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A review of host pulmonary defenses with reference to cattle

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Abstract

Bovine respiratory disease is a leading cause of morbidity and mortality for the cattle industry. Cattle can be protected from respiratory disease by both structural and dynamic defense mechanisms of the respiratory tract, which are critical for prevention of pathogen entry and colonization. Dynamic defenses include those mediated by the innate and adaptive branches of the immune response. The immune response to pathogens is complex, and requires immune cell migration, proliferation, and adaptation. Secretion of molecules, including cytokines, host defense peptides, and opsonins, by cells within the respiratory tract promotes respiratory defense, including pathogen neutralization and phagocytosis. Ongoing discoveries in the field of immunology should guide the development of novel immunotherapeutic interventions for the prevention and treatment of bovine respiratory disease.

Key words: bovine, immunology, respiratory, innate immunity, adaptive immunity

Résumé

Le complexe respiratoire bovin est l'une des principales causes de morbidité et de mortalité dans l'industrie de l'élevage bovin. Les bovins peuvent être protégés de ces maladies par des mécanismes de défense structuraux et dynamiques des voies respiratoires qui préviennent l'entrée et la colonisation des pathogènes. La ligne défensive dynamique inclut les réponses immunitaires acquises et adaptatives. La réponse immunitaire contre les pathogènes est complexe et nécessite la migration, la prolifération et l'adaptation des cellules immunitaires. La sécrétion de molécules par les cellules des voies respiratoires, telles que les cytokines, les peptides de défense de l'hôte et les opsonines, facilite la défense respiratoire autant par la neutralisation des pathogènes que par la phagocytose. Les nouvelles découvertes en immunologie devraient guider le développement de nouvelles interventions immunothérapeutiques pour la prévention et le traitement du complexe respiratoire bovin.

Introduction

Bovine respiratory disease (BRD) is the result of complex interactions between the environment, respiratory pathogens, and the bovine immune response. The respiratory tract has a large epithelial surface that is continuously exposed to pathogens and irritants. Management practices such as commingling of cattle increase opportunities for pathogen exposure. Therefore, pulmonary defense mechanisms are critical to limit pathogen invasion and prevent development of BRD.

The substantial economic impact of BRD has led to a number of investigations into bovine pulmonary immunity that have improved understanding of the pathogenesis of BRD and the interactions between pathogens and the innate (or non-specific) and the adaptive (or specific) branches of the immune response. Defense against pathogen invasion of the respiratory tract involves a combination of structural impediments to colonization and dynamic host defenses. Dynamic respiratory defense encompasses the response of resident immune cells, migrating immune cells, and the airway epithelium (Figure 1) through their secretion of cytokines (Table 1) and antimicrobial molecules. The purpose of this article is to review both the innate and the adaptive immune response of the pulmonary system, focusing on the significance of these immune responses in BRD.

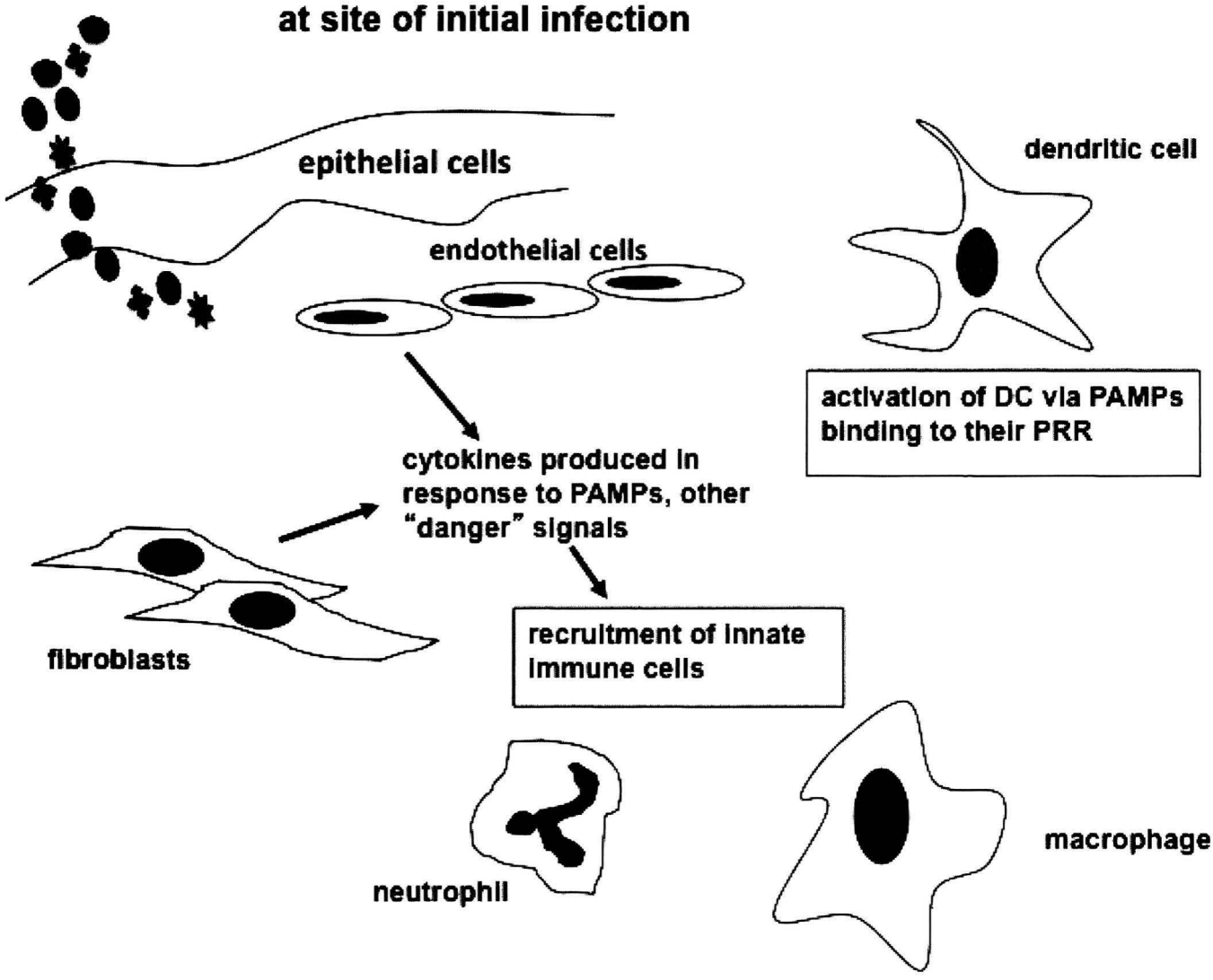

Figure 1. Schematic diagram of the early steps in the immune response to infection across an epithelial surface such as the respiratory tract. PAMP = pathogen-associated molecular pattern. DC = dendritic cell. PRR = pathogen recognition receptor.

