Immunology Review/Refresher with Emphasis on Vaccinology

Chris Chase, *DVM, PhD*
Department of Veterinary Science, South Dakota State University, Brookings, SD

Abstract

Vaccinology involves the preparation and testing of vaccines, the animal's immune response to the vaccine and the outcome against a challenge. This area of veterinary medicine, which offers great promise, is misused, abused and often expected to cure the impossible. Unfortunately, there is no "magic bullet" and the myths of vaccinology outweigh the reality.

Our understanding of the immune response has been greatly expanded in the last 10 years. Terms such as "danger signals" and "dendritic cells" were unknown in vaccinology until the last 5-6 years. In this vaccinology review, I will emphasize the primary immune response and its importance for setting up the long-term memory and duration of immunity.

Résumé

La vaccinologie englobe la mise au point et l'essai de vaccins, l'observation de la réaction immunitaire de l'animal au vaccin et l'issue de cette vaccination face à une infection de confrontation. Parfois, on emploie mal ou à l'excès ce secteur très prometteur de la médecine vétérinaire et on s'attend souvent à ce qu'elle accomplisse l'impossible. Malheureusement, il n'y a pas de « balle magique » et les mythes dépassent souvent la réalité de la vaccinologie.

Notre compréhension de la réaction immunitaire a fait de grands pas ces dix dernières années. Des termes comme « signal d'alarme » et « cellules dendritiques » étaient inconnus en vaccinologie il y a cinq ou six ans. Dans cette revue de la vaccinologie, je mettrai l'accent sur la réaction immunitaire primaire et son importance dans la mise en place de la mémoire à long terme et dans la durée de l'immunité.

Introduction

Vaccinology involves the preparation and testing of vaccines, the animal's immune response to the vaccine and the outcome against a challenge. This area of veterinary medicine, which offers great promise, is misused, abused and often expected to cure the impossible. Unfortunately there is no "magic bullet" and the myths of vaccinology outweigh the reality.

Our understanding of the immune response has been greatly expanded in the last 10 years. Terms such as "danger signals" and "dendritic cells" are relatively new in vaccinology. In this vaccinology review, I will emphasize the primary immune response and effector response — the response we want and need to protect and eliminate infectious disease. I will also discuss new concepts with regard to vaccines, adjuvants and needle-free injection devices.

The Essentials of the Primary Immune Response

The Location-Lymph Node. The primary immune responses have to occur where we have aggregates of lymphoid tissue. About 50% of T or B cells are in lymph nodes. The spleen is also important. Other important lymphoid areas are the mucosal lymphoid follicles, which include places like Peyer's patches, gut-associated lymphoid tissue, and bronchial-associated lymphoid tissue where immune follicles have developed. This organized lymphoid tissue is important because that is where dendritic cells, T cells and B cells all come together. Within the lymph node there are two important areas: one is the medullar area where T cells are localized and interact with dendritic cells, and the other important area is the cortical area, in the B cell follicles, where T cells and B cells interact. Once the primary immune response has occurred, T cells and B cells can go into circulation, get into the bloodstream and circulate.

Step 1. Activation and Travel of the Dendritic Cells (aka as Antigen Presenting Cells) to the Lymph Node.

What are the major cells that affect the primary response? The first is antigen presenting cells that include macrophages and dendritic cells (DC). The dendritic cells arise from macrophage-monocytes lineage. A large percentage of these cells are in the skin or near the skin. Langerhans' cells are in the skin. There are dermal dendritic cells and there are dendritic cells in the laminar propria and the submucosa. These cells are not found in the muscle until an inflammatory response occurs and these cells enter the inflamed area. The other place we find them is in the lymph node itself, but these dendritic cells have a different function. The dendritic cells in the epidermis, dermis and subcutaneous tissue are mainly immature — they are a sur-

