

# Adjuvants in Veterinary Vaccines: Modes of Action to Enhance the Immune Response & Potential Adverse Effects

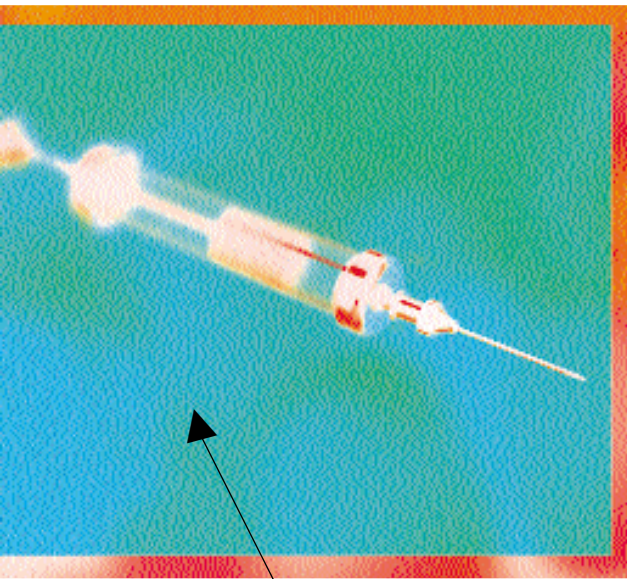
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The word adjuvant derives from Latin. It literally means “aid,” precisely what adjuvants do when added to vaccines. Adjuvant agents enhance the immune response that occurs after vaccine administration. Although they have been used extensively in the last 50 years, there has not been a comprehensive veterinary journal review of adjuvant mode of action and selection rationale until recently<sup>1</sup>. The following is based on information presented in the May/June issue of the *Journal of Veterinary Internal Medicine* under the title, “Adjuvants in veterinary vaccines: modes of action and adverse effects.”

The idea of using agents to enhance vaccines came serendipitously in the 1920s when Ramon reported that horses with vaccination site abscesses had increased antibody titers. Shortly thereafter he and others found that the antibody response to vaccines was enhanced by concurrent injection with a variety of inflammatory substances. Later, in the ‘30s, Freund’s complete adjuvant was developed using mineral oil, water and killed mycobacteria. Although an excellent inducer of cell mediated immunity (CMI) and antibody (Ab) response, it also routinely causes abscesses. Since these discoveries, the goal has been to develop adjuvants that promote a strong immune response without harmful side effects.

An understanding of the protective immune response that occurs after natural contact with pathogens is essential to the development of adjuvanted vaccines that stimulate the immune system to react appropriately. Pathogens include viruses, bacteria, fungi, protozoa and parasites. The tailor-made defensive reaction to pathogens in each of these categories varies, but a common feature during the induction phase of the immune response is that antigen-presenting cells (APC), primarily macrophages and dendritic cells, capture the organism and secrete cytokines, such as interferon and interleukin (IL) while presenting antigen (Ag) to lymphocytes.

The immune responses to intracellular pathogens living in the cytoplasm, in-



tracellular pathogens living in a macrophage, and extracellular pathogens are significantly different. Intracellular Ag in the cytoplasm is presented by major histocompatibility I (MHC I) molecules to cytotoxic T cells. Antigens from intracellular pathogens surviving in macrophages phagosomes are presented by MHC II molecules to T helper 1 (TH1) cells, which secrete the macrophage-activating cytokines interferon gamma, IL-2, and IL-12. In contrast, extracellular Ag is presented by major histocompatibility II (MHC II) molecules to T helper 2 (TH2) cells, which secrete the cytokines IL-4, IL-5, IL-6, IL-10 and IL-13, stimulating B cells to produce Ab, especially mucosal antibody (IgA or IgE). So, as a general rule, intracellular pathogens are attacked by CMI and extracellular pathogens, by the antibody system.

Although crossing of Ag presentation occurs, with MHC I molecules sometimes presenting extracellular Ag and vice versa, once the immune system starts on either the CMI intracellular pathogen response path or the humoral extracellular response path, it does not tend to change course. The cytokines involved are at least partly responsible for this tendency. Interferon gamma inhibits the T helper 2 cell response and one of the cytokines associated with the T helper 2 cell response, IL-10, inhibits the T helper 1 cell response. This is one of the reasons that allergic animals making too much IgE will sometimes have a reduction of allergic symptoms during a viral infection that results in production of interferon gamma, as this cytokine inhibits T helper 2 cells and thus B cell production of IgE.

