems. Reporting is voluntary; veterinarians or owners may contact either the manufacturer or the United States Department of Agriculture (USDA) Center for Veterinary Biologics (CVB), which has regulatory overwatch of animal vaccines: http://www.aphis.usda.gov/animal_health/vet_biologics/. Although servicing the total population, spontaneous systems are disadvantaged in that underreporting is

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common and denominator data are lacking.[2–4] Reports are not screened out, and reporting rates may be influenced by external pressures, for example, the media. Although more vaccines are used overall in large animals than in small, most adverse events reported to CVB are in dogs and cats.[4] To improve vaccine safety studies, other large population databases can be useful in providing selected denominator data and determining background incidence rates.[5,6] Some adverse vaccinal effects are more commonly seen in certain breeds of dogs as discussed in this article, suggesting a genetic predisposition for these effects. This idea is supported by recent studies in human populations immunized against smallpox in which adverse reactions were associated with several gene variants.[7,8]

Adverse vaccinal events are generally uncommon because of good manufacturing practices and procedures used by the biologics industry. Inadvertent pathogen/pyrogen contamination of a vaccine or failure to sufficiently inactivate a live pathogen used for a vaccine can clearly produce an undesired, even lethal, effect. This article focuses on undesirable immune responses from vaccination of presumably healthy pets but does not discuss clinical manifestations of diseases for which the vaccine should have provided protection, for example, vaccine-induced distemper or rabies. Disease initiation by modified live virus or inadequately attenuated biologicals may occur in almost any animal that is sufficiently immunocompromised.

## INNATE IMMUNE RESPONSES TO VACCINES

Vaccines induce both innate and adaptive immune responses, with the latter providing protection from natural disease exposure by immunologic memory. The innate response provides a rapid and necessary, but nonspecific, first line of defense while providing stimulation of the immune system for subsequent development of specific adaptive immune responses. The quality and quantity of immune memory is largely determined by the magnitude and complexity of innate immune signals that imprint the acquired immune response.[9,10]

The innate immune response can be triggered by tissue damage, that is, tissue disruption caused by injection of a vaccine, and by pathogen-associated molecular patterns (PAMPs), which are conserved molecular patterns produced by pathogens but not by the host organism.[11] PAMPs are detected in the host by different pattern-recognition receptors (PRRs), such as toll-like receptors (TLRs), which are expressed on a wide variety of immune cells, for example, neutrophils, macrophages, dendritic cells, natural killer (NK) cells, and B cells, as well as some nonimmune cells such as epithelial and endothelial cells.[12] Engagement of PRRs leads to the activation and secretion of cytokines and chemokines, in addition to the maturation and migration of antigen-presenting cells. In tandem, this creates an inflammatory environment that leads to the establishment of the adaptive immune response.[13,14]

Although an adaptive immune response is required for the primary (label) vaccine antigen (and is the goal of vaccination), other vaccine components serve as immune potentiators to stimulate the innate immune system. These components can include bacterial products, toxins, lipids, nucleic acids, peptidoglycans, peptides, carbohydrates, hormones, or other small molecules. Some components, commonly termed adjuvants, are purposefully added to vaccine formulations to enhance immunogenicity, but many components serve a similar role in vivo. Vaccine delivery systems, such as liposomes, emulsions, and microparticles, can also improve the adaptive response by concentrating and colocalizing antigens and immune potentiators.[13]

The cytokines and chemokines released by cells after activation of PRRs are mediators of inflammation, and include tumor necrosis factor $\alpha$ (TNF-$\alpha$), interleukins (ILs),

histamine, serotonin, complement, and leukotrienes. Different amounts of each mediator can be evoked from ligands triggering different PRRs, creating different cytokine ''profiles''. Cytokine profiles differ not only with the triggering mechanism but likely also between and within host species. Thus, the severity and type of localized inflammatory reactions to vaccines varies depending on the vaccine composition, route of administration, genetic makeup, and other individual differences among the recipients and species.