Innate Immunity

Innate immunity provides the initial defense against invading pathogens. The innate response is rapid and relatively non-specific. Innate immunity can be divided into structural or mechanical defense, and cellular and molecular defense. Cellular and molecular defenses mediate neutralization of pathogens by secreted molecules, including host defense peptides and cytokines, and pathogen phagocytosis.

Mechanical Defenses

Within the pulmonary system, structural impediments to pathogens include conformation of the airways, the cough reflex, and the mucociliary apparatus. The nares are lined by squamous epithelium, which resists pathogen colonization in part through rapid cell turnover.[40] The structure of the nasal turbinates promotes turbulent air flow. Turbulence allows particles, including pathogens, to settle out of the inhaled air and become embedded in mucus on the surface of the epithelium of the upper respiratory tract. Particles that are trapped in mucus are then removed from the respiratory tract, in part through the activity of the mucociliary apparatus.

The mucociliary apparatus consists of ciliated cells, which allow for unidirectional transport of pathogens and inspired debris away from the lower airways, and secretions which trap large (>5 μm) particles and help

Table 1. Key cytokines involved in immune function. IL = interleukin; NK = natural killer; Th = T helper; Ig = immunoglobulin; MHC = major histocompatibility complex; TNF = tumor necrosis factor; TGF = transforming growth factor; IFN = interferon.

Cytokine	Function
IL-1β	Proinflammatory; T cell activation
IL-2	Promotes proliferation of T cells, B cells and NK cells
IL-4	Differentiates T cells to Th2 cells; suppresses Th1 development; promotes IgE expression
IL-5	Eosinophil activation and chemotaxis
IL-6	Proinflammatory; stimulates acute phase response; activates B cells and production of IgA, IgG, IgM
IL-8	Neutrophil chemotaxis
IL-10	Antiinflammatory interleukin; downregulates MHC class II on macrophages
IL-12	Induces differentiation of T cells to Th1; stimulates NK cell proliferation
TNFα	Proinflammatory; activates cytotoxic T cells
IFNγ	Stimulates macrophages and NK cells, induces MHC I and II expression, promotes Th1 differentiation
TGFβ	Antiinflammatory; suppresses MHC II expression

kill pathogens.[7] Secretions are produced by the epithelial cells in the lower airways, and contain primarily water (>95%), with electrolytes, proteins, sugars, glycoproteins, and lipids.[27,44] Secretions are divided into an underlying fluid sol layer, which allows for movement of the cilia, and an overlying gel layer.[1] The gel layer is composed predominantly of mucoproteins, which are secreted into the large airways and help trap large particles. Mucoproteins such as mucin can also bind to bacteria, enabling their removal by immune cells, which are activated upon binding to mucin.[1] Respiratory secretions also contain antimicrobial molecules including enzymes and host-defense peptides, which help to trap and kill pathogens. Host-defense peptides are a diverse class of secreted molecules that contribute to immunity by direct microbiocidal activity or modulation of the host response to pathogens and inflammation. Finally, pathogens and debris trapped in respiratory secretions are cleared by the cough reflex, which promotes rapid expulsion from the lower airways.

Cellular Defense

Cells within the respiratory tract provide a vital function in neutralization of pathogens. These cells include respiratory epithelial cells, lymphocytes, dendritic cells, alveolar macrophages (AMs) and polymorphonuclear leukocytes (PMNs). In cattle, intravascular macrophages residing within the pulmonary circulation also play an important role in the pulmonary immune response.[33] Alveolar macrophages and PMNs phagocytose pathogens and, along with the respiratory epithelial cells, secrete molecules including host-defense peptides and cytokines. These secreted molecules help to neutralize pathogens and recruit more immune cells to the site of infection. Host-defense peptides have direct antimicrobial properties and also influence the immune response through influencing cytokine secretion in response to pathogen invasion. Cytokines are chemical messages that initiate inflammation and coordinate immune function, with certain cytokines being critical for activation of the adaptive immune system.

Respiratory epithelial cells have historically been recognized as an important physical barrier to pathogens. Recent findings suggest that they can also contribute to host defense by secretion of multiple host-defense peptides and cytokines following pathogen recognition. Moreover, respiratory epithelial cells can induce dendritic cell differentiation and migration[30] and can release signaling molecules that promote maturation of B cells and isotype switching to the mucosal immunoglobulin, IgA.[19] Epithelial cells transport IgA produced by submucosal B cells into airways.[19]

Dendritic cells survey the region of the respiratory epithelium for foreign substances such as pathogens. When a dendritic cell identifies a pathogen, it engulfs the pathogen and travels to a local lymph node, where it "presents" pieces of the pathogen (antigens) to naïve T and B cells, which are major contributors to the adaptive immune response (Figure 2). Thus, dendritic cells have a key role in activating the adaptive immune response. Because of these activities, dendritic cells are in a class of cells known as "antigen presenting cells" (APCs).

Lymphocytes are located within the vasculature, parenchyma, bronchoalveolar spaces, lamina propria, and bronchus-associated lymphoid tissue (BALT) and are a critical part of both innate and adaptive immunity. In cattle, lymphocytes are found in relatively high numbers within the vasculature,[32] whereas they make up a relatively small proportion (~3 to 7%) of cells found in bronchoalveolar lavage (BAL) fluid and lung parenchyma.[35,36]

Some lymphocyte sub-populations (i.e., B and T cells) are part of the adaptive immune system and will be discussed later in this review. However, both natural killer (NK) cells and gamma delta (γδ) T cells are classes of lymphocytes that are considered part of the innate immune system. Natural killer cells and γδ T cells differ from T cells and B cells of the adaptive immune system in their ability to recognize and respond to a broad range of infectious agents. In contrast, an individual