veillance system. Once the DC become activated and mature, they move to the lymph node. For an immature DC to mature and migrate to the lymph node, it has to go through a sequence of events. The immature DC in the tissues are in an inactive state, then the DC senses a danger signal. The danger signal is an infection or injury that occurs and activates the cell. 'The danger signal' is required for activation of the inflammatory response to insure a good acquired immune response, memory and duration of immunity. This danger signal is induced (cell debris from necrotic cells, heat shock proteins (HSPs), nucleotides, reactive oxygen intermediates, cytokines [e.g. interferon]) by molecules produced or induced by a pathogenic organism infection and/or are from damaged cells. The molecules associated with pathogens are called pathogen-associated microbial patterns (PAMPS) and are only present on pathogens. These PAMPS are present on a wide range of pathogens, unlike the adaptive immune response that is epitope specific. For example, all gram-negative bacteria contain lipopolysaccharide (LPS), one of the PAMPS, while another PAMP, CpG nucleotides, are present in bacterial DNA. Flagellum present on protozoa and bacteria and double stranded RNA produced by viruses are two other PAMPs recognized by the innate immune cells (APC, dendritic cells, macrophages). These PAMPS are recognized by receptors called pathogen recognition receptors (PRRs or toll-like receptors) on innate immune cells. At least 10 groups of these PRRs have been identified. Non-microbial adjuvants like oil emulsions or saponins may also be recognized by PRRs or other receptors on innate immune cells.

The signal changes the cell's character almost immediately. The cell can then present antigen and produce co-stimulation signals to assist T helper cells. The immature dendritic cell has to go through the danger sequence to properly activate the immune response.

Step 2. Migration of the T and B Lymphocytes to the Lymph Node.

The second set of cells are the T and B lymphocytes, primarily the T helper (Th) lymphocytes. These lymphocyte cells partition in the circulatory systems with about 50% of these lymphocytes in the lymphatic system. Between the lymph nodes, the afferent lymph, the efferent lymph, and the spleen, these cells circulate continuously. They stay in the blood for about 30 minutes. They only stay in a lymph node for about 12 hours, and only stay in the spleen for about five hours. This particular voyage may take a little longer as these cells are constantly moving around.

These lymphocytes have to get from one lymph node to another; that's a key characteristic of lymphocytes. They have on their surface different receptors that can interact with these special "address" molecules that are found on endothelial cells. The lymphocyte has these receptors on its surface, and different combinations of it will help it "home" the lymphocytes to different lymph nodes. There will be one combination if it's going to a mesenteric lymph node and a different combination if it's going to a cervical lymph node. The ability of a lymphocyte to "home" occurs because it has these receptors. The receptors change as the cell matures. The number two player is the T lymphocyte.

The naïve (unstimulated) lymphocyte doesn't express many receptors or adhesion molecules on its surface. It only has one receptor, L selectin, on its surface, which it uses to find special vessels that occur in the lymph nodes called high endothelial venules. All these cells move across on the venous side, because it has much slower circulation. These cells are moving along in the high endothelial venule and once they do that, they can localize and squeeze into the lymph node and migrate into the T cell space in the medulla or middle of the lymph node. The high endothelial venules allow the lymphocytes to get into the lymph node to interact with the dendritic cells.

Once these cells become "educated" by encountering their antigen in the lymph node, they end up having more of these homing receptors on their surface. The receptors provide an address, i.e., "BVDV antigen in the cervical node". Because it has the receptors, the receptors are able to help direct the lymphocyte back into that same area after it circulates around through the body. After the cell is able to mature and divide, it gets new homing receptors specific to different areas, such as the mucosa or the skin. Subsequently, when we need to boost the response or have a secondary response, we can do it because these cells are homed back to the right area.

When antigen is encountered, another important function of the lymph node is to trap lymphocytes in the lymph node. Since the movement of naïve T and B cells to a lymph node is a random event with cells coming in and out of these nodes, the lymph node will shut down and trap the lymphocytes there, increasing the chances that the antigen presenting cell will find a specific T cell. When the lymph node is shut down, the T cells can't leave and are stuck there, increasing the chances they will see the antigen-presenting cell and be able to stimulate the response.

So this occurs in the lymph nodes and the two main players are the dendritic cells and the T cells. There are also B cells, which are responsible for producing antibody, and they need help from the T cells.

Step 3. The Interaction of the Dendritic Cell and the T Lymphocyte in the Lymph Node (The Collision and the Dance 1).

