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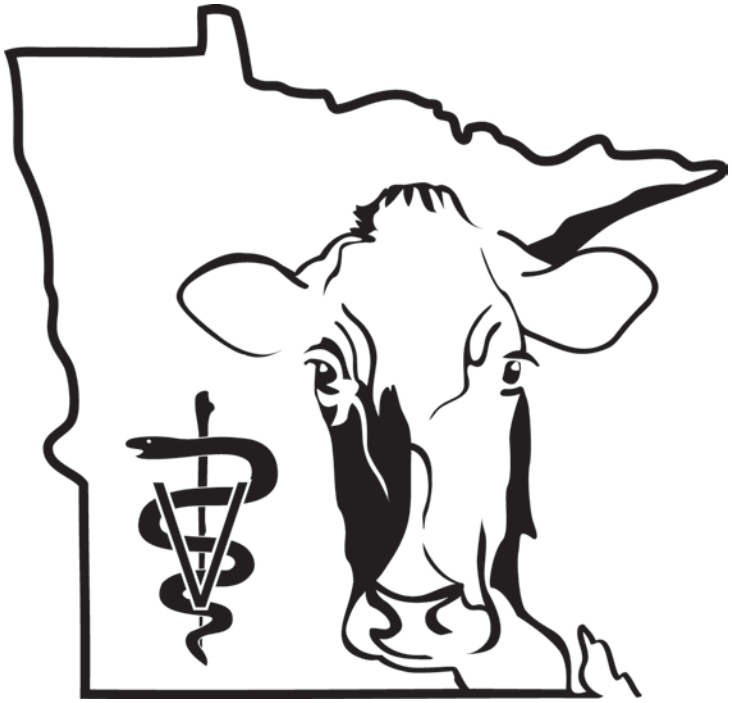

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The Immune Response to Vaccines in Cows- What Gives?

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Primary immune response to vaccines

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 prevent all disease. 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. I will emphasize the primary immune response - the response we want and need to protect and eliminate infectious disease. I will also discuss concepts with regard to vaccines 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 the T or B cells are in the lymph node. The mucosal lymphoid follicles, Peyer's patches, the gut-associated lymphoid tissue, the bronchial-associated lymphoid tissue, are also important lymphoid areas. These organized lymphoid tissues are essential because that is where dendritic cells, T cells, and B cells all come together. Within the lymph node, there are two areas critical for lymphocytes: one is the cortical area where the T lymphocytes are localized and interact with the dendritic cells and the other is the medullar area that contains B cell follicles, where the T- and the B cells interact. Once the primary immune response has occurred, the T cells and B cells can get into the bloodstream and circulate.

Step 1 Activation and Migration of the Dendritic Cells (Antigen-presenting Cells) to the Lymph Node What are the major cells that affect the primary response? The first are antigen-presenting cells that include macrophages and dendritic cells (DC). The dendritic cells arise from macrophage-monocytes lineage. A large percentage of these DC are in the skin or near the skin. In the skin there are Langerhans' cells (a specialized DC) and dermal DC. There are dendritic cells in the laminar propria and the submucosa. These cells are not found in the muscle until the inflammatory response occurs and then 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 DC in the epidermis, dermis and subcutaneous tissue are mainly immature. They act as a surveillance system. Once the DC become activated and mature, they move to the lymph node. For this immature DC to mature and migrate to the lymph node, it has to go through a sequence of events. The immature DC in the tissue are in an inactive state; then the DC senses a danger signal. 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 triggered by molecules produced by pathogenic organisms and/or damaged cells. The

pathogen molecules are called pathogen-associated microbial patterns (PAMPS) and are only produced by pathogens. These PAMPS are present on a wide range of pathogens. For example, one PAMP, lipopolysaccharide (LPS), is present in all gram-negative bacteria, 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 DC. Non-pathogen danger signals include cell debris from necrotic cells, heat shock proteins (HSPs), nucleotides, reactive oxygen intermediates, and cytokines (e.g. interferon). 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 the 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 half of these lymphocytes in the lymphatic system and the other half in the blood. Between the lymph nodes, the afferent lymph, the efferent lymph, and the spleen, these cells circulate continuously. They stay in the blood for only about 30 minutes. They stay in the lymph node for about 12 hours but only stay in the spleen for about five hours. This particular voyage may take a little longer as these cells are constantly moving around.