An adequate innate immune response that guides an appropriate adaptive response is desired, but clinically obvious nonspecific innate responses such as fever, lethargy, swelling, and soreness are not preferred sequelae to vaccination. Although a normal toxicity from vaccination might be expected, it is still preferential to minimize this toxicity for the patient and client's sake. Because various vaccine components can serve as immune potentiators, it is not surprising that a greater exposure (volume of vaccine received per kg body weight) increases the risk of a clinical focal or systemic reaction.[5,15,16] Minimizing the number of vaccines administered in a single office visit can reduce the risk of these undesired vaccine-associated adverse events.

Prevaccination prevention of such adverse events through administration of nonsteroidal antiinflammatory drugs (NSAIDs), for example, acetaminophen or aspirin, is sometimes used in human medicine, but inhibition of cyclooxygenase 2 (COX-2) may attenuate antibody response.[17] Known toxicities of these NSAIDs in cats in particular and in dogs, coupled with challenges in proper dose administration, has generally precluded their similar use in veterinary medicine.

## HYPERSENSITIVITY REACTIONS

### *Type I*

Immediate hypersensitivity (type I) produces IgE-mediated allergic reactions with degranulation of mast cells and basophils. Allergens are proteins, generally with a molecular weight between 10 and 40 kDa, which in low doses induce differentiation of $T_H$ cells into $T_H2$ cells producing IL-4 and IL-5. IL-4 regulates the production of IgE and also enhances the growth of $T_H2$ cells. IgE is typically found in very low concentrations in serum because of its low production, short half-life (approximately 2 days), and sequestration on mast cells and basophils. IgE binds both high-affinity and low-affinity IgE receptors, and high-affinity IgE receptors are typically found only on mast cells and basophils. Mast cells and basophils are the primary histamine-holding cells in the body. When a relevant allergen cross-links 2 specific IgE molecules, signal transduction with calcium influx causes fusion of the exterior cell membrane with membranes of granules containing inflammatory mediators. Preformed granule contents, for example, histamine and heparin, dissolve and are released rapidly (within 5 minutes) while arachidonic acid metabolites, for example, leukotrienes and prostaglandins, are newly generated and released slightly later (5–30 minutes). These mediators increase vascular permeability and cause smooth muscle contraction.

Vaccines contain the active (label) antigens, often adjuvants, antibiotics, preservatives, residual culture medium proteins, and additives. Any vaccine component or excipient could potentially be responsible for an IgE-mediated reaction. In people, allergy to egg protein has been a major cause of allergic reaction after immunization,[18,19] and gelatin (likely of bovine or porcine origin) has also been incriminated as a cause of anaphylaxis.[20,21] Selected vaccines contain antibiotics, and drug sensitivities to neomycin, polymyxin B, amphotericin B, or penicillin have been responsible for vaccine-associated type I reactions. Latex from vaccine vial rubber stoppers and

sorbitol can also evoke reactions. Adjuvants may have more of a secondary role by effecting $T_H2$ cells' response to the primary allergen.[22]

In a retrospective cohort study of more than a million dogs, risk factors were investigated for adverse events documented within 3 days of vaccination.[5] Most events were recorded the same day as the vaccination, with clinical signs consistent with type I hypersensitivity. Greatest risk was associated with the total number of vaccines, that is, milliliters of vaccine, received at the office visit, and a dose-response relationship was evident. The dose response was modified, however, by the dog's body weight, as the (%) increase in adverse event rate for each additional milliliter of vaccine in small (<10 kg) dogs was more than double the rise in rate seen in larger dogs. Even when number of vaccines and quantity were restricted, that is, dogs received only a 1-ml rabies vaccine, small dogs had a greater reaction rate than large dogs and a much greater rate than giant-breed dogs. Multivalent vaccines did not have a higher reaction rate than monovalent vaccines in this study.

