Immunological Basis of Vaccination

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Introduction
Equine veterinarians are faced with new commercial vaccines, new efficacy claims, and potential side effects, combined with an increased awareness of the questionable efficacy of established vaccines. These complex issues require of equine veterinarians a more comprehensive understanding of vaccine immunology in order to be able to judge this information. The future promises further change and controversy. The aim of this paper is to provide a review of the basic immunological processes that are critical to an understanding of the process of vaccination.

Currently a wide variety of vaccines are available for use in horses but the efficacy of these products varies widely despite the fact that many of these vaccines are similar in design, containing killed organisms or toxoids combined with simple adjuvants. A significant reason for this is that this single type of vaccination strategy will stimulate only one array of immune responses. In the case of infections like tetanus, the inactivated toxoid vaccine generates neutralizing antibodies that are highly successful in providing long-term complete protection. However, in the case of viral infections, such as equine influenza virus, current inactivated virus vaccines fail to induce the complete spectrum of immune responses required for lasting and effective protection. The first step to overcoming the limitations of current vaccines is understanding what type of immune responses are required to protect against a specific pathogen.

Components of the Immune Response
Our total immune defenses include both innate responses, such as neutrophils or complement, and adaptive responses, mediated by lymphocytes, which result in immunological memory. Only adaptive responses can be induced by vaccination. The specificity of adaptive responses, mediated by antibodies or by effector cells such as cytotoxic T-lymphocytes (CTLs), is responsible for their capacity to completely protect an animal against a particular pathogen. The principal types of immune effectors, including antibodies and lymphocytes, relevant accessory factors, and examples of infectious agents against which they are most effective are listed in Table 1. There are some over-simplifications; for example, IgG is considered as a single type of immunoglobulin, while in reality there are different sub-classes of IgG with different functions. To understand how these different types of immune responses are induced it is necessary to have a rudimentary understanding of the biology of lymphocytes, as it is these cells that govern the adaptive immune responses that we need to generate with vaccines.
The Lymphocyte Family

Lymphocytes can be divided into different populations with different specialized but coordinated functions, and this family tree is illustrated in Figure 1. The major division within the lymphocyte family is into T cells and B cells. The critical feature separating these cell populations is that T cells have on their surface an antigen receptor called the T-cell receptor (TCR) combined with a signaling molecule called CD3, while B cells express immunoglobulin molecules on their surface and use these directly as antigen receptors. There are two types of TCR, the αβ and the γδ TCR, but for our discussion of vaccine immunology we are only concerned with the αβ-TCR-expressing T cells. There are many other critical cell surface molecules, but understanding these is not essential to our discussion of vaccine immunology. Let’s examine the T cell and B cell families separately.

T cell Function

Beyond this initial distinction of T and B lymphocytes, the T cell family is divided into T-helper cells that express the CD4 surface molecule and cytotoxic T lymphocytes (CTLs) that express the CD8 molecule. The exact reason why CTLs and T-helper cells express these molecules will become clear later, but let’s consider first the basic features of what these cells do and why they are important. The role of CTLs is the easiest to explain. As their name suggests, CTLs kill other cells within the body, and in the case of infectious disease they do this when cells are infected with a virus or an intracellular bacteria (Fig. 2). This specialization is absolutely critical to fighting these types of infections and CTLs are an essential component of immune defenses.

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**Table 1.** Effector molecules and cells of the adaptive immune system, accessory factors, and examples of susceptible infectious agents. (From: Lunn DP, McClure JT. Immunological principles of equine vaccination. In: Colahan PT, Merritt AM, Moore J, Mayhew IG, eds. Equine Medicine and Surgery 5 ed, St. Louis, MO: Mosby Inc., 1999;183–190)

<table>
<thead>
<tr>
<th>Effector Accessory Factors</th>
<th>Infectious Agents</th>
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<tr>
<td>IgG</td>
<td>Complement, neutrophils</td>
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<td>IgA</td>
<td>Alternative complement pathway</td>
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<td>IgE</td>
<td>MAST cells</td>
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<td>IgM</td>
<td>Complement, macrophages</td>
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<td>Perforin, lymphotoxin</td>
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<td>Th1 (CD4+) lymphocytes</td>
<td>Macrophages, B lymphocytes (IgG sub-isotypes)</td>
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<td>Th2 (CD4+) lymphocytes</td>
<td>B lymphocytes (IgA, IgE), MAST cells, Eosinophils</td>
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![Fig. 1. Major divisions of the lymphocyte family. To the left of the diagram different populations of lymphocytes are distinguished by expression of different cell surface molecules. To the right of the diagram the distinctions are functional.](image1)

