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The Immune System – A Military Force

The immune system is vital to the health and wellbeing of any animal species. When this system is mismanaged, it can result in poor immune responses that leave animals susceptible to disease. Conversely, unnecessary immune activation or improper immune regulation results in a loss to efficient production of meat, milk or eggs. The immune system is therefore, not only vital to the animal welfare, but also to efficient and profitable production. There are countless potential feed formulations, functional feed additives, pharmaceuticals and management practices that claim to improve immune function of farm animal species. Therefore, in order to assess all of these possible methods of managing health and productivity of livestock a basic understanding of the immune system is essential.

The Immune System as a Military Force – Training for Battle

We find that the Romans owed the conquest of the world to no other cause than continual military training, exact observance of discipline in their camps, and unwearied cultivation of the other arts of war. - Publius Flavius Vegetius Renatus

When it comes to health and immunity, we can think of our bodies, or those of our livestock, as independent nations and the immune system as that nation's military force. The body is constantly fighting to maintain peaceful homeostasis against numerous potential invaders on multiple fronts. With every element touched, breath taken or bite eaten the body is potentially exposed to enemy troops in the form of bacteria, viruses and parasites. The organs, cells and pathways of the immune system comprise an intricate military like network that guard the host from invasion and wage war on infections.

The first step to building and maintaining an effective military is to have qualified soldiers and leaders. Likewise, immune cells require development and training in order to be effective at maintaining health. The bone marrow is where immune cells are produced and undergo basic differentiation. In this way the bone marrow acts much like a basic training center. Innate cells called granulocytes, are like the infantry soldiers and are common to all immune systems (humans, ruminants, swine, poultry and aquatic species). These granulocytes receive the basic training they need to become infantry soldiers in the bone marrow and then begin to circulate in the blood. If they receive messages indicating there is danger in the tissues they move from the blood into these tissues where they mount rapid, but non-specific attacks on any potential pathogenic invader they encounter.

Conversely, for more evolutionarily advanced cells, like B cells and T cells of the adaptive system (which will be described in detail later), basic training in the bone marrow is insufficient. These cells are higher-level officers of the immune system and as such they require more advanced training in order to play their role in battle, which is to organize very specific attacks on targeted pathogenic enemies.

For the antibody producing B cells, this secondary level training either occurs in the bone marrow in mammals or in a specialized organ called the Bursa of Fabricus in birds (from which B cells derive their name). It is in the bone marrow or Bursa of Fabricus that immature B cells are trained to become functional B cells that can produce antibodies.

For T cells, which act either direct attacks like field officers or conduct specific msissions like special-forces, the secondary level training occurs in the thymus (from which the T cells derive their name). Training in the thymus is especially important in identifying any mutinous T cells that are capable of attacking the hosts own cells. If a traitor T cell is discovered during this training process, it is executed. This ensures that only T cells specific to potential enemies are released into circulation to be available on the battlefields in the tissues.

Specialized training is always best under field conditions. The immune system is no exception. While boot camp occurs in the immune organs before birth/hatch, complete maturation, activation and differentiation of cells often does not occur until after birth/hatch when the immune cells have had time to migrate to the tissues. The tissues offer field condition training, because these tissues comprise the borders of the body where immune cells have the opportunity to encounter microbes (like bacteria, parasites, or viruses) or obtain intelligence information from other cells that have had such encounters. This field training allows the cells of the immune system to diversify and activate, thus improving their attack and defense strategies for future assaults. It should be noted that field training can also be mimicked with vaccination even before birth or hatch (which will be explained in greater detail in later sections).

Specialized organs like the spleen, the lymph nodes and the cecal tonsils (specific to poultry) act like forward operating bases. The spleen is the meeting point for cells that have been training in the blood and perhaps encountered invaders there. The lymph nodes likewise are a meeting point for cells that have had training in in the tissues and the cecal tonsils in poultry are where cells gather training and information from possible enemies in the lumen of the ceca. The location of these basic training centers (bone marrow, bursa of fabricus and thymus) as well as the forward operating bases (spleen, lymph nodes and cecal tonsils) in swine and poultry can be seen in Figure 1.