Several different proteins have been purported as causes of vaccine-associated immediate hypersensitivity reactions in dogs and cats, even though most studies have not measured antigen-specific IgE concentrations. Without this important information, causes remain largely speculative. Most vaccines have been incriminated, but bacterial or spirochete vaccines may pose a higher risk. In Japan, Ohmori and colleagues[23] investigated IgE reactivity against fetal calf serum, gelatin, casein, and peptone in 10 dogs that exhibited allergic reactions at vaccination and compared the results to that of 50 vaccinated but asymptomatic dogs. Seven of 10 dogs with reactions had significantly increased IgE reactivity against fetal calf serum, a component of culture media used in vaccine production. Their analysis of vaccines found high concentrations of bovine serum albumin (BSA) in many vaccines.

A similar continuing study at Purdue University evaluated antigen-specific IgE response to BSA, casein, collagen I, bovine fibronectin, thyroglobulin, laminin, and porcine myosin in vaccinated dogs with or without an allergic reaction. IgE response against specific antigens was demonstrated in both the symptomatic and the asymptomatic group, with significant differences found only between matched samples, that is, littermates.[24] This IgE response in clinically normal dogs is consistent with laboratory studies in dogs[25] and indicates that an elevated antigen-specific IgE response by itself is not sufficient to cause clinical disease.

These study findings strongly suggest that vaccine excipients, probably common to many vaccines and manufacturing processes, are the most frequent allergens in canine and feline vaccines. For dogs, these proteins may be of bovine origin. It is not known whether protein exposure via diet (even exposure in utero or by nursing via the dam's diet) influences the development of specific IgE antibodies. This may, however, help explain allergic reactions occurring at the puppy's first vaccination.

Breed predispositions have been identified in large studies, with greatest risk noted for dachshunds, pugs, Boston terriers, miniature pinschers, and Chihuahuas. Among medium- to large-size breeds, boxers were at disproportionately greater risk.[5] Genetic differences exist, however, within breeds, and multiple genes or genetic regions are likely associated with manifestations of hypersensitivity. Identification of specific gene mutations may be too complex, in the near term, to be of practical significance. Nevertheless, the number of vaccines simultaneously administered to high-risk dogs should be minimized. Whether spacing vaccinations apart (and reducing incidence risk) reduces lifetime (cumulative) risk of a reaction is not known.

For humans, it is now advised that most patients with vaccine allergy can be safely vaccinated,[26] but the guidelines also recommend patient evaluation by an allergist or

immunologist to define the suspected offending antigen. For animals with a history of anaphylaxis after vaccination, skin testing by intradermal inoculation of 0.1 ml of vaccine may elicit urticaria/wheal. Intradermal injections (0.1 ml) of a positive (histamine) and negative (saline) control are also needed for a comparison. If skin testing is not performed, high-risk patients can be premedicated with a $H_1$ antihistamine, for example, diphenhydramine, by subcutaneous or intramuscular administration at least 15 minutes before vaccination. For reasons unclear, not all patients with demonstrated hypersensitivity have reactions at their next vaccination (even without premedication), but owners should be counseled about risk and watchfulness for a reaction.

Clinical manifestations of immediate hypersensitivity in dogs are often related to the skin and general circulation, with signs of facial or periorbital edema, pruritus, wheals, hypotensive shock, or collapse. Vomiting, with or without diarrhea, and respiratory distress are less common in dogs. Cats often exhibit gastrointestinal and respiratory signs, including ptyalism, vomiting, and hemorrhagic diarrhea, as well as dyspnea, collapse, and facial swelling.

Treatment of type I reactions should be tailored to the type and severity of clinical signs. Indicated drugs (used alone or often in combination) include (1) $H_1$ antihistamines to block histamine receptors in immediate phase, (2) rapidly soluble glucocorticoids to block arachidonic pathways in late phase and shock, (3) epinephrine to relax smooth muscle, and (4) intravenous crystalloid fluids to combat hypotensive shock. Although not indicated for all patients, epinephrine and supplemental oxygen should be administered to patients with respiratory distress and cyanosis.

### *Type II*

Type II hypersensitivity reactions are a consequence of IgG and IgM antibodies binding to specific cell surface antigens and producing cytotoxicity. These antibodies can interact with Fc receptors on effector cells such as neutrophils, NK cells, and mononuclear phagocytes, leading to target cell lysis by the effector cell. The attached antibody can also activate the complement pathway. While complement components C3a and C5a attract and activate other effector cells, components C3b, C3d, and the membrane attack complex (C5b-9) are deposited on target cell surfaces. Complement-mediated lysis may then occur, intravascularly destroying the target cell, or the cell may be removed extravascularly through opsonization and phagocytosis by splenic macrophages and Kupffer cells.