![Fig. 2. Cytotoxic lymphocyte (CTL) killing. This sequence shows lysis of a virus infected target cell by a CTL. The target expresses antigens derived from the virus on its cell surface bound to MHC I molecules, which are recognized by the CTL, resulting in binding and release of effector proteins that trigger cell death. The CTL survives the process and can go on to another target.](image2)
If the CD8\(^+\) CTLs are responsible for destroying infected target cells, what is the role of the other major T lymphocyte subset, the CD4\(^+\) T-helper lymphocytes? These lymphocytes, as their name implies, “help” other effector cells to fight off pathogens. It is currently believed that two different subsets of T-helper cells, characterized by their cytokine production profile, may be responsible for determining the nature of the immune response to infectious agents.\(^1\) The subsets are the T-helper 1 subset (TH1) which stimulates cytotoxic and inflammatory functions, and the T-helper 2 subset (TH2) which stimulates strong antibody and allergic responses (Fig. 3). These two types of T-helper subsets and the cytokines they produce tend to suppress each other. As a result, in an immune response to a particular pathogen, either the TH1 or the TH2 will predominate and give rise to either an inflammatory/cytotoxic or a humoral immune response.

Therefore, if an appropriate immune response to a pathogen is to be produced, vaccination must induce the appropriate T-helper response. An example of such a circumstance may be *Rhodococcus equi* infection in foals. Like other intracellular pathogens, such as *Salmonella* sp, this organism survives by parasitizing macrophages.\(^2\) In order to overcome such infections, the macrophage requires help from inflammatory TH1 cells that can activate the macrophage by secretion of cytokines such as IFN-\(\gamma\) and GM-CSF. In contrast, a TH2 response may be ineffective in combating these intracellular organisms, and may actually be counterproductive by suppressing TH1 activity. This may explain the lack of efficacy of killed vaccines against intracellular pathogens.

Several factors have been identified which may influence whether a TH1 or TH2 type response will predominate and these include the type of antigen presenting cell, dose of antigen, the type of adjuvant, immunization route, and the cytokines present during antigen presentation. The type of T-helper cell induced by vaccination will determine whether the vaccine is either helpful or possibly even detrimental, in protecting against disease. Therefore a key issue in vaccine development is the ability of different vaccination strategies to stimulate specific T-helper subsets.\(^3\)

**B cell Function**

The role of the B cell family is the production of a complex array of antibodies. In the horse all the major antibody classes have been identified: IgM, IgG, IgA, and IgE. Importantly, the IgG class can be divided into a number of subclasses, the best characterized and most important of which are IgGa, IgGb, and IgG(T). Each of these classes and subclasses are specialized for a specific role as indicated in Table 1, and discussed in detail below in the context of IgA and mucosal immunity. The structure of an antibody molecule is shown for IgA in Figure 4, an antibody which is specialized for activity at mucosal surfaces. The other major immunoglobulins involved in fighting microbial disease are IgM, which is responsible for a rapid initial response to either infection or vaccination, and IgG, which increases after IgM but is produced in larger amounts and with a higher affinity for its antigen. In addition, IgG responses can be very long-lasting and it is IgG that is responsible for recall responses on re-exposure to a pathogen or booster vaccination.

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The maturation of B cells, illustrated in Figure 5, provides an example of the process of lymphocyte
development. There are two key processes that occur here. The first is a rearrangement of the germ line DNA of the B cell in order to determine the exact structure of the immunoglobulin molecule antigen binding site it will later express on its surface or secrete. This process is mirrored in T cells when they determine the antigen binding specificity of the TCR they will subsequently express. This is a critical step in all lymphocyte development as it is responsible for the ability of the immune system to recognize a vast array of foreign antigens. Interestingly, a failure in this process, due to mutation in an enzyme gene, is responsible for the failure of B and T cell development that we see in Arabian severe combined immunodeficiency foals (SCID). A second key step in B cell development is the process of deciding what type of immunoglobulin class to produce. All B cells start out making IgM and IgD, but later make a final commitment to one specific class or subclass of immunoglobulin. This decision depends in large part on cytokine signals from T-helper cells. Finally, activated B cells either mature into short-lived antibody secreting plasma cells, or become long-lived memory B cells.