To develop an adaptive immune response these cells must interact. The two sets of events that must

happen, a collision and a dance, occur in the lymph node. The cells are moving around at different velocities. T cells are fast; they move at 12 microns per second. B cells move at about six microns per second, and dendritic cells move at about three microns per second. The first collision is between the antigen carrying DC and the naïve T cell. The second event occurs immediately after the collision — the dance where the cells have to stay together for some length of time. The first dance to take place happens after this collision occurs between the dendritic cell and the T cell. The longer they stay together, the better. This dance can take place from just a few minutes to a couple of hours. If it is a couple of hours, that's much better. The longer the DC and T cell can dance together, the better the adaptive immune response will be.

Step 4. The Interaction of the T Lymphocyte and the B Lymphocyte in the Lymph Node (The Collision and the Dance 2).

After the T cell has done its dance with the dendritic cell, the second series of collision and dance events has to happen. The T cell has to have a collision with a B cell that recognizes the same antigen and dance with a B cell just like the DC and T cell.

Why is the length of the dance important? This whole series of signaling events or cross talk between the two cells doesn't occur quickly. Looking at the surface of an antigen-presenting cell, it has antigen presented in a MHC II molecule; it is looking for a T cell. Then a T cell comes along that will recognize that same antigen. Additional surface molecules must also interact between the cells. These cells end up docking together here. Now a signal goes from the lymphocyte to the antigen-presenting cell and the other way, too; signaling goes both ways. This takes a matter of hours. Then new receptors come up, another signal is sent, and more receptors are expressed on the surface and cytokines are released. It is better if you have two hours rather than just two minutes, because the more of these signaling events that happen, the more specific and longer the adaptive immunity will be.

The most important step of the acquired immune response is the T cells interacting with DC. This is the first event that has to occur. This is strictly a numbers game. What's the chance that a T cell will react with an antigen-presenting cell that's expressing its antigen? With T cells, the number of antigen specific cells is fixed. Somewhere between one out of every 5,000-10,000 will react against that specific antigen, so that cell that is circulating and doing has to run into another antigen-presenting cell that has that antigen on the surface.

The variable here is actually the DC or antigen-presenting cell. If there are more antigen-presenting cells in the lymph node presenting the antigen on the surface, the chances increase that the one out of 10,000 will actually see its antigen. This is the real key in looking at adjuvants and increasing the effectiveness of our vaccines. If the number of antigen-presenting cells and the amount of antigen on their surface that are present in the lymph node can be increased, the odds increase that the naïve T cell will find its antigen presented on that antigen-presenting cell.

Summary - The Primary Immune Response

The primary immune response can only occur if the antigen containing DC and antigen-specific T-cells *randomly* bump into each other. By increasing number of antigen containing dendritic cells in the lymph node, the chances of activation of the antigen-specific T-cell are increased. In the end, we want as many antigen-containing dendritic cells as possible to get to the node. The danger activated DC express 5-10 times as much MHCII on their surface. Increased MHC expression means there are increased amounts of antigen on the surface of the DC, making them much better targets to dance with these T cells and get them activated.

The whole idea of collision and dance is very important. Two collisions and two dances need to happen if the complete T cell and B cell immune response is to occur. This is the key to how vaccines work, because if these collisions and dances don't occur, there will not be good stimulation, memory and duration of acquired immune response.

The take-home from all this science is that if antigen presentation is increased, the magnitude, character and duration of the acquired immune response will also be increased. Some cells already present in the lymph node will interact with the antigen-presenting cells and actually divide, but they won't have the same character or memory as cells that come from the outlying areas into the lymph nodes. This has led to the belief that if delayed delivery methods (in which the antigen doesn't get to the lymph node too quickly) are employed, the acquired immune response can be increased. The other side of the coin is that if in the course of activating the dendritic cells we introduce a lot of inflammation, we may impede the progress of DC. An effective primary response walks on a tightrope. The dendritic cells need an inflammatory response to mature, but a severe inflammatory response with extensive swelling will disrupt the lymphatics and trafficking of the DC to the lymph node.

The Animal's Response: Cellular Basis of Vaccine Protection

Lymphocytes are the key effector cell. Although they are dependent on APC to initiate the acquired immune response, lymphocytes are the cells responsible

for vaccine memory and for the subsequent immune response. Lymphocytes respond to presented antigens by production of cytokines (by T and B cells) or antibodies (by B cells). These have many actions, including control of the adaptive immune response by secondary action on the participating cells, and, in the case of cytotoxic T cells, in killing virally-infected host cells. B and T cells use three lines of attack to fight infection and disease. These approaches can occur alone but often the immune system uses some combination of all three.