Immune-mediated cytotoxicity in companion animals is typically directed toward host platelets and/or erythrocytes, and dogs are much more commonly affected than cats. The diagnosis of immune-mediated cytotoxic disease is poorly defined in small animal practice, often becoming a diagnosis of exclusion. Available assays for antierythrocyte or antiplatelet antibodies have limited accuracy because of false-negative and false-positive results. A positive test is supportive of the diagnosis, but test sensitivity can be influenced by reagents and temperature.[27]

Immune-mediated thrombocytopenia, or idiopathic thrombocytopenic purpura (ITP), is an uncommon but known adverse vaccinal event following human immunization. The incidence is best recognized after measles-mumps-rubella immunization, although it has been reported after administration of other vaccines, such as hepatitis B, influenza, and varicella.[28,29] Postvaccinal ITP appears to be more likely after vaccination for viral diseases in which thrombocytopenia occurs during natural infection, for example, measles. Thrombocytopenia after routine immunization of children is usually benign, resolving within 1 month in most children.[30]

Immune-mediated hemolytic anemia (IMHA) or aplastic anemia from destruction of red cell precursors is considered an extremely rare sequela to human immunization.[29]

Although isolated cases have been reported,[31] it is unknown if the incidence is greater than the background rate for the disease.

Thrombocytopenia has been reported after modified live canine distemper virus vaccine administration in dogs, but the condition spontaneously resolved.[32] Whether the decreased platelet count was due to transient immune mechanisms or infectious mechanisms was unknown. Severe immune-mediated thrombocytopenia with petechiae has been stated to occur within 2 weeks of vaccination,[33] but cause or frequencies are unreported. It is unusual in practice to evaluate platelet counts within 2 weeks of vaccination, thus minor and transient decreases are rarely detected. More severe disease, necessitating glucocorticoid therapy, when seen in practice typically does not present with a history of recent vaccination. That would be expected because, under a uniform distribution, the 3 weeks following vaccination constitute only 5.8% (and 2 weeks only 3.8%) of an annual period. Better surveillance is needed to improve the understanding of the relationship of this disease to vaccination, but improved diagnostic tests are also required to identify an immune mechanism.

Vaccination has been a purported cause of IMHA in dogs, in spite of its rarity in cats and humans. This possible association was suggested by a case-control study in which 15 of 58 IMHA cases (26%) had been vaccinated in the previous 30 days compared with 5% of the 70 control dogs.[34] The second highest rate was among dogs (13 of 58) that were vaccinated more than 12 months before IMHA diagnosis. This association was not supported by a later case-control study which found no significant difference between groups.[35] Five (10%) of 52 cases had been vaccinated in the month before diagnosis, as had an equal number of control dogs. The largest number (17) of cases had been vaccinated 12 months or more before diagnosis, compared with 5 controls. Other investigators also failed to find an association between vaccination and IMHA using a case-control study.[36] Vaccination histories were not detailed in any of these studies.

Case-control studies are a reasonable and economical method to investigate rare events, but they need to be thorough. In different studies, and even within a study, dogs had been previously exposed to a myriad of vaccine antigens by way of different vaccinations from different manufacturers. Lack of detailed vaccination histories for the cases and controls reduces the ability to discern the predisposing factors (what loaded the gun?) as well as the precipitating, or antigen-specific, causes (what pulled the trigger?) of these adverse events. Due to the large number of marketed biologicals, large studies would likely be required to detect differences between groups. Vaccination may be an inciting cause of IMHA in some dogs, but probably not in most cases of IMHA. The extent to which that risk is increased with selected vaccine antigens is unknown.