The cell that interacts with the dendritic cell is the T lymphocyte. These lymphocytes have to get from one lymph node to another. On their surface, they have 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 receptors will help the lymphocyte "home" to different lymph nodes. There will be one combination of receptors if the lymphocyte is going "home" to a mesenteric lymph node and a different combination if it's going to "home" 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 naïve (unstimulated) lymphocyte doesn't express many receptors or adhesion molecules on its surface. It only has one receptor, L selectin, on its surface, and it uses it 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 e.g. "PRRSV antigen in the cervical node". Because the lymphocyte 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 a booster response is needed for a secondary response, cells that have been restimulated and boostered can home back to the right area.

Another important function of the lymph node is holding or sequestering lymphocytes, which occurs following antigen exposure. Because 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, 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 trapped, increasing their chances that they will see the antigen-presenting cell and be able to stimulate the response.

Step 3 The interaction of the dendritic cell and the T lymphocyte in the lymph node (The Collision and the Dance, Part 1) The most important step of the acquired immune response is the T cells interacting with DC. This is strictly a numbers game. What's the chance that a T cell will react with a DC 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 cells 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 lymphocyte will actually "see" its antigen. This increase in the number of antigen-presenting cells and the amount of antigen on their surface is the one of the keys in the ability of adjuvants to increase the effectiveness of vaccines.

To develop an adaptive immune response, these T cell and DC must have two interactions. The two sets of interactions, a collision and a dance, occur in the lymph node or the mucosal lymphoid follicle. The cells are moving at different velocities. T cells are fast; they move at 12 microns per second and dendritic cells move at about three microns per second. The 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. This "dance" can last just a few seconds to a couple of hours.. The longer the DC and T cell can dance together, the more activated the T cell becomes, the more the T cell divides and produces memory T cells and the better the adaptive immune response will be.

Why is the length of the dance important? The series of signaling events or cross talk between the two cells doesn't occur quickly. Looking at the surface of a dendritic cell, it has antigen presented in a MHC II molecule; it is looking for a T cell. 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.

Step 4 The interaction of the T lymphocyte and the B lymphocyte in the lymph node (The Collision and the Dance, Part 2) After the T cell has done its dance with the dendritic cell and has been activated, the activated T cell has to have a collision with a B cell that has recognized the same antigen. These two cells are also moving at different velocities- T cells are speeding along at 12 microns per second while B cells move at about six microns per second. Following the collision, the T cell and B cell have to dance to activate the B cell, just like the DC and T cell did to activate the T cell. The activated B cell divides and produces memory B cells for future antigen memory and plasma cells that produce the antibodies that will be present in the blood and the mucosal surfaces.

Summary-

The primary immune response The primary immune response can occur only if the antigen-expressing DC and antigen-specific T cells *randomly* bump into each other. By increasing the number of antigen-expressing dendritic cells, the chances of the activation of the antigen-specific T cell are increased. In the end, we want as many antigen-containing dendritic cells as possible get to the node. The danger-activated DC expresses 5-10 times as much MHCII on their surface. Increasing MHC expression means that 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- one between the DC and the T cell and the second between the activated T cell and an antigen-activated B cell 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 message for primary immune response is that if antigen presentation is increased, the magnitude, character, and duration of the acquired immune response will also likely be increased. Some of the cells that are already present in the lymph node will interact with the antigen-presenting cells and will actually divide, but they will not 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 does not get to the lymph node too quickly) are employed, the acquired immune response can be increase. The other side of the coin is that if in the course of activating the dendritic cells we introduce too much inflammation, we may impede the progress of DC. An effective primary response walks a tightrope. The DC 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.