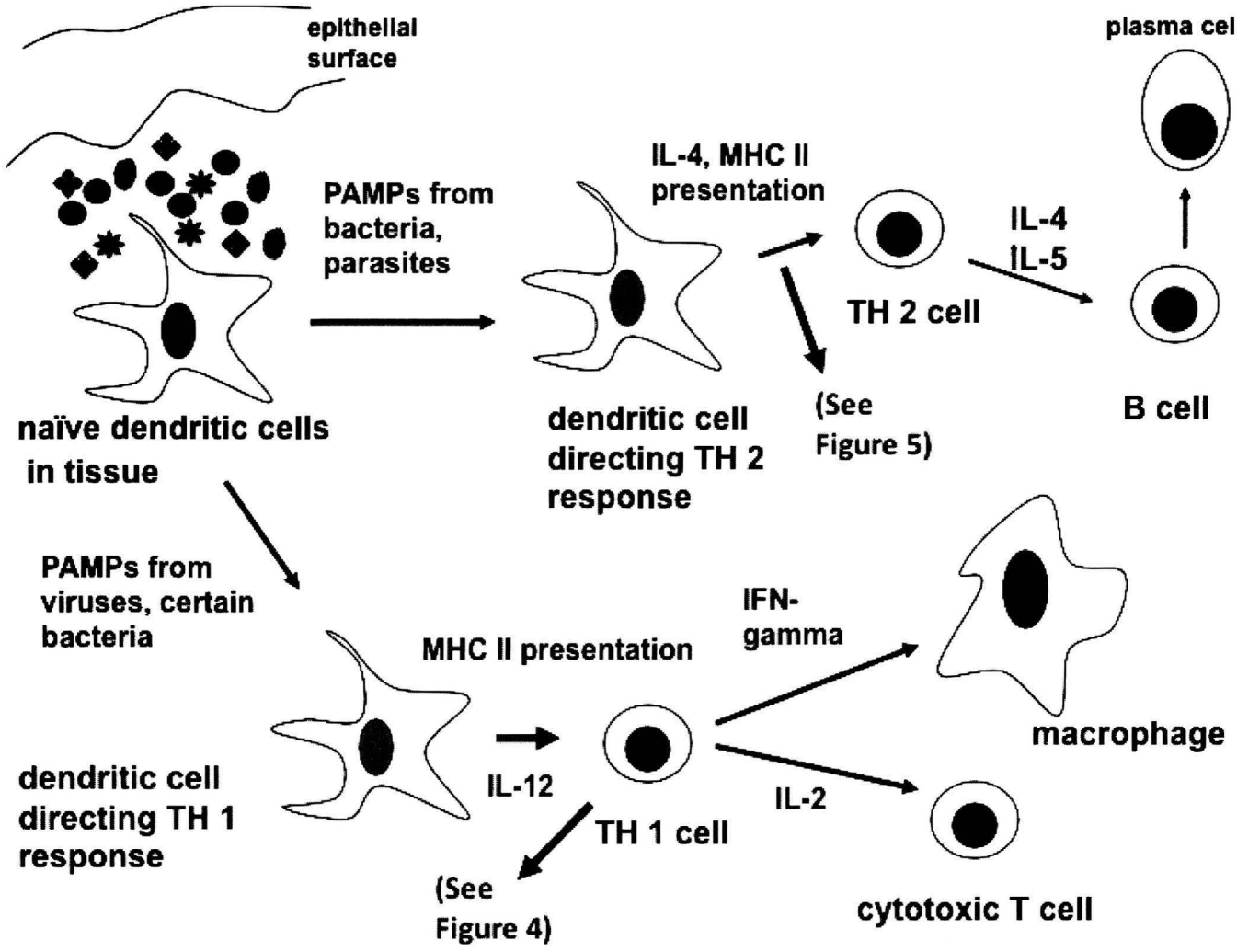

Figure 2. Activation of helper T (Th) 1- and Th 2-mediated immunity following dendritic cell response to pathogen exposure. PAMP = pathogen-associated molecular pattern. MHC = major histocompatibility complex. IL = interleukin.

T cell or B cell can respond only to a single antigen, which generally represents only a single pathogen. The breadth of the NK cell response is due to the cells' ability to recognize changes in an important molecule on the surface of all nucleated cells, the major histocompatibility complex Class I (MHC I) molecule. Infection of a cell with a virus or other intracellular pathogen will result in altered expression of MHC I on the surface of the infected cell. Natural killer cells recognize these changes, and kill or destroy cells expressing abnormal MHC I. Natural killer cells also recognize the absence of MHC I molecules on cells, and kill cells lacking MHC I. This is useful because some viruses, such as bovine herpesvirus-1 (BHV-1), cause infected cells to decrease (downregulate) their expression of MHC I.[55]

Interestingly, γδ T cells have no MHC restriction, which means they do not respond specifically to MHC molecules.[9] While γδ T cells are typically considered part of the innate immune response, they have some features more typical of the acquired immune response, such as memory functions,[38] and the ability to regulate T cell activation.[22] Thus, γδ T cells can be thought of as "in between" innate and adaptive immunity. It is believed that γδ T cells have unique functions in cattle compared to other domestic species or humans. In cattle, γδ T cells can be activated by pathogen-associated molecular patterns (PAMPs[28]) or by the cytokine, interleukin-12 (IL-12[41]) to produce interferon-γ (IFNγ), a cytokine which is necessary for effective immunity to viruses and other intracellular pathogens. Also, γδ T cells have the ability to kill infected cells, which appears to be age-dependent in cattle.[22]

Polymorphonuclear leukocytes, particularly neutrophils, play a critical role in microbial defense.

Although PMNs are typically found in small numbers (<10%) within the alveoli,[35,36] cytokine release from respiratory epithelial cells and immune cells following pathogen invasion results in recruitment and activation of PMNs. Polymorphonuclear leukocytes phagocytose and kill invading extracellular pathogens. Cytokines bind to receptors on PMNs, causing these PMNs to release even more cytokines. Although the concentration of neutrophils within the lower airways is low, the pool of marginating neutrophils within the pulmonary vasculature consists of ~40% of the body's total PMNs,[58] and recruitment of neutrophils to the airways in response to respiratory infection is an important mechanism of host respiratory defense.