The role of other autoantibodies and disease following vaccination is debated.[37] The mere detection or measurement of autoantibodies does not infer clinical disease. Does antibody production after vaccination account for canine immune-mediated thyroiditis and clinical hypothyroidism in dogs? A small experimental study showed that anticanine thyroglobulin antibodies were increased in dogs receiving a rabies vaccine, but not in dogs receiving only a multivalent distemper vaccine. When followed for almost 6 years, however, there was no difference in thyroid histopathology between vaccine groups and unvaccinated controls.[38,39]

### *Type III*

Type III hypersensitivity reactions develop from acute inflammation triggered by the presence of immune complexes in tissues. Type III reactions differ from type II

reactions in that type III reactions involve antibodies directed against soluble antigens in serum or tissues, producing antigen-antibody complexes. The antigen-antibody complexes subsequently invoke a variety of inflammatory processes as the antibodies engage Fc receptors on neutrophils, lymphocytes, basophils, and platelets. This process releases vasoactive amines, causing endothelial cell retraction, increasing vascular permeability, and allowing immune complex deposition on the vascular wall. Immune complexes also activate complement pathways, releasing peptides C3a and C5a and chemotactic factors. Macrophages are also stimulated by the complexes to release cytokines, such as TNF-$\alpha$ and IL-1, further inciting inflammation.

Clinical signs associated with type III reactions often become apparent with the rise of neutralizing antibody titers. Anterior uveitis, or blue eye, in dogs was associated with administration of modified live canine adenovirus type 1 (CAV-1) vaccines,[40] due to immune complex deposition in the anterior chamber and endothelial damage to the cornea. This problem has been virtually eliminated by the use of cross-protecting adenovirus type 2 (instead of CAV-1) in canine vaccines.

In many naturally occurring infectious diseases, immune complexes are deposited in the glomeruli. Glomerulonephritis has been noted in dogs and cats secondary to viral, rickettsial, and Dirofilarial infections. In spite of this, glomerulonephritis has not been attributed to complex deposition secondary to vaccination in dogs or cats. Renal disease is common in older cats, albeit usually interstitial, and recurrent vaccination has been postulated as a possible insidious cause. The use of feline kidney cell lines in production of vaccine for cats supports the biologic plausibility of vaccine-induced antibody formation against kidney cells, but experimental evidence is lacking. Although parental vaccination against feline viral rhinotracheitis, calicivirus, and panleukopenia can induce detectable antibodies against cell lysates, no renal disease was detected in a 56-week follow-up study.[41,42]

In people, immune complex deposition and associated joint disease can be a frequent but late complication of autoimmune disease, that is, rheumatoid arthritis. Although the role of vaccination in inciting or exacerbating this disease in humans has been debated,[43] it has not been proven. Due to the very low incidence of autoimmune disease in companion animals, a possible impact of vaccination on immune complex-related joint disease in dogs or cats remains unknown. A described immune-mediated polyarthritis in related young Akita dogs has several clinical signs similar to human juvenile rheumatoid arthritis, but lack of long-term follow-up in these dogs precluded determining any role of immune complex disease.[44] As noted with virtually any diagnosis in a young pet of vaccination age, a temporal association can be found but true pathophysiologic mechanisms secondary to vaccination remain unknown. This temporal relationship has been noted in a small case series of idiopathic immune-mediated polyarthritis,[45] but was not found in a larger group.[46]

### *Type IV*

Type IV or delayed hypersensitivity, according to the Gell and Coombs classification, takes more than 12 hours to develop and involves a cell-mediated immune response rather than antibody response to antigens. Delayed hypersensitivity therefore indicates the presence of antigen-specific CD4 T cells. After activation, these T cells release proinflammatory cytokines, such as interferon-$\gamma$, TNF, IL-3, and granulocyte-macrophage colony-stimulating factor, which attract and activate macrophages. Chronic stimulation of T cells and cytokine release can result in the formation of granulomas, composed of macrophages and lymphocytes.