We will focus on the antibody response to this pathogen, as this is probably a critical component of protective immunity to influenza. Until recently only killed vaccines were commercially available for equine influenza, but in young horses in particular they gave short-lasting protection at best. In contrast, equine influenza virus infection leaves horses protected for at least 6–12 months. To work out why vaccinal immunity compared so poorly with infection-acquired immunity, Dr. Kay Nelson performed an experiment to study local and circulating antibody responses and protection resulting from a conventional commercial vaccination or natural influenza virus infection. It was found that 3 months after administration of two doses of a conventional vaccine, ponies were left unprotected when subjected to a challenge infection. In contrast, 3 months after being given an initial influenza virus infection, ponies were completely immune to a repeat challenge infection. The key local and serum antibody responses to infection or vaccination are shown in Figure 6. A critical difference between infection and vaccination was that infection induced high levels of IgA in nasal mucosal secretions whereas vaccination induced no IgA antibodies. In addition there were marked differences in the isotypes of IgG induced by infection compared to vaccination, with natural infection inducing IgGa and IgGb responses and conventional vaccines inducing IgG(T) responses. It is interesting to note that in the horse the IgGa and IgGb sub-isotypes are capable of mediating important anti-viral activities such as complement fixation and antibody-dependent cellular cytotoxicity, while IgG(T) responses can actually inhibit complement fixation and are better adapted to neutralizing toxins such as those produced by the Clostridia sp.

Fig. 5. Maturation of B lymphocytes. Different stages of B lymphocyte development can be recognized by expression of immunoglobulin molecules. This maturation requires a series of gene rearrangements in order to select the genes which will encode the antigen binding part of the immunoglobulin molecule (variable region), and subsequently to select the genes that determine the class or subclass of the antibody molecule. Initially, immature B cells express IgM (the majority of peripheral blood B cells), but after antigen exposure the B cell becomes activated and may express any of the immunoglobulin classes or subclasses. This decision depends in large part on cytokine signals from T-helper cells. Finally, activated B cells either mature into short-lived antibody secreting plasma cells, or become long-lived memory B cells.

Fig. 6. Immune response to influenza virus. Equine immunoglobulin responses in two groups of four influenza naıve ponies to either influenza virus infection or conventional inactivated vaccine administration. The graphs show the mean nasal mucosal IgA response to a viral infection (administered on Day 0), or the IgG subclass responses to infection or a series of two vaccinations (second dose on Day 0).
Not all killed equine influenza vaccines perform as poorly as did the one used in this study, but this example highlights that vaccines must induce the right kind of immune responses, and in the right places. Now that we understand the components of the immune response, there is one more basic immunological concept that must be grasped, and that is how the immune system sees an invading pathogen. This process is called antigen presentation.

**Antigen Presentation**

T lymphocytes don’t respond directly to antigens present on the surface of a virus, for example. Instead they recognize small processed antigen fragments that are present on the surface of either infected cells, or on cells that are specialized for capturing foreign antigens and presenting them on their surfaces. This latter type of cell is termed an antigen presenting cell (APC), and examples include macrophages and the highly efficient dendritic cells (Fig. 7). After processing the antigen, short antigenic peptide fragments are presented on the cell surface bound to Major Histocompatibility molecules (MHC), which can be divided into the MHC class I molecules which are present on all cells, and the MHC class II molecules which are only present on specialized antigen presenting cells.

Antigens are processed for presentation by either the endogenous pathway resulting in presentation by MHC I molecules, or by the exogenous pathway resulting in presentation by MHC II molecules as illustrated in Figure 8.10 This means that CTLs can recognize any cell in the body that is infected with an intracellular pathogen and is displaying components of that pathogen on its surface bound to MHC I molecules. In contrast, T-helper cells recognize antigens only on the MHC II expressing APCs, such as macrophages.

At this point, an important practical implication for vaccine development is apparent. CTLs have a critical role in eliminating viral infection because they are adapted to seek out and destroy infected cells. These cells can only respond to antigens presented by MHC I molecules. However, killed or inactivated antigens in vaccines are phagocytized by the antigen presenting cells and are therefore far more likely to be presented by MHC II molecules. Therefore many conventional vaccines are unlikely to be able to induce the CTL responses that are essential for defense against some viral infections.
In addition, in the case of T-helper cells, several factors involved in antigen presentation can influence whether a Th1 or Th2 response is induced. It is apparent, therefore, that appropriate and efficient antigen presentation is an essential requirement for any vaccine.