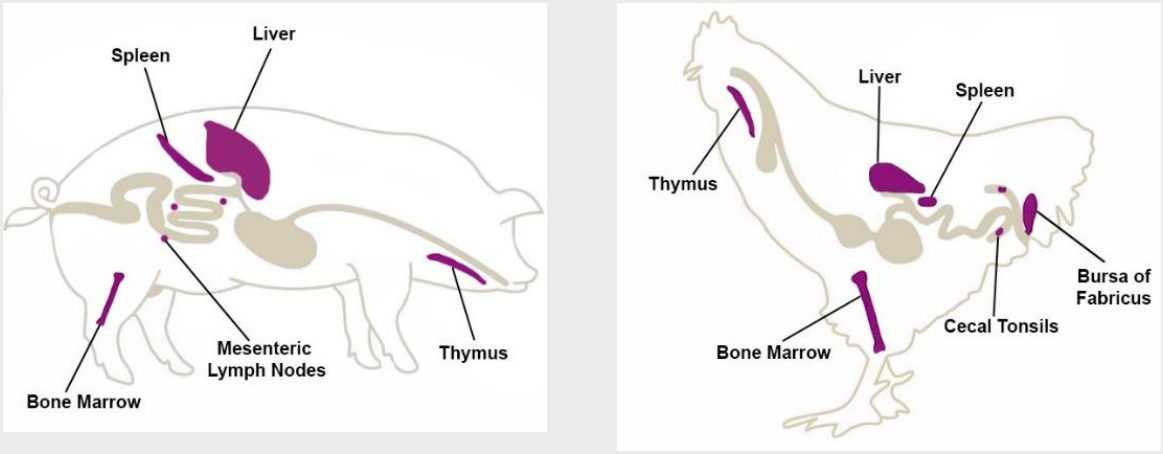

Figure 1: **The immune organs in swine and poultry.** The bone marrow in both species is where all immune cell originate. The thymus, common to both species, is required for training of the T cell officers. The bone marrow in swine and bursa of fabricus in poultry is where B cell generals are trained. The spleen is the immune organ filtering the blood, while the liver is involved in the acute phase response to infections. The lymph nodes in swine and cecal tonsils in poultry act like forward operating bases, receiving messages from the mucosal tissues and gut lumen about possible pathogenic invaders.

The Lymphatic System and Lymph Tissues – The Importance of Infrastructure for Defending Borders

We shall defend our island, whatever the cost may be. We shall fight on the beaches, we shall fight on the landing grounds, we shall fight in the fields and in the streets, we shall fight in the hills; we shall never surrender. -Winston Churchill

Just as an army requires infrastructure to transport soldiers, provisions and equipment, the immune system requires infrastructure to transport cells, nutrients and messenger molecules. The lymphatic system along with the circulatory system, act as this infrastructure for the immune system. For example, the lymph and blood are how immature cells are transported from the bone marrow to the thymus, bursa or tissues for training.

The immune system also requires an infrastructure in order to transfer messages from the front lines of the body where there are conflicts with pathogens, to the upper level adaptive immune cells. The front lines of the body are called the mucosa-associated lymphoid tissues (MALT). MALT tissues line for example the respiratory, gastrointestinal and reproductive tracts. These front line tissues are the borders of the body that are most frequently in contact with the outside world and therefore most at risk.

The tissues of the MALT act as a physical barrier, like a defensive wall to the outside world. The gut for example, is comprised of individual epithelial cells held together by protein bonds known as tight junctions. These junctions, as the name indicates, are tight to prevent invading bacteria, viruses or parasites from breaching this wall and causing harm inside the body. Conditions resulting in a weakening of this wall result in a 'leaky gut' which allows both harmful and commensal bacteria from the gut lumen to come through the wall and induce inflammation (Wijtten et al., 2011; Pearce et al., 2013; Ghareeb et al., 2015).

Tight junctions alone are not able to keep out invaders, so the MALT borders also produce land mines and trip wires to create an inhibitory zone outside the wall as extra protection. For example, specialized goblet cells secrete peptides known as mucins. These mucins create a mucus layer that helps prevent pathogens from getting close to the epithelial cell wall and additionally help to continually wash away potential organisms (Faderl et al., 2015). The epithelial cells of the MALT borders additionally secrete of antimicrobial peptides, like defensins to kill potential pathogens before they can breach the epithelial barrier (Robinson et al., 2015). Another mechanism of pathogen control at the MALT front lines is the secretion of a specialized kind of antibody called secretory immunoglobulin A (sIgA) to neutralize specific threats when they are still beyond the borders in the airways, gut lumen or reproductive tract (Mantis et al., 2011).

The MALT borders are also guarded by specialized cell troops, namely macrophages and dendritic cells. Upon encountering invaders, these cells engulf them through a process called phagocytosis. Once a pathogen has been phagocytosed it can be killed with weapons like reactive oxygen species that damage the pathogen's cell wall or DNA. During this process the macrophage or dendritic cell is also collecting pieces of the invader, known as antigens. These antigens are carried back to meeting points, called follicles, in the forward operating bases like peyers patches or lymph nodes. In these follicles the antigens are presented to T and B cells as vital information about the battles being fought at the mucosal borders (Habtezion et al., 2016). Macrophages and dendritic cells are, therefore, categorized as Antigen Presenting Cells (APCs).

The APCs could be described as the surveillance and intelligence officers of the immune system.

They are not only collecting information about current pathogen attacks, but are also gathering intelligence from beyond the wall to prepare for possible attacks. Some APCs reside just below the epithelial cells and can extend arm like structures, known as dendrites, between epithelial cells to grab at microbes beyond the wall (Foti and Ricciardi-Castagnoli, 2005). Specialized cells in the gut, known as Microfold or simply M cells also help the APCs gather intelligence from the other side of the wall. These M cells are staggered throughout the gut between the epithelial cells

and are gates of transport for messages from the gut lumen to the APCs (Foti and Ricciardi-Castagnoli, 2005). Please see figure 2 for a visual summary of the gut associated lymph tissue as a representative of a MALT.

The information gathered by these APCs at the wall is not only used to convey messages to the T and B cell officers, but is also used to call in basically trained soldiers of the innate system from the blood. These messages sent by the immune cells in the MALT also cause dilation of blood vessels so that granulocytes can more easily reach the tissues where they are needed. This dilation results in the redness and swelling of inflammation.