## CUTANEOUS VASCULITIS OR GRANULOMATOUS REACTIONS

Dermatopathies have been reported to occur several weeks or months after vaccination. In 1986, pathologists reported a case series of 13 dogs with focal alopecia at sites of rabies vaccination.[47] Lesions were characterized by nonsuppurative inflammation and adnexal atrophy in the dermis and periarteriolar aggregates of lymphocytes and plasma cells in the subcutis. The arteritis was postulated to result from local formation of antigen-antibody complexes. Skin biopsies from 3 dogs were tested and had low-to-moderate intensity rabies-specific fluorescence in the walls of dermal blood vessels; skin biopsies from rabies-vaccinated asymptomatic dogs were not examined for comparison. Of the 13 affected dogs, 10 were poodles, and vaccines from at least 2 manufacturers were identified from case histories.

Subsequently, a pathology report of focal granulomatous panniculitis in 8 cats and 2 dogs documented deep dermal aggregates of macrophages, lymphocytes, plasma cells, and eosinophils at subcutaneous sites of rabies vaccination.[48] Four of the 10 cases also had discernible foreign material within macrophage cytoplasm, interpreted as vaccine-related material. More extensive immunologic tests were not performed.

Three mature dogs of different breeds with rabies vaccination-site alopecia later developed multifocal (pinnal margins, periocular areas, tail tip, and/or paw pads) cutaneous disease.[49] Ischemic dermatopathy was diagnosed based on reduced number and lymphocytic cuffing of dermal vessels, as well as a folliculocentric vasculopathy. Complement (C5b-9) deposition was observed in vessels of skeletal muscle in 2 of the dogs. The histologic changes in the dogs were noted to be indistinguishable from familial canine dermatomyositis. The specific antigenic stimulus for the complement-mediated microangiopathy was unknown, but microbial superantigens, as noted from disease after natural viral or bacterial infections, were postulated.

Clinical signs associated with ischemic vasculopathy were improved after oral pentoxifylline administration. Pentoxifylline is a methylxanthine derivative formulated for vasculopathic disease in people. It inhibits platelet and leukocyte adhesion to endothelial surfaces, improves erythrocyte flexibility, and reduces erythrocyte fragmentation, thus improving tissue perfusion. It may also have antiinflammatory effects by inhibiting TNF-$\alpha$ production.[50]

As noted with other adverse vaccinal events, specific vaccine components and mechanisms that serve as the predisposing or precipitating causes of this condition are unknown.

## VACCINATION SITE–ASSOCIATED SARCOMAS

Fibrosarcomas and, to a much lesser degree, other soft tissue sarcomas have received much attention in feline practice and small animal vaccinology since the 1990s. Pathologists first reported an increase in the incidence of sarcomas diagnosed at vaccination sites in cats, with a speculated relationship to increased rabies vaccinations.[51,52] Contributing or associated factors at that time included an increasing cat population in the United States, advancements in feline practice, promotion of new feline vaccines, including feline leukemia virus (FeLV), and new local laws mandating vaccination of cats against rabies. Without a national database or mandatory reporting of adverse events, subsequent studies could only estimate prevalence. Estimates had wide (>10-fold) variation, ranging from as many as 1 in1000 vaccines administered to less than 1 in 10,000 vaccines.[53] Sarcomas in cats occur at rates much lower than immediate hypersensitivity, but are devastating in outcome because of their poor response to surgical or medical therapy.

Individual and collective efforts, including a national task force, sought to define the pathogenesis of this disease. Although initially associated with rabies vaccination sites, later studies found that FeLV vaccination posed equal or greater risk than rabies.[54,55] Sarcoma formation, however, has also been associated with other vaccines, and even with injection of nonbiologicals. A possible ''smoking gun'' emerged with the identification of aluminum in some of the described tumors.[52] Aluminum, as aluminum hydroxide or aluminum phosphate, is used as an adjuvant in some vaccines. Although there are other types of adjuvants, the particulate structure of aluminum makes it a readily identifiable marker of previous vaccination.