#1. Elimination of extracellular pathogens. In response to infection, B-cells mature into plasma cells that secrete antibodies. B cells recognize pathogens using membrane-bound antibody as the antigen receptor. At time of first vaccination, there is no antibody in blood and the level does not begin to increase until 10-14 days after infection. The level of antibody rises slowly, peaks and then gradually declines. This is a primary immune response. On subsequent exposure to the same pathogen, the level of antibody begins to increase within 24 hours and reaches a high level in a few days that can last for weeks or months. This is the secondary or anamnestic response. The immune system produces antibodies that recognize many different types of structures on the pathogen. Serum from an immune animal contains many different types of antibodies, each recognizing different epitopes on the surface of the pathogen. Antibody is secreted on mucosal surfaces and diffuses through tissues to target extracellular pathogens. Binding of antibody to pathogens activates two mechanisms to eliminate pathogens: 1) complement activation with opsinization and/or lysis of the pathogen and 2) phagocytosis by neutrophils and macrophages with intracellular killing.

#2. Elimination of intracellular pathogens in macrophages. Even when antibody and complement opsonize pathogens and phagocytosis occurs, some pathogens are not killed but survive and multiply in macrophages, e.g. *Mycobacterium bovis, M. paratuberculosis* and *Listeria monocytogenes*. Killing of the pathogen occurs through the assistance of T helper cells (T_h cells). T_h cells recognize macrophages containing intracellular pathogens using their T cell receptors. They help macrophages to kill pathogens by synthesizing cytokines that stimulate bacterial killing mechanisms in macrophages.

#3. Elimination of pathogens by cell-to-cell killing. Viruses are obligate intracellular pathogens that can infect many types of cells. During viral replication, virus proteins appear on the surface of the infected cell. A second subset of T cells, cytotoxic T cells (T_c), recognizes these viral antigens on the infected cells. These cells then secrete molecules that kill the virally-infected cells.

Vaccines: Developments in the Types of Vaccines

Modified-live virus. Modified-live virus (MLV) vaccines have been used because of the good antibody response, longer duration of immunity, fewer doses needed per animal and lower cost. These vaccines are administered intramuscularly, intranasally or subcutaneously. MLV vaccines have drawbacks because they can contain adventitious agents and the MLV bovine viral diarrhea virus (BVDV) and infectious bovine rhinotracheitis virus (IBR) are immunosuppressive. Although the return to virulence in MLV viruses has been minimal, mutations will occur and there is some risk of new strains arising. The selective pressure from an animal's immune response to MLV vaccines may lead to new virus strains.

Recombinant MLV vaccines. Recombinant MLV vaccines fall into two groups: gene-deleted MLV vaccines or vectored MLV vaccines. The gene-deleted vaccines were first developed for pseudorabies in pigs. Gene-deleted infectious bovine rhinotracheitis (IBR) vaccines are used in Europe. Using recombinant technology, specific genes are removed from vaccine strains of the virus, reducing virulence. Most importantly, the organism is now "marked", so vaccinated animals can be differentiated from naturally infected animals. Vectored MLV use a different virus as the backbone or vector for the vaccine (i.e. poxvirus or adenovirus). These vaccines are created by recombinant technology where the vector genes are deleted and one or more genes from the pathogen are inserted into the vector. The vector with the pathogen insert is then administered as the vaccine. The vector can be severely attenuated so that it will not be shed from the animal, or be host-restricted so that it will not replicate itself within the animal's tissues. These are now commercially available for Newcastle disease in poultry, canine distemper in dogs and West Nile virus in horses. These vaccines seem to have many characteristics of an ideal vaccine. The vectored MLV are free of adverse side effects, stable, adaptable to mass vaccination, and like the gene-deleted vaccines, allow for differentiation between a vaccinate and an infected animal. At this time there are no vectored vaccines available for cattle.

Inactivated vaccines. Inactivated vaccines contain chemically or physically treated bacteria, toxins and/or viruses so there is no danger of replication in the vaccinated animal of the pathogen or adventitious agents that maybe present in a MLV. Improved adjuvants have increased the scope and duration of inactivated virus immunity. They have several disadvantages including cost, a larger number of doses required per animal and a shorter duration of immunity. Inactivated vaccines gen-

erate cell-mediated responses. Hypersensitivity reactions (allergic) also occur more often with inactivated vaccines.