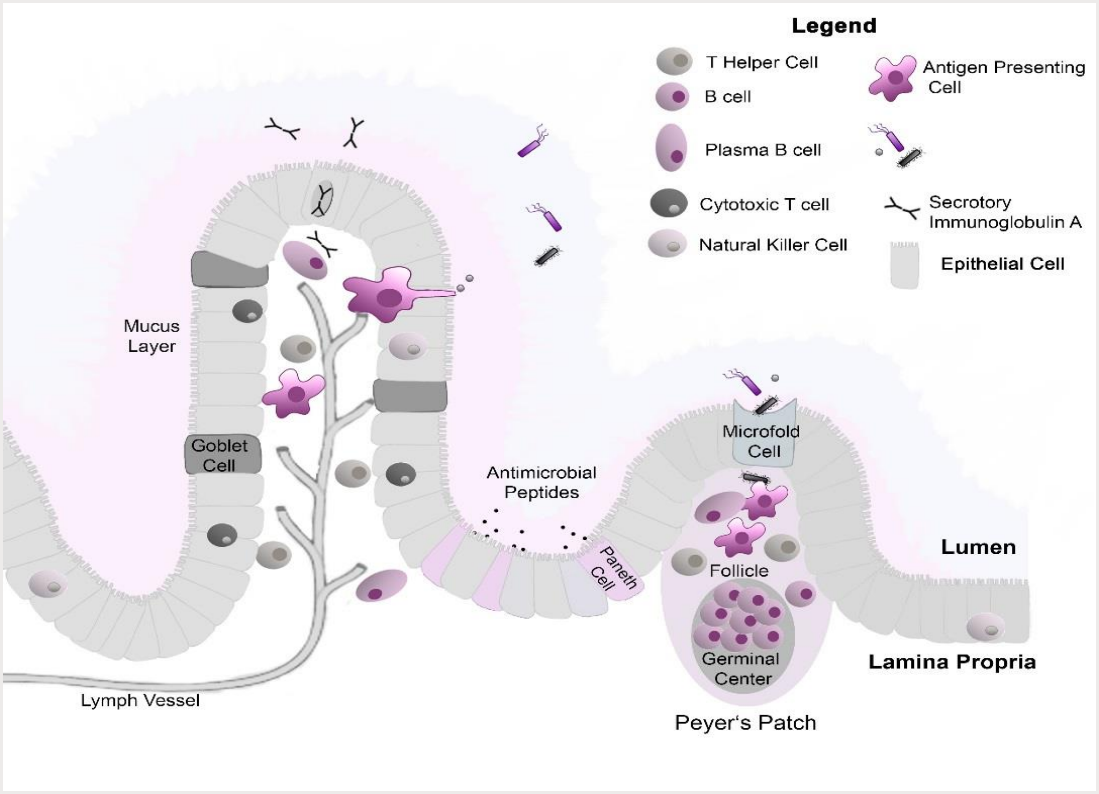

Figure 2: The Gut Associated Lymphoid Tissue. The intestinal wall is comprised of many individual epithelial cells held together by tight junction proteins. Interspersed are goblet cells that produce mucins comprising tightly adherent and loosely adherent mucus layers. Most bacteria in the lumen do not reside in close proximity to the epithelial cells, but remain in or are washed away by these mucus layers. Antimicrobial peptides are secreted by specialized Paneth cells found in the crypts, while secretory immunoglobulin A (sIgA) is produced by plasma B cells and transported through epithelial cells into the lumen. Mucus layers are visibly thinner near microfold cells, whose role is to sense antigens from the lumen and instruct antigen presenting cells (macrophages and dendritic cells) in the underlying tissues. Particularly in specialized tissues known as Peyer's Patches, these microfold cells are often associated with a follicle, a hub where antigen presenting cells can instruct T helper cells and subsequently B cells about potential threats beyond the wall in the lumen. Lymph vessels additionally carry information from the front line GALT to lymph nodes or other lymph tissues for further involvement of the adaptive system lymphocytes (T and B cells). Cytotoxic T cells and NK cells are also present between epithelial cells in case a host cell becomes compromised by an intracellular pathogen and needs

The Local Mucosal Immune System – Soldiers at the Front Line

Older men declare war. But it is the youth that must fight and die. - Herbert Hoover

The cells of the innate system make up the basic defenses in any species with an immune system. As mentioned in the last section about infrastructure, granulocytes do not reside in the tissues but rather in the blood and are only called to action when they receive messages from the APCs known as chemoattractants or chemokines. The granulocytes make up the main group of innate cells moving into tissues during infections and are very effective in defense against pathogens that may have invaded the tissue borders, but remain outside of host cells in the extracellular spaces between like many initial bacterial infections. Granulocytes are not able to distinguish between specific enemies, they identify targets based on general markers that are associated with pathogens, known as pathogen associated molecular patterns (PAMPs).

When a granulocyte encounters such an enemy they have several ways of destroying them. Some granulocytes can produce antimicrobial peptides like the epithelial cells while others behave like the APCs as they phagocytose and kill enemies with reactive oxygen species (Fournier and Parkos, 2012). A particularly interesting weapon that specific granulocytes known as neutrophils (heterophils in poultry) use to disarm an enemy are called NETs. NET stands for neutrophil extracellular trap and occurs when the neutrophil excretes its own DNA to create a net like structure that traps and neutralizes pathogens (Fournier and Parkos, 2012). The described processes by which granulocytes kill pathogens (specifically NETs) also results in the death of the granulocyte itself. Therefore, much like a military infantry, these cells generally have a shorter life span and higher mortality rate than the upper ranks. The pus that sometimes accompanies infections (especially bacterial) is generally an accumulation of these dead soldiers as well as dead pathogens on the battlefield, at the site of the infection.