As discussed before, adjuvants enhance antigen presentation and potentiate the immune response. The degree and manner by which this response occurs varies with the structure and properties of the adjuvant and with the adsorption mechanism.[56,57] One theory is that overzealous inflammatory reactions to vaccine adjuvants promote vaccine-associated sarcomas. Adjuvanted vaccines produce histologically and sometimes grossly evident inflammation after vaccination,[58] but an association between overt localized reactions postvaccination and later sarcoma development had not been demonstrated.[16] Furthermore, no difference in sarcoma rates at sites of adjuvanted versus nonadjuvanted vaccine was reported in a large cohort of cats.[59]

Oncogenesis may be more related to inappropriate (and less overt) inflammatory reactions from which some fibroblasts undergo malignant transformation. Oncogenes may code for and overexpress growth factors or their receptors. Immunoreactivity for platelet-derived growth factor, epidermal growth factor, and their receptors and transforming growth factor β has been demonstrated in vaccine-associated sarcomas.[60] These investigators also found overexpression of *c-jun*, coding for translational protein AP-1 and implicated in stimulation of quiescent fibroblasts and oncogenesis.

The increased incidence of sarcomas may be due, largely or in part, to increased immunologic stimulation (via well-intended, repeated vaccination) of a genetically at-risk feline population. Immunohistochemical staining of feline vaccine-associated sarcomas revealed that most tumors had antibody staining for p53 mutation,[61] with nucleotide polymorphisms in the p53 gene sequence subsequently detected and associated with prognosis.[62] Tumor suppressor gene p53 encodes a nuclear protein involved in cell cycle regulation. Cells with mutated or absent p53 proceed unregulated through the cell cycle, creating aberrant clones and resulting in tumorigenesis. Specific p53 genotypes are likely associated with cancer phenotypes, and in humans, p53 mutation carriers have a greater than 100-fold risk of developing soft tissue sarcomas compared with noncarriers.[63]

Whereas much of the specific mechanisms related to immune response and genetic interaction remain to be determined, some veterinarians note that ''the suggestive term 'vaccination-site fibrosarcoma' has been used a little too indiscriminately and has biased the veterinary and lay community alike.''[64] This may lead to reduced vaccination against infectious diseases and subsequent loss of individual as well as herd immunity.

## NEUROLOGIC COMPLICATIONS

Vaccine-induced neurologic disease is typically caused by the use of modified live virus vaccine and the recrudescence of a neurotropic agent, for example, rabies or canine distemper virus, producing clinical signs of that specific viral disease. The vaccine virus that is responsible for the disease can often be isolated from the sick patient. Multiple vaccines, or concurrent natural exposure to other pathogens, may

exert an immunomodulating effect and increase susceptibility for this uncommon phenomenon.[65]

Immune-mediated neurologic disease is a rare adverse vaccinal event in human medicine. Guillain-Barré syndrome (GBS) is an autoimmune disease resulting from antibodies that cross-react with epitopes on peripheral nerves, for example, gangliosides, leading to nerve damage. GBS clinically presents as an acute flaccid paralysis, characterized by varying degrees of weakness, sensory abnormalities, and autonomic dysfunction.[66] About two-thirds of cases occur several days or weeks after a naturally occurring illness, often respiratory or enteric infections.[67] Vaccines have been temporally associated with the development of GBS in humans, with strongest evidence for swine flu (H1N1) vaccine in 1976–77 and older rabies vaccines.[37,68] This association has not been demonstrated with recent influenza vaccines.[69] Although polyradiculoneuropathies occur in companion animals and coonhound paralysis has been considered as an animal model of GBS,[70–72] reported associations between vaccination and this type of disease are quite rare in dogs or cats.[73,74] Specific immune mechanisms were not elucidated in these isolated case reports. In spite of a proposed autoimmune mechanism, glucocorticoids have not been shown effective in altering clinical signs of polyradiculoneuropathy; the immunosuppressive drug cyclophosphamide may alleviate disease severity.[33]

## VACCINE-ASSOCIATED HYPERTROPHIC OSTEOPATHY (METAPHYSEAL OSTEODYSTROPHY)

Painful swelling of the distal radius/ulna (or less commonly, other long bones) with radiographic changes consistent with hypertrophic osteodystrophy (HOD) have been noted in young dogs within a week or two of vaccination. Because of the location of radiographic changes, this disease has also been termed metaphyseal osteopathy. Although also documented in small breeds, growing dogs of large or giant breeds seem more commonly affected. Great Danes, Irish setters, German shepherds, and Weimaraners are reported to have increased risk of HOD,[75] and the disease in Weimaraners has been more extensively investigated.[76–80] The described breed and familial tendencies support a genetic basis to the disease, but specific genes or genetic markers have not been identified.