Subunit vaccines. Subunit vaccines use just the immunogenic portion of an organism. These immunogenic proteins can be purified directly from the pathogen and incorporated into vaccines. Another approach is to produce purified antigen subunits by recombinant DNA techniques. This DNA can then be placed in bacteria, yeast, or other cells that will produce the protein. These protein antigens can be produced in large, pure quantities. *Mannheimia haemolytica* vaccines that contain leukotoxin are an example of a subunit vaccine.

Synthetic peptides. Synthetic peptides use even smaller pieces of the protein then subunit vaccines. These peptides contain just 10-20 amino acids of the most important protective sites on the antigen that are called epitopes. These synthetic epitopes can be made into a vaccine if they are large enough to be immunogenic. Experimental peptide vaccines have been shown to be effective against external parasites such as ticks.

DNA vaccines. DNA vaccines use recombinant DNA techniques to make DNA plasmids that contain the pathogen genes that produce a protective immune response. The plasmid is directly injected in the animal and the DNA plasmid gets in the host's cell where the host actually makes the protein. These DNA vaccines have not been marketed for cattle but a West Nile virus DNA vaccine for horses has been approved. DNA vaccines usually require an initial vaccination with a MLV or inactivated vaccine and the DNA vaccine is usually used as a booster.

Vaccines: Adjuvants

Adjuvants influence the magnitude and quality of the immune response. Adjuvants function by targeting the antigen, influencing antigen processing, activating immune cells, promoting cytokine release and regulating cell receptor expression. Adjuvants can influence the antibody class (i.e. IgA vs IgM), the T-helper response, the cytokine profile and cytotoxic T lymphocyte response. Although they have been used primarily with subunit and inactivated vaccines they are now also being used with modified-live vaccines to enhance immune targeting

What do adjuvants do? There are three logistical functions for adjuvants. The first is activation of the immature antigen presentation cell to a mature cell through the danger signal pathway. The second function is transport — the adjuvant must facilitate antigen uptake by antigen presenting cells (APC) and these APC must deliver the antigen to the draining lymph nodes. This may be achieved by facilitating antigen uptake by APCs, or by increased influx of APCs into the injection site or lymph node. Whichever is the case, the result is the same: an increase in the antigen-loaded APCs for stimulation of naive T cells. The third function is storage and has been called the "depot effect". The depot theory was based on the antigens being stored or sequestered at the site surrounded by APC. This represented what was seen with oil-based adjuvants that developed the so-called sterile abscess. Subsequent research with newer adjuvants has looked at a cellular depot effect. This theory states that these complexes are taken up by APC and can be presented over time so the APC will provide stimulation over time. This process sustains T cell activation and extends an otherwise short-lived response.

Adjuvant Components

Vehicles. The goal of vehicles is to enhance the immune response by sustaining the release of the antigen in the animal. *Metabolizable oils* are mixed with water and form micelles (water-oil-antigen complexes). These micelles form a bilayer that contains the antigens and provides sustained release. *Liposomes* are a mixture of isolated phospholipids and cholesterol that form a bilayer structure with an internal aqueous space containing the antigen. They are synthesized and can be administered with immunostimulants. *Microcapsules* are synthetic polymeric materials that release antigen slowly over time. Degradable polymers like poly-lactide co-glycolide (PLG) can release antigen over 30 days.

Immunostimulants. Immunostimulants fall into four classes: bacterial products, saponins, cytokines and aluminum salts. *Bacterial products* include bacterial cell wall components (TDM and MDP-Tubercle [Mycobacterium] bacilli, MPL-Salmonella) and toxins (cholera toxin-CT). *Saponins* are potent immunostimulants that induce the humoral and cellular immune response. Quil A and QS 21 are two members of this group that are derived from the tree bark of a South American tree. A vaccine mixture called ISCOMS is made by making liposomes in the presence of saponin and antigen. *Cytokines* must be given at the appropriate time and site. Cytokines have multiple effects and the type of response desired must be targeted. *Aluminum salts* (alum) have been used for many years in veterinary vaccines. With these adjuvants, the antigen is adsorbed onto the surface of an insoluble salt. Not all antigens are adsorbed well to these adjuvants. The aluminum complex attracts APC, activates complement and results primarily in an antibody response.