The alveolar macrophages are the primary phagocytic cell within the lung, comprising approximately 80 to 85% of cells found in bovine BAL fluid.[25,35,36] Alveolar macrophages are critically important because they initiate the inflammatory response that initiates the host response to infection through their production of proinflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α). These proinflammatory cytokines induce changes in the vasculature that allow PMNs and other cells to migrate into the airways to participate in the immune response. Alveolar macrophages also play an important role in clearance of PMNs that have died during the fight against pathogens, and resolution of inflammation in pneumonia.[18,31]

Previous work suggests that pulmonary intravascular macrophages also play an important role in immunity against bacterial pathogens in cattle.[33] In ruminants, pulmonary intravascular macrophages can produce thromboxane,[52] which may contribute to the thrombosis and infarction that occurs with some types of bacterial pneumonia.[33] In addition, pulmonary intravascular macrophages have been shown to be a source of TNFα, a proinflammatory cytokine, in a model of *Mannheimia haemolytica* pneumonia. Furthermore, depletion of intravascular macrophages attentuates pulmonary inflammation associated with pulmonary *M. haemolytica* infection.[50]

In addition to lymphocytes, PMNs and macrophages, eosinophils can also play a role in the immune response. Eosinophils are primarily associated with mucosal surfaces,[47] and are activated by pathogens via PAMPs or directly via complement. Following activation, eosinophils release their protein granules, proteinases, and cytokines, influencing innate and adaptive immune responses.[47] Although best known for their role in allergic and parasitic diseases, eosinophils also have other functions, including direct antibacterial activity.[47] Furthermore, eosinophils have been shown to help resolve acute inflammation.[26] Interestingly, in one study, calves with lower eosinophil counts at arrival to research facilities had a higher risk of being diagnosed with BRD during a 42-day receiving period.[43]

Cellular Signaling: Pathogen Recognition Receptors

Dendritic cells, respiratory epithelial cells, and AMs all have pathogen recognition receptors (PRRs) on their surface that recognize PAMPs. These PAMPs are conserved peptides, carbohydrates, lipids, or nucleic acid fragments that are common to a variety of infectious agents. Recognition of PAMPs thus allow immune cells to respond to a wide range of pathogens, even if the pathogen has never been previously encountered by the host. Some of the most well-studied pathogen recognition receptors are the Toll-like receptors (TLRs) and the nucleotide-binding oligomerization domain (NOD)-like receptors.

The TLRs are identified by a sequential numbering system (Table 2). Toll-like receptors 1, 2, 4, and 6 are present on the outer surface of cells, and are activated by microbial surface antigens. In contrast, TLRs 3, 7, 8, and 9 are intracellular, and recognize nucleic acids from phagocytosed microbes or microbes that are generated within cells, such as replicating viruses.[54] Activation of a TLR sets off a chain reaction of events within the cell (referred to as intracellular signaling) that promotes increased transcription of inflammatory cytokines and surface expression of other molecules necessary for the response to infection (Figure 3). Airway epithelial cells have been shown to primarily express toll-like receptors 2 through 6.[24,46]

The NOD receptors are intracellular receptors that are primarily expressed in lymphoctyes and APCs.[16] Similar to TLRs, NOD-like receptors activate pathways which increase production of inflammatory cytokines and chemokines. NOD-like receptors also upregulate production of antimicrobial peptides and induce apoptosis.[16]

Table 2. Identified toll-like receptors (TLRs) and their respective pathogen-associated molecular pattern (PAMP).

Toll-like receptor	PAMP recognized
TLR 1	Triacyl lipopeptide of mycobacteria
TLR 2	Peptidoglycan, lipotechoic acid, mycobacterial lipoarabinomannan
TLR 3	Double stranded RNA
TLR 4	Lipopolysaccharide (LPS)
TLR 5	Flagellin
TLR 6	Diacyl lipopeptides of mycoplasma
TLR 7	ssRNA
TLR 8	ssRNA
TLR 9	CpG (bacterial DNA)
TLR 10	Unknown

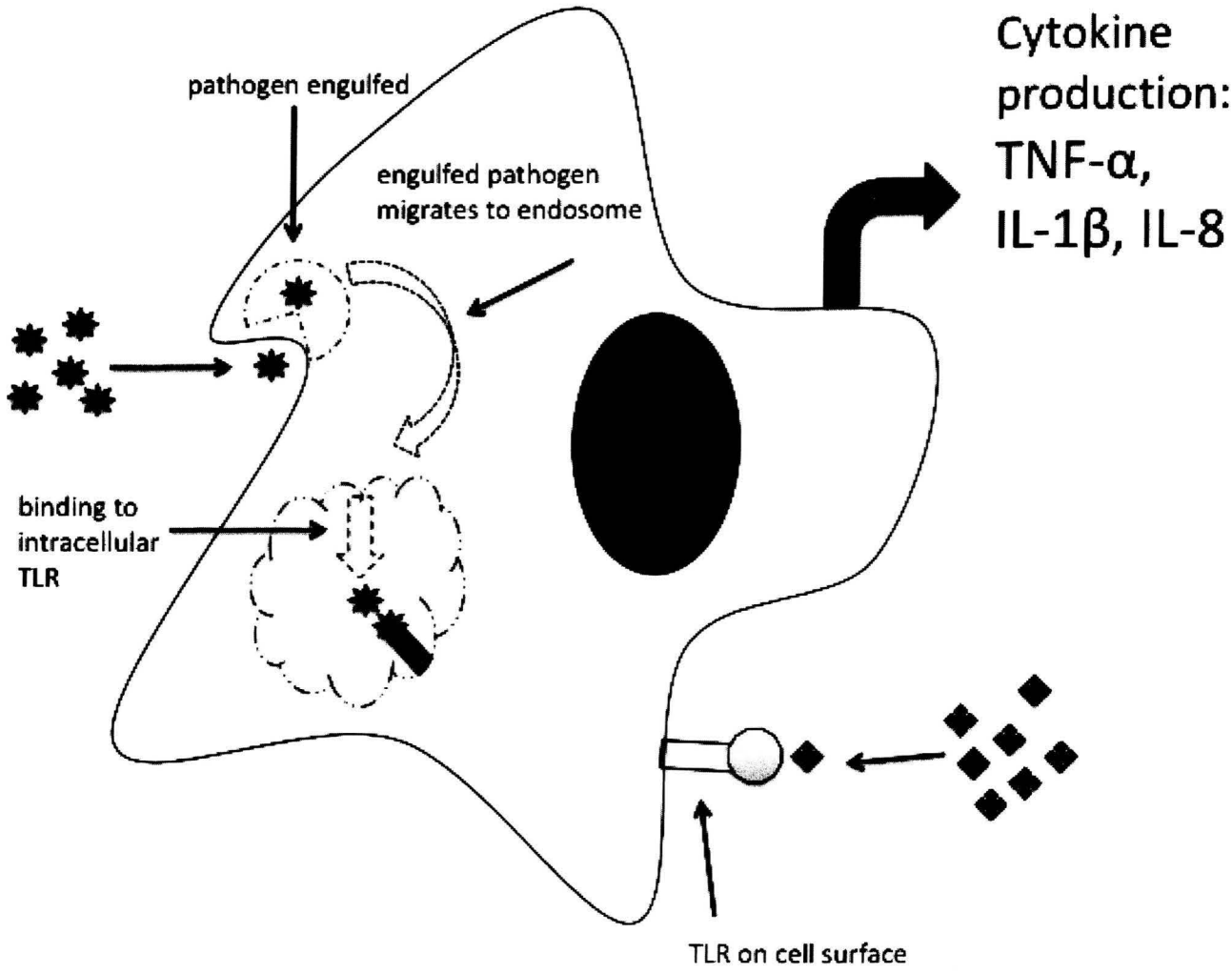

Figure 3. Schematic of cellular toll-like receptor (TLR) response to intracellular (top of cell) and extracellular (bottom of cell) pathogens and subsequent cytokine secretion. TNF-α = tumor necrosis factor-α. IL = interleukin.