Looking closer at the immune response to pathogens reveals a complex system with a variety of defensive tools coming into play depending on the pathogen's mode of assault (Figure 1). IgA attacks pathogens on the

mucosal surface. IgE is more effective once an organism invades the mucosa. Gamma delta T cells are important when the pathogen attacks epithelial cells. Cytotoxic T cells kill virus infected cells synthesizing foreign proteins. IgG neutralizes toxins, agglutinates pathogens, and opsonizes them making them easier to phagocytize, thus IgG is essential to fight off bacterial septicemia.

The type of immune response mounted to a particular pathogen is determined, to a large degree, by the cytokine messengers that are triggered by certain elements of the pathogen. Using adjuvants that induce the production of the same cytokines that are produced when a natural infection occurs is a good start to the trial and error process of adjuvant selection. The classical problem with the early adjuvants was that the amount of adjuvant necessary for immunity resulted in an excessive inflammatory response and abscesses.

The natural immune response to pathogens can provide a template for the selection of adjuvants. Modified live vaccines (MLV) produce similar signals to natural disease. The bacteria or virus may migrate and replicate at a site that is identical or similar to the location of replication during natural infection. Killed vaccines, on the other hand, produce an unnatural set of signals. Adjuvants included in vaccines help stimulate an immune response that mimics the response to natural infection by a wide variety of mechanisms. If the adjuvant is very good at including the correct type of immune response, even though it is an artificial set of signals, there may be very good immunity. To improve the immunity of a modified live vaccine, it may be necessary to have an adjuvant that sends artificial signals, which are the correct signals so that vaccine induces a

very strong correct type of immune response. However, it is necessary to know the immune response desired. Adjuvants fall into three main classes: microbial components, chemicals and mammalian proteins.

The three proinflammatory cytokines, IL-1, IL-6 and tumor necrosis factor (TNF), are induced by endotoxin and a variety of bacterial components (Figure 2). Whole heat-killed bacterial preparation is a crude way of producing adjuvant. Mycobacteria were used to produce the oldest adjuvants of this type.

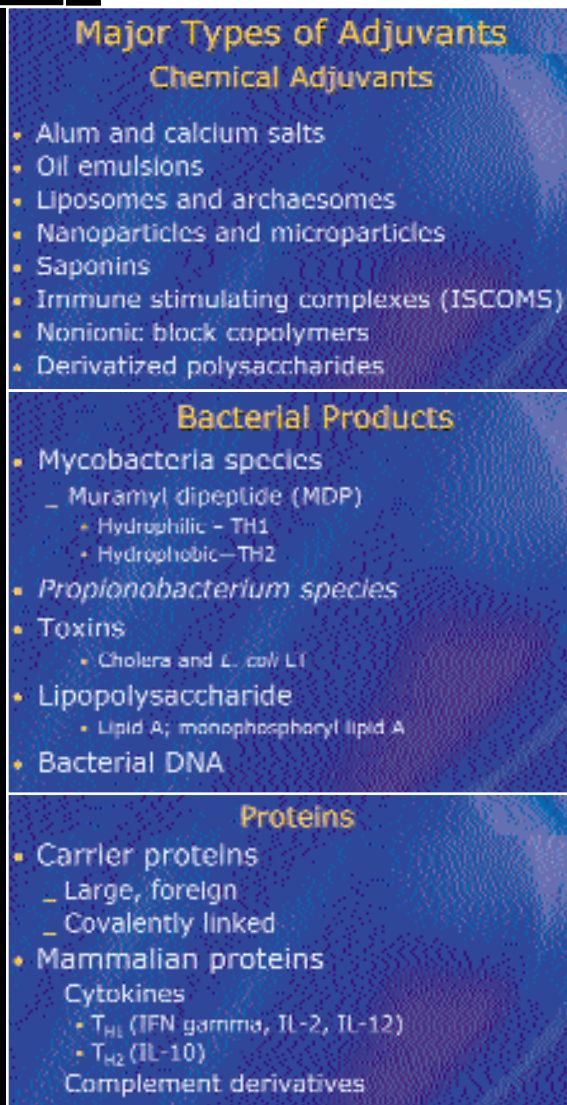
Figure 1

Pathogenic Mechanisms	Defense Mechanisms
• Adherence to Mucosa	• Mucosal Antibody (IgA)
• Parasites	• IgE
• Exotoxin/Endotoxin	• Neutralizing Antibody
• Viremia	• Neutralizing Antibody
• Septicemia	• Opsonizing Antibody
• Intracytoplasmic Growth	• Cytotoxic T cells
• Growth in phagosome	• T <sub>H1</sub> Cytokines
• Infect epithelial cells	• Gamma Delta T cells

Research into reduction of the side effects associated with these adjuvants resulted in the production of muramyl dipeptide (MDP). Derivatives of MDP were produced to decrease toxicity. The hydrophilic derivatives induce primarily TH2 responses, and the lipophilic derivatives induce mainly TH1 reactions.