Mucosal Immunity

The components of the mucosal immune system are no different from the immune system in the rest of the body, but this "compartment" of the immune system is so important that it deserves special consideration. In the equine influenza study above there was a strong mucosal IgA response following infection, and there is excellent evidence that this type of immunity is critical for protection from many pathogens that invade mucosal surfaces. The mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts are continuously exposed to foreign antigens, including potentially infectious bacteria and viruses. The adaptive mucosal immune responses that have evolved to protect the body against these challenges have distinct and specialized characteristics.11 The principal immunoglobulin produced by the mucosal immune system is secretory IgA, which is the most abundant immunoglobulin class in the body. Specialized antigen uptake cells in the Peyer’s Patches of the intestinal tract or nasopharyngeal lymphoid tissues, termed microfold or M cells, transport antigens to underlying mucosal associated lymphoid tissues or MALT (Fig. 10).12,13 In the MALT antigen processing and presentation takes place, resulting in immunoglobulin class-switching and activation of antigen-specific IgA-positive B cells.13 T-helper cells are critical to this process.14 After homing of these IgA-B cells to effector sites such as the lamina propria of the gut and respiratory tract, second signals from antigen presentation cells and from T-helper cells result in further differentiation into IgA producing plasma cells.

Secretory IgA protects the body from bacteria and viruses principally by immune exclusion, i.e., by physically preventing attachment to mucosal surfaces.11,15 After release of secretory IgA by plasma cells into the interstitium, it is transported across the epithelial cell and released at the luminal surface (Fig. 11). During its transit through the epithelial cell it is even possible that IgA can neutralize intracellular infections.16 Overall the mucosal immune system can function as an independent immunological organ, equipped with specialized tools to deal with the particular antigenic challenges faced by mucosal surfaces. For many diseases, including influenza virus17 and Streptococcus equi18,19 infection, a mucosal immune response may be the most effective type of immune protection.

Effective Vaccination

We have reviewed many of the key functional elements of the adaptive immune response, and some of the regulatory processes that control them. In particular, we have identified three types of T lymphocytes that mediate immunity: the CTL which can destroy virus infected cells; the Th1 lymphocytes which can provide pro-inflammatory signals to activate cell mediated immunity (e.g., macrophage activation); and the Th2 lymphocyte which can drive antibody production. Appropriately stimulating these regulatory and effector T lymphocyte responses is an essential function of an effective vac-
Modified Live Vaccines

A modified live vaccine (MLV) will produce proteins in the cytosol which will be presented by MHC I molecules and induce CTLs. MLV viral vaccines can be produced by attenuation in cell culture, by use of variants from other species (e.g., smallpox and vaccinia), or by development of temperature-sensitive mutants. The mutations in MLVs are often poorly defined and reversion to virulence is a constant threat. In future, MLVs may be available in which specific mutations are produced, using recombinant DNA technology, which has predictable effects and cannot be reversed. Examples of such approaches are the development of experimental equine herpesvirus (EHV-1) vaccines with deletions of specific glycoprotein genes. Current equine MLVs include a highly efficacious Equine Viral Arteritis Vaccine, and two relatively recent intranasal vaccines, one against S. equi and one against equine influenza virus.

Recombinant Vector Vaccines

Both bacteria and viruses can be engineered, using recombinant DNA technology, to be carriers for defined antigenic polypeptides or peptide epitopes from other pathogens. However, this is technically far more complicated for bacteria, given their much larger and more complex genomes. The advantage of such vectors is that they allow introduction of genetic material encoding pathogen antigens into host cells, with subsequent protein production and antigen presentation by both MHC I and MHC II pathways. A critical prerequisite to using this technology is a knowledge of the protective antigens of the specific pathogen of interest (see subunit vaccines below). An example of suitable vector is canarypox virus, which can infect mammalian cells but is unable to produce viral progeny, and has been used as a vector for EHV-1 and equine influenza virus genes. This type of technology obviously has great promise and is an area of active investigation. There are some safety concerns which are similar to those of classical MLVs, with the additional risk of contamination with adventitious agents and vector pathogenicity.

Dead Vaccines

Dead or killed vaccines remain attractive because of their relative ease of preparation, lack of pathogenicity, and inability to replicate and spread between hosts. However, dead vaccines typically require multiple doses and regular boosters, and efficacy frequently depends on use of potent adjuvants.

Inactivated Pathogen Vaccines

Inactivated whole pathogen vaccines are the most common form of equine vaccine in current use.
Inactivation is achieved with agents such as thimerosal or phenol in the case of bacteria, and formalin or beta-proprionolactone for viruses. Historically such vaccines have frequently proven highly immunogenic, although their limitations in protecting horses from respiratory pathogens represent an ongoing and serious problem.5,7,26 Quality control in the production of such vaccines can overcome some of these limitations,27 but development and use of effective adjuvants represents the most important tool for overcoming their limitations (see below).28,29

Protein Vaccines
Protein vaccines include naturally produced components of pathogens, such as the M-protein vaccine for S. equi which is non-pathogenic and may promote fewer injection site reactions than whole bacterial products. The most commonly used protein vaccine in horses is tetanus toxoid, which is prepared by formalin inactivation of tetanus toxin and incorporation with an alum adjuvant.