In the case of viruses or parasites that invade host cells, another type of innate cell known as Natural Killer cells are essential warriors. NK cells act as the internal security forces, because they can detect traitorous host cells that have been compromised by the enemy and kill them before the infection can spread further. Like the granulocytes, NK cells are not specifically trained so must look for general markers that a cell has been compromised. The markers of a compromised cell can be either an antigen presented on the cells surface or the lack of a specific signal that identifies the cell as a being from the same host. In essence, these NK cells are looking for cells that are wearing the wrong uniform and are therefore clearly not from their own troops. If such a marker is recognized, the NK cell kills the traitorous cell in order to prevent the spread of infection to healthy neighboring cells or tissues (Figure 3). NK-cell induced killing is often referred to as the 'kiss of death' as it involves physical contact to induce apoptosis or transfer cytotoxic molecules from the NK cell to the infected cell in order to destroy it (Krzewski and Strominger, 2008).

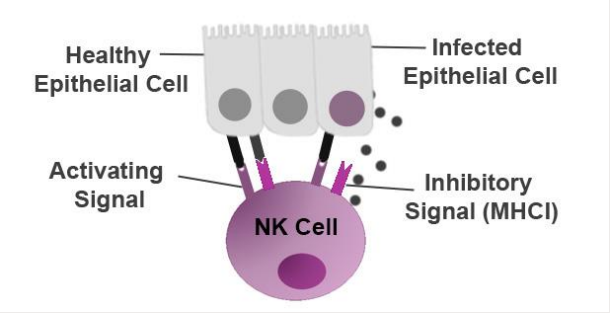

Figure 3: Natural Killer Cell Kiss of Death (adapted from Janeway's Immunobiology 8th Ed.). Natural Killer (NK) cells interact with epithelial cells by adherence of both an activating signal and in the case of a healthy cell an inhibitory signal (major histocompatibility I, MHCI) that instructs the NK cell that the epithelial cell is healthy and from the same host. In the case of an infected epithelial cell, the inhibitory signal is missing or altered. The NK cell cannot identify the epithelial cell as one of its own and proceeds to kill the infected cell with a 'kiss of death' by either sending messages to induce apoptosis of the cell or producing pore forming and cytolytic compounds.

The Adaptive Immune System – An Organized Hierarchy

Ten soldiers wisely led will beat a hundred without a head. - Euripides

The innate system as described in the last chapter is very good at providing a general response to local infections and in some organisms, like shrimp, it is the sole source of immune function. However, this innate immune system is really only comparable to a well-trained militia. A second arm of the immune system, known as the adaptive immune system, has developed (Figure 4). The adaptive system exists in more evolutionarily advanced organisms like vertebrate fish, poultry, swine, cattle and humans. The addition of a more intricate chain of command can result in better organised attacks initially, but most importantly allows for improved training and defense tactics through systematic military education. This is also true of the more intricate level of protection offered by the adaptive arm of the immune system. The cells of the adaptive immune system are the B and T cells (lymphocytes), previously mentioned. These cells act as high-ranking officers to better instruct innate cells throughout an infection and as veterans to store a memory of the attack for a faster, improved response to any similar battles in the future.

B cells are further removed from battles both physically and functionally than the innate cells. They are only able to respond to a very specific antigen message that are relayed from the MALT borders by other cell. For these reasons, B cells may seem ineffective compared to the innate cells that fight at the front lines with any foreign cell they encounter. However, much like an older military general may not at first glance look as menacing as a young infantry soldier, neither B cells nor generals should be underestimated. Rather than fighting with basic instructions at the front line tissues like the innate cells, B cells process very precise information they receive to initiate highly specific attacks that the innate cells are not able to.

While each B cell is very specific and only able to respond to one antigen, there are instances where the antigen is present on more than one strain of a given pathogen. However, in these cases the B cell is still limited to respond only to this very specific range of organisms. These antigens are shown to the B cell most often through a T helper cell that received the information from an APC (Honda and Littman, 2016). Upon activation, the B cell will begin to replicate itself to increase the number of cells specific to that antigen (Honda and Littman, 2016). The activated B cells will also make antibodies (also known as immunoglobulins, Ig) which act like heat seeking missiles when released into circulation. The antibodies assist the innate immune system in fighting infection by neutralizing the specific pathogen and further exposing them to cells like macrophages so that they may be destroyed. The term humoral immunity is used to describe immune responses that involve these B cells and the antibodies they produce.

Field officers are the link between the battlefield troops and the military generals. They instruct the active troops and also relay information to the higher-ranking officers in the chain of command. T helper cells (Th) similarly support the defense tactic for the immune system by instructing both innate cells and B cells. While some Th cells reside in the tissues, many are located in the follicle forward operating bases and do not directly interact with the pathogens themselves. Therefore, they must receive messages from the MALT front lines from the APCs (macrophages and dendritic cells) in order to plan specific attacks and further instruct B cells. Th cells are often referred to as $CD4^+$ T cells in the literature, because they utilize a very important receptor known as CD4 in their recognition of pathogens presented to them from the APCs.

As field officers, Th are posted at different fronts and will face different enemies they must be able to instruct their troops in different ways. Th cells are similarly confronted by diverse assaults and therefore diverse subsets of Th cells exist. A few common examples of Th cell types are listed below; however, it should be noted that this is a brief and simplified view as their roles are diverse and sometimes overlapping.