Bacterial Killing Following Phagocytosis

Phagocytosis of bacteria or other pathogens by AMs and PMNs is critical to effective host defense. Both AMs and PMNs can kill phagocytosed pathogens via oxygen-independent or oxygen-dependent (respiratory burst) mechanisms.[23] Oxygen-independent mechanisms involve proteolytic enzymes released from cytoplasmic granules subsequent to phagocytosis. The respiratory burst occurs when bacteria, pulmonary surfactant proteins, lipopolysaccharide (LPS; endotoxin) or other PAMPs interact with the host cell, allowing for activation of the nucleotide adenine diphosphate hydrogen (NADPH) oxidase. NADPH oxidases lie within the plasma membrane of leukocytes and within the membrane of the phagosome. Upon activation, NADPH oxidases generate a superoxide radical. Superoxide may be converted to several other reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), hydroxyl radical, and hypochlorous acid.[23,48] Hypochlorous acid may react with nitrogen intermediates to form reactive chloramines.[48] All of these ROS and reactive nitrogen species, if not neutralized via antioxidants and antioxidant enzymes, can damage bacteria via oxidation or nitration of bacterial proteins and lipids. Additionally, if these ROS and reactive nitrogen species are not controlled, host cells can be damaged.

Secreted Molecules: Host-defense Peptides

Host-defense peptides (HDPs) are molecules secreted by a variety of cells that help to combat pathogens. Host-defense peptides include enzymes that digest microorganisms (e.g., lysozyme), peptides that bind metals essential for pathogen survival (e.g., lactoferrin), and peptides that disrupt membranes, causing

increased permeability of pathogen membranes (e.g., defensins and cathelicidins).[49] In addition to their direct antimicrobial properties, HDPs may enhance innate immunity by modulating immune cell response. Host defense peptides can also modulate the host immune response by inhibiting proinflammatory cytokine release by macrophages and oxidant production by neutrophils.[53]

Defensins are cationic HDPs secreted by respiratory epithelial cells and neutrophils that have broad-spectrum antimicrobial activity. There are 2 classes of defensins, α and β. Alpha-defensins are constitutively produced by phagocytic cells, while β-defensins are produced by respiratory epithelial cells.[34] Respiratory epithelial cells of cattle produce 2 β-defensins, lingual antimicrobial peptide and tracheal antimicrobial peptide.[1] Production of the β-defensins may be constitutive or may be induced by proinflammatory cytokines or LPS.[34,58] Defensins primarily act by forming pores in microbial membranes. Defensins also promote the immune response by enhancing production of cytokines by immune cells, increasing expression of cell surface molecules involved in the host response to infection, and recruiting dendritic cells.[34]

Secreted Molecules: Opsonins

Opsonins are molecules that bind to infectious agents and thus enhance the ability of leukocytes to phagocytose these agents. Antibodies are key opsonins that are produced by B lymphocytes. Other opsonins, including serum amyloid A and complement factors, are produced within the liver. Finally, some opsonins may be produced locally in the respiratory tract by alveolar epithelial cells. Opsonins in bronchoalveolar lavage fluid include collectins, fibronectin, and C-reactive protein.[39] Examples of collectins include surfactant proteins A (SP A) and D (SP D) and mannose-binding lectin (MBL).[58] These molecules can neutralize several pathogens including viruses, bacteria, fungi, and various allergens.

In addition to their role as opsonins, SP A and SP D also upregulate pathogen recognition receptors on immune cells, modulate inflammation, and serve as chemoattractants for phagocytes to clean up cellular debris.[54] Surfactant proteins A and D are produced by alveolar type II epithelial cells and non-ciliated bronchiolar epithelial cells, also known as Clara cells.[57]

Secreted Molecules: Cytokines and Chemokines

Cytokines are produced by all cells, including immune cells and airway epithelial cells. All immune functions are mediated by cytokines, and a thorough review of cytokines and their function is beyond the scope of this manuscript. However, some basic points warrant mention in the context of respiratory defense. Cytokines may be classified as pro- or antiinflammatory (Table 2). Proinflammatory cytokines include TNFα, IL-1β, IL-6, IL-8 and IFNγ.[37]

Interferon γ is a type II interferon. Interferon γ activates macrophages and promotes immunity to intracellular pathogens, including viruses.[37] Natural killer cells appear to be the primary source of IFNγ during early host defense, while production by T-lymphocytes predominates during adaptive immunity.[45] Type I interferons (most other interferons, including α and β) play an important role in modulating viral infections through inhibition of viral replication and immune stimulation.[10] Type I interferons also promote differentiation of monocytes into APCs, and modulate production of cytokines and cellular response to IFNγ.[10]

TNFα activates phagocytosis, oxidative burst, and bacterial killing. TNFα is also important in recruitment of PMNs.[31] Antiinflammatory cytokines include IL-10 and TGF-β;[37] these cytokines play an important role in modulating inflammatory and immune responses to prevent host tissue injury or autoimmune reactions. Chemokines are cytokines that promote cell movement. In the context of respiratory disease, chemokines promote immune cell migration to the site of infection or inflammation.[6] There are 4 classes of chemokines: C, CC, CXC, and CX3C. Following binding to their respective receptors, chemokines enhance expression of cellular adhesion molecules and promote movement of immune cells out of the circulation and into the tissues.[6] Different chemokine types will promote migration of different immune cells. For example, IL-8 is a chemokine which is a potent stimulator of neutrophil migration in bovine bacterial (pasteurella) pneumonia.[15]

Adaptive Immunity

The adaptive immune system provides an antigen-specific (and thus pathogen-specific) response that is mediated by B and T cells. Because it requires activation through antigen recognition and presentation by dendritic cells, the adaptive immune response is delayed relative to the innate immune response. However, the specificity of the adaptive immune system is important in the elimination of encapsulated bacteria, viruses, and intracellular pathogens.[58] The adaptive response is characterized by immunological memory, which allows for enhanced response following subsequent and repeated exposure to a specific pathogen.