Although recent vaccination is often reported in symptomatic puppies, the disease occurs in unvaccinated dogs.[79] With the disease most common in young dogs, it is not surprising that vaccinations were recently administered. Modified live canine distemper virus vaccines have also been associated with the disease,[33] but controlled studies have not evaluated relative risk compared with other vaccines. Without a control or comparison group, the exact role of vaccination will remain difficult to determine. Vaccination in a genetically susceptible dog possibly provides the immunologic stimulus to manifest clinical disease. Different vaccines (with their associated components) and the frequency/spacing of administration may modify the occurrence of disease.[77]

Clinical signs besides metaphyseal swelling and lameness can include fever and lymphadenopathy, with leukocytosis noted on complete blood count. Pyoderma and diarrhea are less commonly observed. Because postvaccinal concerns have been typically associated with the onset of juvenile bone disease and/or pyrexia, decreased neutrophil phagocytosis has not been suspected in these dogs, even though reported in young Weimaraners with recurrent infections.[81] Immunologic studies in Weimaraners found affected dogs to have lower concentrations of one or more serum immunoglobulins (IgG, IgM, and IgA); accurate vaccination histories

were available on 10 dogs, and 9 had developed clinical signs within 5 days of a vaccination.[80] More extensive immunologic studies in postvaccinal affected dogs and in postvaccinal asymptomatic dogs (for comparison) are lacking. Investigators evaluating the findings, as well as response to therapy, have suggested that the clinical signs are manifestations of a form of immune dysregulation rather than a multifocal inflammatory disease.

Best recommendations for treatment are hindered by the lack of randomized clinical trials. Such trials should, in theory, be large enough to equally distribute between treatment groups patients that will likely vary in genetic predisposition, quality and quantity of immune stimulus, and degree and nature of immune dysregulation. This biologic variability somewhat explains differences in published treatment recommendations. Primary complaints of lameness with joint (or near-joint) swelling and radiographic changes in bone have supported guidance to administer NSAIDs,[82,83] which are effective in some dogs. Concurrent fever and leukocytosis in affected dogs also raises concern of an infectious process and an understandable reluctance to use corticosteroids. Nevertheless, glucocorticoids are the recommended treatment and are likely to give a superior response,[33,76,80] particularly when HOD presents soon after the immune stimulus of a vaccination. Antiinflammatory doses of glucocorticoids (0.5–1.0 mg/kg/d prednisolone) may be adequate for some cases, but high-dose pulse therapy (an immunosuppressive dose of 2–4 mg/kg/d tapered within a week to physiologic doses) can produce dramatic improvement in moderate and severe cases by rapidly downregulating steroid receptors and by inhibiting cytokine synthesis.

Are these dogs with suspected immune dysregulation at risk for other immune-related diseases after vaccination? Dogs with multiple manifestations of immunodeficiency, for example, stomatitis, and recurrent fever, will likely have disease problems regardless of vaccination. There is no long-term study of dogs with only HOD after vaccination. Recurrence of HOD appears to be unlikely after the dog's growth phase, and the (relative) immune stimulus from vaccination is likely reduced as a result of the increased body mass at adulthood. Nevertheless, restricting the number and type of vaccines administered to these dogs is prudent.[33]

## SUMMARY

Adverse vaccinal events, or perceived vaccine-associated adverse events, are relatively uncommon after canine and feline vaccination. Nevertheless, undesired immune sequelae occur, often evoking great concern from owners and attending veterinarians. Because of the low incidence of these events and the large number of potential antigenic causes, exact mechanisms may be difficult to elucidate. Good scientific studies, genetic studies to identify populations and breeds at risk, improved vaccine quality, and modified vaccination protocols will likely work together to further reduce these events in the future.

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