Th1 cells are the officers mediating responses to intracellular pathogens that have invaded cells, including many types of bacteria and viruses. Th1 cells stimulate inflammation to bring more cells to the site of infection as well as to activate and differentiate these cells to respond in the appropriate way. Th1 cells can also signal for B cells to produce antibodies, though they may be less effective at this task than other Th cell types. Examples of pathogens to which Th1 cell responses are prominent are bacterial *Salmonella enterica* infections in swine (Meurens et al., 2009), and avian influenza viruses infections in poultry (Karpala et al., 2011)

Th2 cell officers initiate responses to extracellular pathogens, such as parasitic worms. This type of Th cell is very effective in stimulating specific B cells to start producing antibodies. Th2 cells are additionally very apt at signalling B cells to switch the Ig structure they are producing to a form (IgE) that is optimal for large pathogens. Th2 cells also attract specific groups of granulocytes known as eosinophils, which are important in fighting complex parasitic organisms. Examples of infections where Th2 cells are involved are the parasitic worm infections caused by *Ascaris suum* and *Trichuris suis* in pigs (Roepstorff et al., 2011) or *Heterakis gallinarum* in poultry (Schwarz et al., 2011).

Th17 cells are often discussed in context of auto-immune diseases in humans. However, these cells are also generally involved in inflammatory responses to pathogens breeching MALT front lines. The Th17 cells organize the deployment of granulocytes, specifically neutrophils, to the site of infection to prevent invaders from disseminating into the rest of the

body. In this way, Th17 cells reinforce barriers like the skin or intestine that may be compromised during the infection. An example of an immune response where Th17 cells are involved is enterotoxigenic *E. coli* infections in post-weaning piglets (Luo et al., 2015).

Cytotoxic T cells, also sometimes called $CD8^+$ T cells in literature, are also a part of the adaptive immune system, but with a more active function than the $CD4^+$ T helper cell subsets. Their main role is to identify host cells that have been compromised by an intracellular infection. Like the NK cells of the innate immune system, cytotoxic T cells are able to kill host cells infected by viruses or parasites while leaving healthy cells alive. The difference between cytotoxic T cells and NK cells is the specificity the cytotoxic T cells have in identifying infected cells and differentiating them from healthy ones. The cytotoxic T cells act as the special forces of the immune system, conducting targeted seek and destroy missions of compromised host cells. The specificity of cytotoxic T cells additionally allows them to create a memory of how to most effectively fight that particular infection again in the future. Once activated by an APC, a cytotoxic T cell can act alone to initiate attacks on compromised cells. However, the cytotoxic T cells are much more efficient if they are guided by activated Th cells that send them a strong 'license to kill' signals.

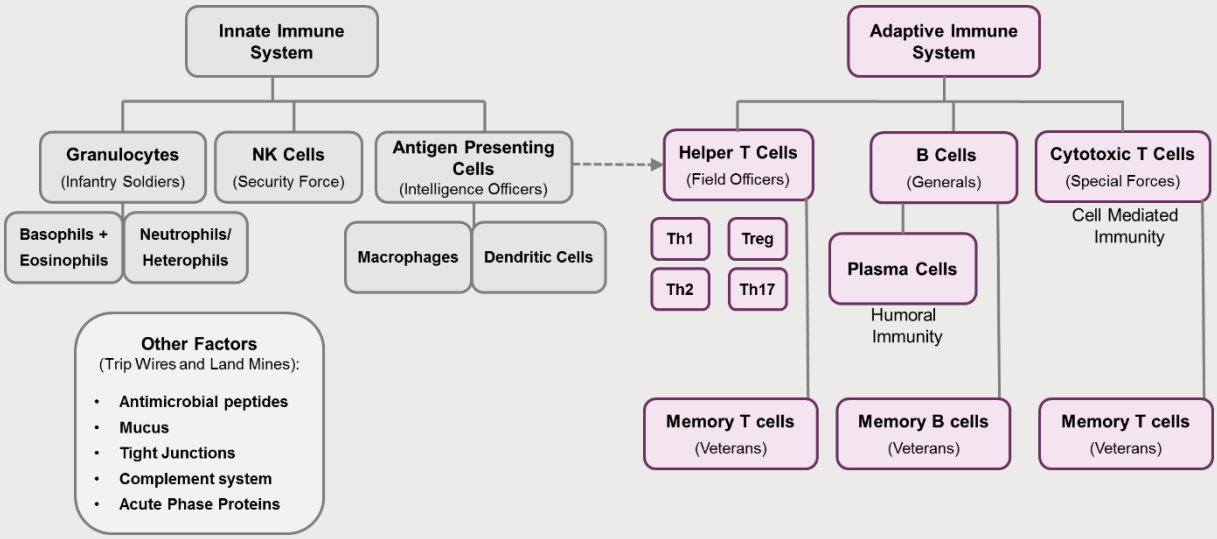

Figure 4:. The classification of immune cells. The immune system of mammalian and avian species comprises the innate system and adaptive system. The innate system contains cells with non-specific pathogen recognition and killing, including the granulocytes, natural killer cells and antigen presenting cells. This system also includes several other non-specific factors that offer a first line of defense to the body. The cells of the adaptive system are highly specific with each lymphocyte (T or B cell) having specificity to only one antigen. These T helper cells are instructed by the antigen presenting cells of the innate system and then differentiate into various functions (Th1, Th2, Th17, Treg). T helper cells of the adaptive system then interact with B cells to create humoral immunity through production of antibodies by plasma B cells or cell mediated immunity through activation cytotoxic T cells. Memory subsets of helper T cells, B cells and cytotoxic T cells are created for faster and more efficient responses upon subsequent challenges.