Cell-Mediated Immunity

T cells are responsible for the component of the adaptive immune response termed "cell-mediated immunity" (CMI), which protects the host from intracellular pathogens such as viruses, certain bacteria (such as mycobacteria or salmonella), or certain parasites. T cells secrete cytokines that amplify the CMI response.

Certain T cell-secreted cytokines can also activate the other component of the adaptive immune response, the "humoral" (or antibody-mediated) immunity. T cell populations are divided broadly into either CD4+ (helper T cells, Th) or CD8+ (cytotoxic T cells).[51] The CD4+ helper T cells "help" by their production of cytokines that activate and improve the response of other T cells, B cells, and macrophages, among other cells. However, helper T cells only produce their cytokines after they are first activated by an antigen presenting cell (APC), such as a dendritic cell. Macrophages and B cells can also serve as APCs. These APCs are alike in their expression of MHC II molecules on their surface, which "present" to the helper T cell pieces of pathogens (antigens) that the APCs have previously phagocytosed. In addition to MHC II, APCs have other molecules on their surface, known as co-stimulatory molecules, which further activate the helper T cell. Cytokines secreted by the APC at the time of interaction with the helper T cell are a third factor required for a helper T cell to become properly activated. This 3-step requirement helps to limit inappropriate activation of T helper cells, which might result in harmful and excessive inflammation or autoimmune responses.

Like helper T cells, cytotoxic T cells must also be "shown" that an infection has occurred by another cell. However, in contrast to helper T cells, cytotoxic T cells are shown antigen on MHC I molecules, which are expressed by virtually all nucleated cells. Cells infected with intracellular pathogens have changes in MHC I that are recognized as abnormal by cytotoxic T cells. Once a cytotoxic T cell recognizes a cell expressing abnormal MHC I, that cell is killed. Thus the cytotoxic T cell helps clear the body of cells that are infected or recognized as abnormal.

Lymphocyte subsets are not uniformly distributed throughout all body systems or secretions. In cattle, there are increased proportions of activated lymphocytes and memory lymphocytes in BAL fluid compared to systemic circulation, which likely represents a response to local (pulmonary) antigen exposure. Notably, there is a higher proportion of CD8+ cells within BAL fluid, and a higher proportion of CD4+ cells in peripheral circulation.[36] Gamma-delta T cells are present in higher concentrations in peripheral blood compared to lung parenchyma or BAL fluid.[35,36]

As noted above, helper T (Th) cells are critical for the induction of adaptive immunity. In mice, Th cells have been divided into different subsets, with Th1 and Th2 subsets being identified initially, and additional subsets (Th9, Th17, Th22, and T-effector cells) being more recently recognized.[2] The induction of a Th1 or Th2 response depends upon the cytokine environment at the time the Th cell is first stimulated by a dendritic cell.

A Th1 response is primarily induced by interleukin 12 (IL-12), which is secreted by activated macrophages and dendritic cells (Figure 4). A Th2 response is induced by interleukin 4 (IL-4).[8] Th1 cells are identified by secretion of IFNγ but not IL-4. Th2 cells do not produce IFNγ; instead, they produce IL-4 and IL-5, which induce B cells to produce IgE, and which stimulate eosinophil migration[2,8,37] (Figure 5).

Th1 responses support protective immunity against such intracellular pathogens as viruses or certain bacteria, including mycobacteria and salmonella, while Th2 responses support protective immunity against extracellular pathogens, especially parasites. Th2 responses also play an important role in allergic responses. In addition to directing a response through stimulation of either Th1 or Th2 proliferation, cytokines may suppress development of the alternate Th cells.[8] Thus, a strong Th2 response, as might be induced by nematode infestation, could suppress the development of a Th1 response to a viral infection occurring concurrently.

It should be noted that cattle do not appear to have strict separation of their Th responses as has been described in mice. While cattle do develop Th1 cells in response to intracellular infections, and Th2 cells in response to extracellular parasites, they also develop Th0 cells in response to both types of infection, which produce both IFNγ and IL-4.[11,12] However, cattle have been found to produce relatively more IFNγ than IL-4 in response to chronic intracellular infections,[12] and relatively more IL-4 than IFNγ in response to chronic extracellular parasite infections.[11] This suggests that in cattle as in mice, IFNγ is important for the development of an immune response to intracellular infections, and IL-4 is important for the development of an immune response to extracellular parasites.

The practical relevance of the division between Th1 and Th2 cells is that vaccines can be designed to induce a relatively dominant response of one type or the other, in part through the choice of adjuvants included. Thus, a vaccine against viral pathogens should be designed to induce a stronger Th1 response, characterized by dominance of IFNγ production during the response. Vaccines that induce strong Th2 responses to viral pathogens have been produced inadvertently; such vaccines have led to enhanced disease following viral infection, as in the case of inactivated bovine respiratory syncytial virus (BRSV) vaccines.[21,29]

Humoral Immunity

Humoral immunity (that is, immunity mediated by immunoglobulins) plays an important role in host defense because immunoglobulin can opsonize pathogens, neutralize toxins, activate complement, and mediate killing of infected cells. Humoral immunity is mediated by B cells, which produce immunoglobulins.