Another bacterial component, lipo polysaccharide (or endotoxin), is a good immune stimulator but has too many side effects. One of its derivatives, monophosphoryl lipid A, has reduced side effects and shows potential as a good adjuvant.

Bacterial DNA can act as an adjuvant. CpG oligonucleotides (polymers made up of a few nucleotides rich in the dinucleotide CG) mimic bacterial DNA and may not only induce Ab production, but also shift immunity toward CMI. Theoretically, titration of the concentration of a CpG adjuvant could provide a method of controlling the balance between CMI and humoral immunity.



A couple of the oldest adjuvants, alum and oil, are examples of the chemical adjuvant class. They work by trapping Ag at the injection site, forming a depot, thus providing a steady supply of Ag for local APC to respond to. Microparticle adjuvants, made from polymers similar to those used in suture material, use this same mechanism to form long-term (one to six months) depots.

Some adjuvants, including alum, carbohydrate polymers, and liposomes, improve uptake by APC. Alum does this by creating protein aggregates that are more easily phagocytized. Carbohydrate polymers guide Ag to APC by carbohydrate receptor attachment. Liposomes are membrane-bound vesicles of cholesterol and phos-

pholipid. Ag is incorporated either within the lumen or in the membrane. Archaeosomes are liposomes that come from a unique, ancient group of microorganisms, the Archaea. Although classified with bacteria, they are genetically and metabolically different from all other bacteria. They thrive in extreme environments and appear to be the survivors of a primeval group of organisms that bridged the gap between bacteria and eukaryotes. Archaeosomes can induce both the TH1 cytokine interferon gamma and the TH2 cytokine IL-4. Liposomes and archaeosomes are being used with an increasing frequency.

Saponins are extracted from plants and purified to Quil A and other substances. Immune stimulating complexes (ISCOM) are liposomes that contain saponin. ISCOM and saponins are immunomodulators, can induce TH1, TH2 and CTL responses. Saponins and Quil A are commonly used in veterinary vaccines. ISCOMS have been used in several experimental veterinary vaccines.

Some proteins are used as adjuvants. Protein carriers can be linked to small Ag and improve their immunogenicity. Cytokine proteins can shift the immune response and are being investigated, although several problems remain to be solved before they will be routinely incorporated into vaccines.

According to the latest Compendium of Veterinary Products<sup>2</sup> there are 67 USDA licensed feline vaccines, 38 of which have killed Ag. These products cover 11 diseases. There is a choice between killed or MLV products for five of the diseases (calicivirus, chlamydia, panleukopenia, rabies and rhinotracheitis). MLV is the only kind of vaccine available for Bordetella and feline infectious peritonitis and killed vaccine is the only option for the other four diseases (feline immunodeficiency

virus, feline leukemia, giardia and *Microporum canis*).

Information regarding adjuvants used by the companies for production of their vaccines is often proprietary and closely guarded. The optimal adjuvant depends on the animal species, pathogen, Ag, route of administration and type of immunity needed. Although much is known about individual adjuvants, development of the ideal adjuvant or combination of adjuvants for a particular vaccine is still fraught with the inherent inaccuracy of trial and error experimentation. The science has advanced considerably since Freund developed his famous adjuvant. There is still a long way to go. Solving the difficult problem of injection-associated feline sarcomas has illuminated the need for further epidemiological studies and vaccine research. The ability to increasingly design and select adjuvants based on specific qualities of the Ag, characteristics of the target species, and needs of the situation has put the animal vaccine industry on the brink of tremendous innovation.



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#### References

1. Spickler AR, Roth JA. Adjuvants in veterinary vaccines: modes of action and adverse effects. *J Vet Intern Med.* 2003 May-Jun;17(3):273-81.
2. Bayley AJ, Inglis S, Stahle D, et al, ed. *Compendium of Veterinary Products.* 7th Ed. Ames, IA: Iowa State Press; 2003.