Memory Function of the Adaptive Cells – A Memory of the Battle will Improve Future Tactics

The two most powerful warriors are patience and time. -Leo Tolstoy

The adaptive system is not only responsible for helping organize an initial response to attack by infectious organisms, but is also responsible for remembering the battle. This memory allows for an extremely fast and efficient defensive attack upon any subsequent interaction with the specific infectious agent it previously faced. During an adaptive immune response, both T cells and B cells will begin to create memory subsets. As T and B cells are very specific to one kind of pathogenic organism, these memory cells must be formed for every individual bacterial, viral or pathogenic enemy encountered.

The memory T and B cells are like war veterans, they have an in depth knowledge of the specific enemy that was encountered so they are both quick to act upon subsequent exposure and are also able to quickly and efficiently instruct new cells on how to fight effectively. Memory B cells also become stronger fighters upon each subsequent pathogen

encounter, through a process much like natural selection. The B cells with the strongest binding affinity for a specific antigen and those that can adapt to further strengthen their capabilities are better able to survive the fight with that pathogen and become the memory cell veterans for the next round of attacks. This results in not only faster, but stronger responses each time a pathogen is encountered.

Vaccines were developed in order to induce memory cell production for faster responses without the need for an initial infection. Vaccinations artificially expose immune cells to pathogens in order to specifically train them to gain a memory of a battle that was never actually fought. During training exercises for military personnel the enemy's behaviour, climate, language and tactics are mimicked in a safe environment to prepare soldiers for the conditions they will encounter when they are dispatched to a war zone. This allows for as close to veteran experience as is possible without actual life-threatening battle. Vaccination utilizes the same principles. Pathogenic bacteria or viruses are either killed or mutated in a way that removes their ability to cause harm and infect, but that does not greatly inhibit the immune system's ability to recognize them. When these inactivated, killed or partial pathogens are introduced to the immune system via vaccination, the adaptive immune cells are trained to produce a fast and competent response if the real pathogen is encountered.

The memory T and B cell veterans are not only beneficial in improving the animal's immune response to future attacks, but also for protecting the offspring of the animal. Antibodies produced by female mammals are transferred to their offspring through milk, while antibodies produced by female birds are transferred to their offspring through the egg yolk. These antibodies, called maternal antibodies, protect the young animal while their own immune system is still developing and being trained through exposure to different microbes in the environment. Vaccinating breeder flocks or sows can therefore increase the protective maternal antibodies these mothers can pass to their offspring. Additionally, technologies like *in ovo* (in egg) vaccination allow us to train the cells of chickens before they even hatch and have a chance to encounter any pathogens. Both strategies improve the animal's chances of survival when battling specific pathogens they may encounter during the early phases of rearing.

Pathogen Adaptation – An Enemy in Hiding

You must not fight too often with one enemy, or you will teach him all your art of war. -Napoleon Bonaparte

Although the creation of memory response through vaccination is an efficient tactic, animal producers do not vaccinate against all potential pathogens. This is due to the cost of the vaccine, the cost of the staff needed to vaccinate and the loss of profit resulting from performance losses that may occur as the animal responds to the vaccine. Just as a military does not have unlimited resources to train and equip their troops, the immune system does not have unlimited resources to train and equip their cells. Each vaccination will require energy and amino acids in order to mount the response required to create memory. Vaccinating for all potential pathogens could mean great losses to performance as resources are diverted from growth and metabolism towards generation of memory B cells, T cells, antibodies and cytokines. For example, while live infectious bursal disease vaccines can be very advantageous in preventing infection in poultry flocks, they can also be costly to the system as there are additional requirements for nutrients like specific amino acids, for the bird to mount a protective response to the vaccines (Maroufyan et al., 2013; Tan et al., 2015).

Another vital reason why vaccination is not a stand-alone strategy for disease prevention is that some pathogens are smart enemies that can adapt to defenses that are commonly used to overcome them. In the context of bacteria, viruses and parasites this adaption often comes in the form of changing the antigens they express on their surfaces so that the memory cells of the immune system can no longer recognize them. This adaptation strategy is like simply changing out of a military uniform into camouflage to hide any obvious distinguishing markers from the immune system defenses. Some examples of pathogens that are very good at adapting and creating new surface markers or even new strains to

avoid immune detection are *Mycoplasma* spp. (Citti and Rosengarten, 1997) and influenza viruses (Kim et al., 2016; Kucharski et al., 2016).

Another strategy that pathogens use to avoid the immune system, is to induce the hosts own regulatory mechanisms of the immune system in order to weaken the active defense strategies against them. Some bacteria like *Salmonella* spp. (Wigley, 2014) and many parasites like *Eimeria* spp. (Miska et al., 2013; Arendt et al., 2016) are very good at these immune suppression tactics. These pathogens attempt to prevent the recruitment of innate cells, the activation of cytotoxic T cells, or B-cell production of antibodies.

Regulatory and Healing Mechanisms of the Immune System – Prevention of Homeland Destruction

If we don't end war, war will end us. -H. G. Wells

The body, like any wise nation cannot sustain a continuous state of all-out war without great detriment to the nation itself. When the battle is won, troops must fall back, rest, recuperate and rebuild the infrastructure. Similarly, if the immune system induction of defense strategies like fever and inflammation are not regulated, they can begin to destroy healthy host cells and tissues. This can eventually lead to the death of the entire organism. For instance, when bacteria, viruses or parasites enter internal systems like the bloodstream, brain, internal organs or abdominal cavity they can cause a systemic inflammatory response (Delano and Ward, 2016). The body wide inflammatory response is so strong, that in an attempt to kill the invading bacteria, the host is unable to withstand the intensity of the defense and dies. This can be related to what may happen if a nation's military decided to fight a war with nuclear arms; potentially killing the enemy, but also its own country and people. This scenario not only hopefully highlights the importance of self-regulatory mechanisms, but also acts as an important reminder of why the front line MALT like that of the gut, airways and skin must be so efficient in protecting the internal systems from invasion of outside forces.