Delivery Methods: Needle-free devices

Types of needle-free devices. Needle-and-syringe devices have been the predominant method for vaccine and drug delivery for cattle. Although needle-and-syringe devices are inexpensive and easily adaptable to different settings, needle-free technology offers significant advantages compared to conventional vaccine delivery methods including enhanced safety for the operator and the animal, enhanced immunogenicity, higher carcass quality because of reduced trim from needle induced abscesses and is beef quality assurance (BQA) compliance friendly. Needle-free injection devices (NFID) can be divided into two types based on the source of power—spring-powered or compressed gas powered. Spring-loaded devices are compact and lower cost but suffer from limited range of force and reduced versatility. Spring-loaded devices have been primarily used for subcutaneous administration of drugs. Gas powered devices (jet injectors) have sustained force generation, greater flexibility and the ability to deliver larger volumes. The main disadvantage is their reliance on an exhaustible energy source. Jet injectors have been used for mass vaccinations and can deliver the target molecule at variety of tissue depths from the dermis to the muscle depending on the force generated by the jet injector. The vast majority of cattle vaccine trials have used gas-powered jet injectors.

NFID and the immune response. The mechanism for an enhanced immune response to antigen delivered via a needle-free injector seems to hinge on the larger dispersion pattern invoked by these devices. More efficient exposure of antigen to cells of the immune system has been demonstrated to facilitate increased immunogenicity. Skin, (including the epidermis and dermis) and the subcutaneous tissue, as opposed to muscle, is one of the largest immune organs of the body, and is rich with antigen presenting cells (APCs) such as dendritic cells (DCs). Delivery of antigen to this area increases the targeting of APCs and results in an enhanced immune response. Studies in mice and people have demonstrated that often a larger quantity and wider variety of antibodies are induced by antigen delivered dermally rather than via intramuscular injections. This is due to increased numbers of DCs in dermal tissues that are APC. The wide dispersion pattern of the antigen using transdermal delivery allows increased surface area contact with APCs compared to conventional needle injections delivered to the muscle that results in bolus dispersion. As discussed above, dendritic cells are the primary APCs to bridge the innate and adaptive immune systems. They can initiate primary T-cell response and efficiently stimulate memory response.

Conclusions

NFID. In addition to delivering vaccine that results in a protective immune response, needle-free vaccine delivery offers significant advantages over conventional needle vaccine administration, including improved carcass quality by eliminating broken needles and reducing carcass bruising and abscesses; smaller volume of vaccine; reduced mechanical spread of infectious disease; and improved safety for workers by eliminating accidental needle sticks when using traditional syringe-needle administration

Suggested Reading

Baizer L, Lacey C, Hayes J, *et al*: Needle-free injectors: New technologies. *The Drug Delivery Company Report. Report 2001/02*:51-54, 2001.

Catron DM, Itano AA, Pape KA, Mueller DL, Jenkins MK: Visualizing the first 50 hr of the primary immune response to a soluble antigen. *Immunity* 21:1-20, 2004.

Davis DM: Intrigue at the immune synapse. *Scientific American* 294:48-55, 2006..

Dufour V: DNA vaccines: new applications for veterinary medicine. *Veterinary Sciences Tomorrow*, Issue 4, http://www.vetscite.org/publish/articles/000020/article.html, 2001.

Itano AA, McSorley SJ, Reinhardt RL, Ehst BD, Ingulli E, Rudensky AY, Jenkins MK: Distinct dendritic cell populations sequentially present antigen to CD4 T cells stimulate different aspects of cell-mediated immunity. *Immunity* 19:47-57, 2003.

Mitragotri S: Current status and future prospects of needle-free liquid jet injectors. *Nature Reviews: Drug Discovery* 5:543-548, 2006.

Schijns VEJC: Activation and programming of adaptive immune responses by vaccine adjuvants. *Veterinary Sciences Tomorrow*, Issue 3, http://www.vetscite.org/publish/articles/000027/article.html, 2000.

Schultz RD: Veterinary vaccines and diagnostics. *Advances in Veterinary Medicine*, Volume 41, Academic Press, San Diego, 1999.