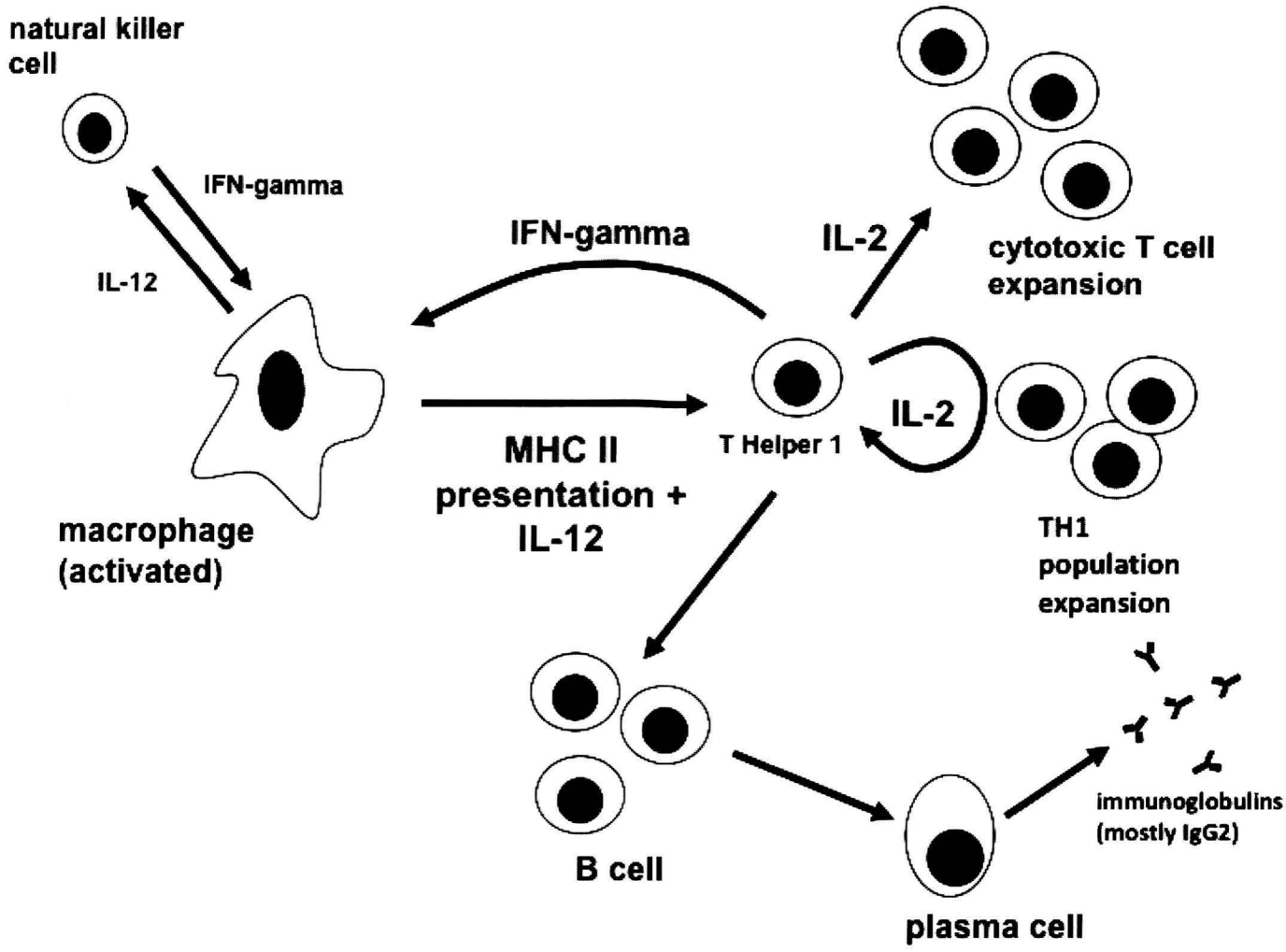

Figure 4. Interactions important in the generation of a helper T cell type 1 (Th1) response, which is appropriate for protective immunity to intracellular infections. Cytokine secretion may promote activation or clonal expansion of immune cells. Note that macrophages can only activate a Th cell after that Th cell has first been activated by a dendritic cell in the lymph node draining the area of infection. Macrophages and Th cells later interact as shown here elsewhere in the body, such as at the site of infection. IL = interleukin. MHC = major histocompatibility complex. Th1 = T helper 1 cells. IFN = interferon.

Upon activation, B cells proliferate and become plasma cells or memory cells. Plasma cells secrete very large quantities of immunoglobulin; they can be thought of as "immunoglobulin factories". In contrast, memory B cells produce less immunoglobulin, but they persist for months or years, allowing for rapid expansion following future exposure to a pathogen, leading to a massive pathogen-specific immunoglobulin response.

Immunoglobulins on the surface of the respiratory epithelium are produced by B cells in the tissues underlying the respiratory epithelium. The concentration of immunoglobulin-producing B cells under the airways varies with age. In a study investigating immunoglobulin-producing B cells using immunohistochemistry, the number of cells increased with age from birth to 16 months of age.[3] However, cattle at 16 months of age had greater numbers of immunoglobulin-producing B cells than adults.[3] In addition, anatomic location within the respiratory tract is important, with immunoglobulin-producing B cells being most numerous in the larger airways and decreasing in numbers in smaller airways. Immunoglobulin isotype production has also been quantified in cattle. In cattle greater than 4 months of age, IgA-containing cells were the most common cells underlying the respiratory epithelium (~65% of total), followed by IgG1 (~20%), IgG2 (~10%), and IgM (<10%)-containing cells.[3] Although present in small concentrations within airways, IgE is recognized