Several specific cell types are very important to the process of immune regulation. Regulatory T cells (Tregs), are the peacekeepers of the immune system. These cells are induced during the end of a response to infection and are responsible for sending out cytokine messengers that inhibit the T cell officers (Th1, Th2 and Th17), T cell special forces (cytotoxic T cells) and B cell generals from initiating responses (Goodman and Pizarro, 2013). Certain phenotypes of macrophages and dendritic cells reside in MALT are also important in down-regulating immune activity to maintain homeostasis (Farache et al., 2013). These regulatory cell phenotypes are responsible for the cessation of active immune responses once an infection has been successfully cleared and they are no longer needed.

The disruption in immune regulation also delays wound healing (Leoni et al., 2015). For example, after an infection has been fought, macrophages may tell innate infantry soldier cells to retreat while cleaning up the debris left behind from their fallen comrades and enemy bacteria (Leoni et al., 2015). Meanwhile, specialized cells called platelets have been clotting the blood in the affected area damaged by the infection. This dams up broken blood vessels and closes wounds that may act as weak points for entry of additional pathogens. In the gut, wound healing involves the proliferation of the epithelial cells that make up the intestinal villi and strengthening of the barriers between these cells to reinforce the security wall that protects the internal host from the contents in the intestinal lumen. Signals from specialized wound healing macrophages as well as regulatory dendritic cells and T cells all communicate with the epithelium in order to induce epithelial cell proliferation to replace intestinal cells that were damaged or lost in battle (Iizuka and Konno, 2011; Leoni et al., 2015).

Immune Homeostasis – Preventing Friendly Fire

Great is the guilt of an unnecessary war. -John Adams

The regulatory mechanisms described in the previous section not only instruct the immune system to regroup and retreat after a war, but are also important in maintaining homeostasis when harmless outsiders arrive at the borders. For

example, commensal bacteria in the gut are harmless and often very beneficial foreigners in the gastrointestinal system. They can provide essential goods and services, like the production of vitamins or short chain fatty acids from components of the feed that may otherwise be indigestible by the hosts own digestive mechanisms. Specific probiotic strains of bacteria can act as highly skilled expats. In return for a home that is safe and bountiful, these probiotics can aid in the digestion and the protection of the system from harmful invaders through numerous different mechanisms (O'Toole and Cooney, 2008).

Mechanisms that regulate immune responses are very important in maintaining good diplomatic relations with friendly foreigners like beneficial bacteria. For example, a lack of regulatory mechanisms in the gut would result in an immune response to commensals, probiotics or even molecules from the feed that are present in the lumen. Not only could this harm the diverse microbiota that would otherwise support proper function of the body, but it could also result in unnecessary damage to self-tissues (Omenetti and Pizarro, 2015). Commonly encountered examples of this overreaction are observed in human auto-inflammatory disorders such as Inflammatory Bowel Disease and Asthma. A similar occurrence can be observed in piglets after weaning as intestinal inflammation is induced during this period, but is often not attributed to any identifiable pathogen (Pie et al., 2004; Huang et al., 2012; Pedersen et al., 2012). The failure of piglets to thrive in this post-weaning period is not simply due to stress-induced anorexia, but also a result of an unnecessary immune response. This auto-inflammation has been shown to be reduced when piglets are inoculated with immune modulators that induce regulatory cell types (Whelan et al., 2014).

While the immune system generally provokes images of vaccine induced protection or localized defense, the regulatory arms should be recognized and appreciated as a vital part of animal health. Research into manipulation of these regulatory pathways may elucidate ways in which excessive and damaging inflammation, as can be seen in the intestines of post-weaning piglets, can be avoided. Additionally, while strong responses to pathogens are the quickest ways to defeat these enemies, the quicker the win can be recognized and the troops retreated the faster the body can restore normal function and redistribute energy from immunity back to metabolism and growth.

Resource Allocation to the Immune System – Wartime Rationing

Those who are at war with others are not at peace with themselves. -**William Hazlitt**

Having an active military is necessary to the safety and autonomy of any nation. However, continuous mobilization and activation of the armed forces can be detrimental to the infrastructure, resources and overall wellbeing of a country and its people. The body in parallel suffers the same consequences with aberrant activation and mobilization of the immune system. As we strive towards healthier and more efficient production of animal protein for human consumption, we should consider how to control over-stimulation of immune responses that negatively affect performance while providing everything the animal needs in order to successfully ward off detrimental pathogenic invasion.

Performance losses incurred during health challenges are largely the result of the energy and nutrients expended to mount a strong enough immune response to defeat the disease. Recent meta-analysis studies in poultry and swine show that not only is feed intake reduced in incidences of infection or poor sanitation, but also the maintenance requirements of the animals appear to increase (Pastorelli et al., 2012; Remus et al., 2014). Perhaps most interestingly was that despite treatment, mostly with antibiotics, these performance aspects were still negatively impacted by the infections (Remus et al., 2014). These studies suggest that even when the immune system is supported with early detection and medical treatment, resources are still likely being diverted from the metabolic processes required for growth in order to support immune function. This is supported by the observation that the metabolic pathways prioritized change in inflammatory states, resulting in increased catabolism of tissues to meet nutrient requirements of the immune system (O'Neill et al., 2016). Such findings provide evidence that the animal's biological systems have likely evolved to quickly redistribute nutrients and energy away from growth to the more urgent requirements of the immune system to ensure survival in times of crisis when pathogenic invasion occurs.