Recombinant Subunit Vaccines
The explosion of knowledge in the field of recombinant DNA technology has led to the identification, and in some instances, synthetic production of many of the specific antigens that are important for immunity to pathogens. Such vaccines can include recombinant polypeptides, or peptide-based vaccines containing a single antigenic epitope. Unfortunately these purified proteins may be poorly immunogenic by themselves, and particularly so in the case of peptide vaccines, and cannot overcome the barriers that prevent MHC I presentation without the use of appropriate adjuvants.

Adjuvants
Comprehensive explanations of the many types of adjuvants is beyond the scope of this paper; however, several excellent reviews have been published.3,4,30,31 including one review of vaccine adjuvants in use in veterinary products.32 The success of killed vaccines frequently depends on the adjuvant system used, as adjuvants can determine the form of immune response that will be stimulated through stimulating either the Th1 or Th2 regulatory lymphocyte subsets. Current adjuvants, such as alum, tend to stimulate Th2 responses while Freund’s Complete Adjuvant (FCA) is an example of an adjuvant that stimulates Th1 responses. However, although FCA can induce Th1 responses, it cannot be used in commercial vaccines due to its side effects. Another critical function of adjuvants is to gain access to the cellular compartments that allow for MHC I presentation and CTL induction. One of the most promising adjuvants for this purpose is the Immune Stimulating Complex (ISCOM). ISCOM adjuvants have been associated with greatly increased antigen-specific antibody responses, and a wide range of T cell responses including the induction of cytotoxic T-lymphocytes.33 There is already evidence of the efficacy of influenza virus ISCOM vaccines in horses.28,34,35 An important component of ISCOMs is Quil A, a component of a plant sapo-

One particular type of adjuvant deserving special mention are the mucosal adjuvants. Mucosal immunity has a critical role in resistance to wide variety of pathogens such as equine influenza virus7 and S. equi.36 Generating mucosal IgA responses with killed vaccines is challenging, and the only effective mucosal adjuvants are the bacterial exotoxins of enteric bacterial pathogens such as cholera toxin (CT) or the labile toxin of E. coli.37 This adjuvant effect may depend on several known actions of CT, including enhancement of antigen presentation, promotion of B lymphocyte isotype differentiation, stimulation of CD4+ T-helper lymphocytes, and induction of local and systemic memory responses. A disadvantage of using CT is that it can produce cholera diarrhea in humans,11 although it is well tolerated in other species. Recently, encouraging results have been reported in horses vaccinated intranasally with inactivated equine influenza virus combined with cholera toxin B subunit.38

DNA Vaccines
DNA vaccination results in the in vivo synthesis of antigenic proteins in a manner identical to that occurring in natural infection.25 This endogenous production results in presentation of antigens by MHC I and presentation to CD8+ T cytotoxic lymphocytes, and uptake and presentation of soluble proteins by MHC II to CD4+ T-helper lymphocytes. As a result DNA vaccination has been shown to induce both potent CTL and antibody responses. Investigations of the use of DNA vaccines in horses are at an early stage, but it has already been demonstrated that they are effective at protecting horses from influenza virus infection and induce appropriate antibody isotype responses,39,40 and there is provisional evidence of their potential for EHV-1 vaccination.41,42

Passive Vaccination
Passive vaccination is accomplished by administering preformed antibodies either as a plasma transfusion or in a concentrated form, as in commercially available tetanus antitoxin. This strategy can be highly effective in diseases for which there is no available form of active antitoxin (e.g., R. equi) or in high-risk situations when there is inadequate time for protection to be generated by active vaccination. Generally passive vaccination should be avoided when possible due to the risk of transmission of infection in serum-derived products. A prime example of this is the association with acute hepatic necrosis with a previous administration of tetanus antitoxin.43
Summary
Understanding the basics of T cell and immunoglobulin function, antigen presentation, and mucosal immunity makes the immunological reasons behind vaccine success or failure far easier to grasp and better equips us to evaluate new and old products. As new and innovative vaccines reach the marketplace, this knowledge will have increasing value for equine veterinarians as we try to distinguish fact from fiction.

References