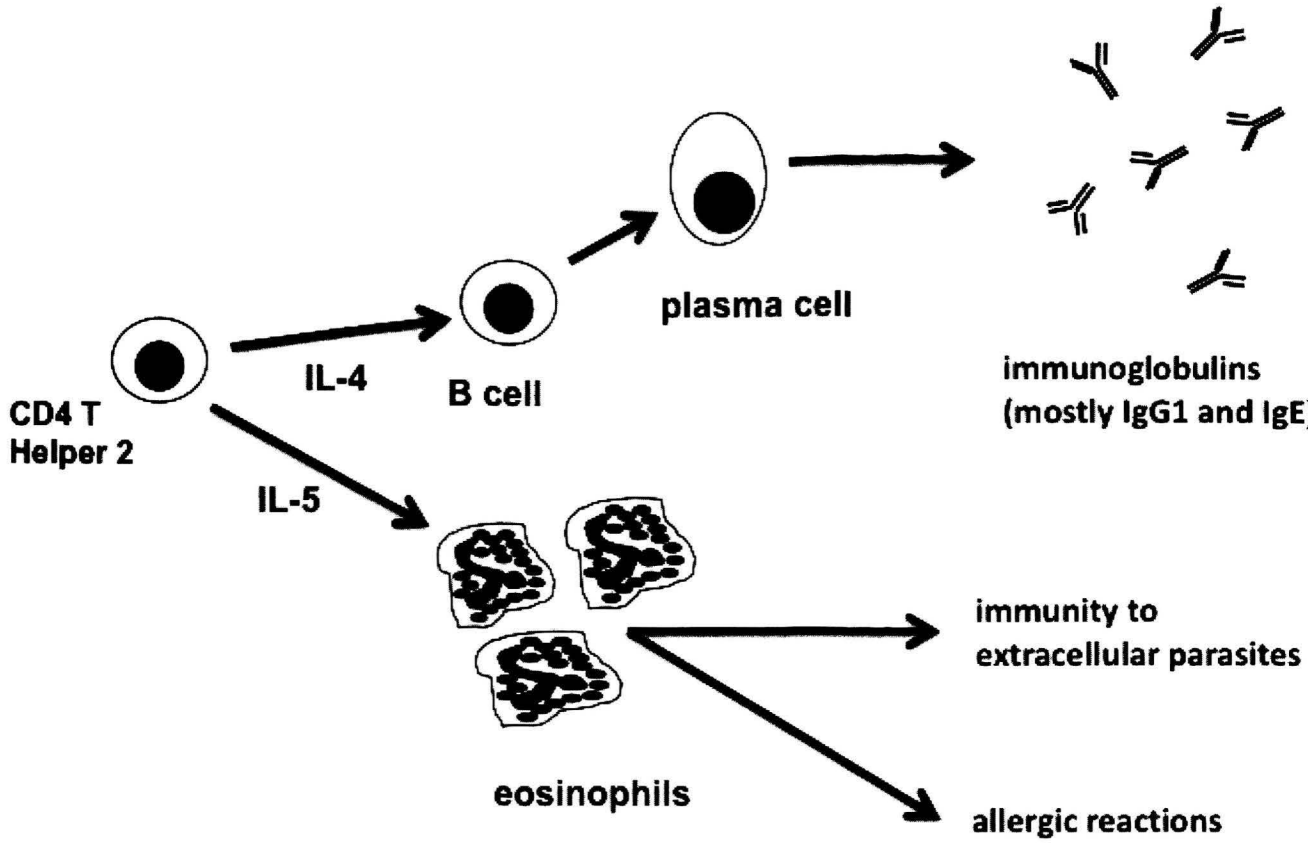

Figure 5. Interactions important in the generation of a helper T cell type 2 (Th2) response, which is appropriate for protective immunity to extracellular parasites and bacteria, and which can also result in allergic responses. Th2-mediated immunity allows for activation of B cells to produce certain isotypes of antibody, and activation of eosinophils. IL = interleukin. CD = cluster of differentiation.

as an important component mediating disease severity in bovine *Histophilus somni* and BRSV infections.[17,20]

Both immunogolobulin isotype and epitope (antigen) specificity are important for immunoglobulin efficacy.[13] In a study comparing immunoglobulin isotype concentrations in serum, BAL fluid, and nasal wash fluid, IgA was found to be the predominant immunoglobulin within the nasal passages. Bronchoalveolar lavage fluid had similar concentrations of IgG and IgA, while in serum, IgG was the predominant isotype.[56] These findings are consistent with the role of IgA in local mucosal immunity.

Immunoglobulin functions are diverse, and may promote or suppress inflammation and influence cell-mediated immunity. Immunoglobulin effect on the inflammatory state is dependent upon immunoglobulin isotype, presence of stimulatory or inhibitor receptors, and size of the immunoglobulin-antigen complex formed.[14] Furthermore, cross-linking of immunoglobulin receptors can alter the production of inflammatory cytokines and alterations in antigen presentation.[14] Complement activation leads to production of inflammatory complement-split products.[14] Immunoglobulin may also promote phagocytosis and promote Th1 activation through cross-linking of the immunoglobulin (Fc) receptors.[14]

Application to Treatment and Prevention of BRD

Understanding the interaction between the bovine immune response and recovery from BRD is critical for development of new treatments, preventative health protocols, and management or husbandry recommendations. Evidence that depletion of pulmonary intravascular macrophages decreases pulmonary inflammation suggests that modulation of immune response may attenuate disease severity.[50] To date, recombinant cytokine (IL-1β and/or IL-2) therapy has been shown to potentiate immune response to vaccination;[42] however,

such treatment did not impact the clinical course of disease following infection with BHV-1 and *Mannheimia haemolytica*.[5] However, administration of IFN-α prior to infection with BHV-1 and *Mannheimia haemolytica* did ameliorate disease.[4] Further research is needed to determine how cytokine (or anti-cytokine) therapy, or other treatments that modulate the immune response, can be most effectively applied to decrease the impact of BRD. Because the field of immunology is rapidly changing, it is likely that veterinarians will regularly learn of new developments related to the bovine immune response that are applicable to the control of BRD.

Conclusion

Pulmonary defense against invading pathogens is complex, and involves static structural barriers as well as dynamic responses by the cells of the immune system. Although the immune system is classically divided into innate (non-specific) and adaptive (specific) immunity, overlapping roles of immune cells and signaling molecules are increasingly recognized. Further investigation into the roles of the innate and adaptive immune responses may allow for the development of novel immunotherapeutic interventions applicable for the prevention and treatment of bovine respiratory disease.

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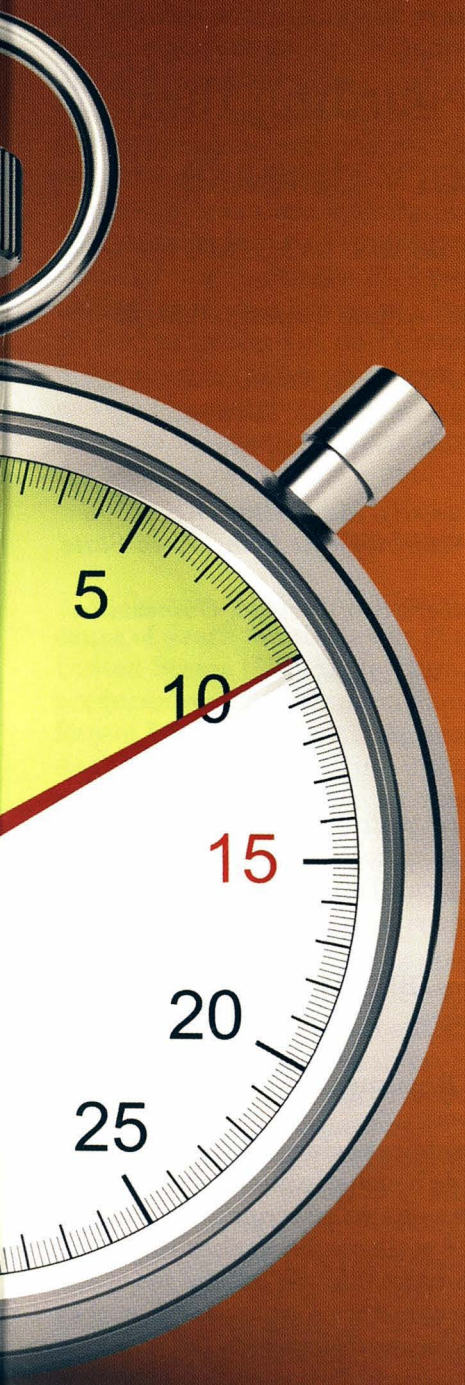

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