In addition to resources being allocated to the immune system and away from protein deposition, immune stimulation can lead to a reduction in feed intake. A local infection can lead to an increase in inflammatory cytokines in the blood and the production of acute phase proteins in the liver. These inflammatory cytokines and acute phase proteins activate what is known as the hypothalamic-pituitary-adrenal axis which results in hormonally driven reduction in feed intake (Barb et al., 2001; Quinteiro-Filho et al., 2012; Bazhan and Zelena, 2013).

The performance losses observed due to disease are two fold when infections occur in the gut. Not only do losses in feed intake and diversion of nutrients to the immune system occur in intestinal infections, but the damage to the gut also impairs digestion and absorption of nutrients (Su et al., 2015). Much like the scorched earth warfare tactic, pathogen induced intestinal damage reduces the infrastructure by which the immune system can obtain resources it requires to fight. An example of an intestinal condition with diverse negative impacts on growth is that of Necrotic Enteritis. Necrotic Enteritis is a multifactorial disease affecting broiler chickens globally. It often begins when broilers are infected with various species of *Eimeria* parasites that disrupt digestive and immunological functions that predispose the animal to bacterial infections (Timbermont et al., 2011; Rochell et al., 2016). The causative agent of Necrotic Enteritis, pathogenic strains of *Clostridium perfringens* bacteria are then able to proliferate and produce toxins that lead to necrotic lesions in the gut (Skinner et al., 2010; Timbermont et al., 2011). These lesions further impair the feed intake, nutrient absorption and feed efficiency of the affected broilers as nutrients are diverted from growth to immune responses (Skinner et al., 2010; Timbermont et al., 2011). It is therefore unsurprising that gut health challenges are some of the most costly to the livestock industry. Necrotic Enteritis for instance, has been estimated to cost up to US$1480 per flock of 20,000 over an entire production cycle (Skinner et al., 2010) and US$6 billion annually to the global poultry industry (Wade and Keyburn, 2015). This highlights the need to fully understand the immune processes involved in livestock infections, especially those of the digestive tract, in order to establish management and feeding practices that support these immune responses with minimal losses to performance.

Functional Feed Additives – A Sustainable Arsenal

He who wishes to fight must first count the cost. -Sun Tzu

It is clear that there are costs involved in mounting and maintaining an immune response. However, we have a diverse arsenal of weapons and tactics at our disposal to support a well-functioning, but energetically efficient immunological military force. For animal husbandry this arsenal includes many aspects including management, nutrition, feed additives and pharmaceuticals.

Of course, in the decision making process it should be understood that each changing aspect also comes with costs. Changing management practices may involve updating physical structures and systems within a production facility and retraining staff. Nutritional strategies involved in health may reduce efficiency of animal protein deposition and/or increase the cost of feed. Non-nutritive additives, like probiotics or prebiotics, likewise increase overall feed costs. Immune modulatory feed additives may unnecessarily reallocate energy resources to the immune mechanisms even in times of peace, where no pathogen challenge exists. Prophylactic in-feed antibiotics (where not already governmentally banned) disrupt the normal physiological and immunological development of animals and increase antibiotic resistance in bacterial population, which may pose a real threat to future treatment of disease in both animals and humans. Pharmaceuticals not only add to productions costs, but are administered upon signs of clinical infection and therefore production losses due to disease that have already been incurred. Vaccines are not available for all potential diseases and stressors that may negatively impact animal welfare and performance.

Obviously, there is no one clear solution that applies to all species, diseases or producer's needs. With new products and strategies continually being developed, each individual producer must be able to critically compare all available options. The selection of a suitable feed additive should include a cost to benefit analysis that includes not just the cost

of the additive itself, but weights any losses to performance that may be caused by immune stimulation to any losses that could otherwise be incurred by the health issues they aim to prevent.

In addition to the many options available to improve animal health there are numerous stakeholders involved in the decision making process such as the government regulatory agencies, feed producers, livestock managers and consumers. This makes selection of strategies to balance the cost of disease and cost of treatment or prevention exponentially more complicated. While understanding the intricacies of the immune system may appear to confuse an already complex equation of cost analysis and welfare outcomes, a basic understanding of the mechanisms involved within the animal in maintaining healthful homeostasis is a vital part of the evaluation.

We aim to continue reviewing the various health issues facing the animal agriculture industry and the diverse strategies to deal with them in future AminoNews issues. Therefore, we hope this review has imparted a fundamental understanding of how the immune system operates to prevent and fight infection. We believe this will assist in the critical evaluation of the risks associated with different challenges your operation faces and the suitability of the diverse strategies available. After all, management practices, nutrition strategies and feed additives are merely assets to the most important player in the war on disease; the animal's immune system.

Abbreviations

APC – antigen presenting cell

Ig – immunoglobulin

IgE – immunoglobulin E

M cell – microfold cell

MALT – mucosa-associated lymph tissue

NET – neutrophil extracellular trap

NK cell – natural killer cell

PAMP – pathogen associated molecular pattern

sIgA – secretory immunoglobulin A

Th cell – T helper cell

Treg – regulatory T cell

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