Ynte Schukken

Chief Scientific Officer at GD Animal Health.
Professor of Herd Health and Epidemiology at Cornell University (USA)

New insights into the immunology of the Bovine Mammary Gland

Ynte Schukken[1,2], Brianna Pomeroy[1] and Anja Sipka[1]

[1] Cornell Program for applied research in mastitis, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.
[2] GD Animal Health, Deventer, the Netherlands

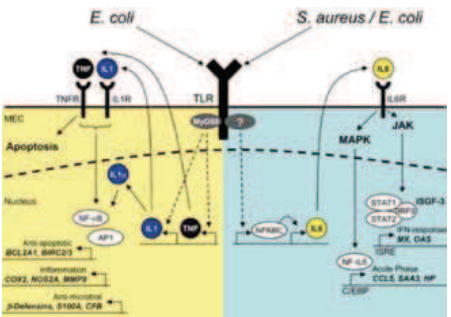
Introduction

Many different bacterial species have the ability to cause an infection of the bovine mammary gland and the host response to these infections is what we recognize as mastitis. Mastitis is a highly prevalent disease in dairy cows and economically costly to the dairy industry worldwide (Bannerman, 2009). Clinical mastitis is characterized by visible changes in milk from the gland including presence of clots, heat, pain or swelling of a gland. Clinical mastitis is generally caused by an intramammary infection (IMI) with a bacterial pathogen. The distribution of bacterial pathogens has been studied extensively and in many countries. In general the bacterial causes of mastitis are distributed fairly equally among staphylococcal, streptococcal and gram-negative bacterial species (OldeRiekerink et al., 2008, Barkema et al., 1998). Although intramammary infections occur at any time during the life cycle of the adult dairy cow, there is a predominance of clinical mastitis cases in early lactation (Barkema et al., 1998, Green et al. 2005).

Immune response

The innate defense mechanisms of the mammary gland include physical barriers such as the teat sphincter, chemical barriers such as teat canal keratin and lactoferrin, and more proper components of the immune system such as macrophages, dendritic cells, mast cells, neutrophils, eosiniphils and natural killer (NK) cells (Werling et al. 2006). Pathogen recognition is the first and mandatory step in the immune defense against the invading pathogen. Mammals are equipped with a battery of trans-membrane receptors sensing the presence molecular components of pathogens. The 13 different mammalian toll-like-receptors (TLRs) represent the best-described family of such 'pattern recognition receptors' (PPRs) (Akira and Takeda, 2004). TLR signal transduction pathways activate transcription factors such as NF- B, IFR, and activating protein-1 (AP1) (Akari and Takeda et al., 2004). MyD88 (myeloid differentiation primary-response protein 88) dependent pathways are associated with early-phase NF- B response whereas as MyD88 independent pathways are associated with late-phase NF- B response (Figure 1). These NF-B factors may subsequently enter the nucleus and bind to target promoters. The adaptive immune response to IMI has mostly been studied in relationship to either E. coli or *S. aureus* IMI. Commercial vaccines are available for both these organisms. Vaccination with a core J5 *E. coli* vaccine is commonly practiced on dairy farms in the USA (Gurjar et al. 2013) and a commercial combination vaccine that includes J5 *E. coli* as a S. aureus bacterin is currently being marketed throughout the world. In a recent field study, the efficacy of this vaccine in terms of reducing transmission of *S.aureus* infection was estimated to be approximately 45% (Schukken et al. 2014).

Figure 1: Activation of the immune response in Mammary Epithelial Cells. *E. coli* activates the expression of the three master cytokine IL-1, TNF-α and IL-6. *S. aureus* only drives significant IL-6 expression *via* a MyD88 independent signal transduction.



In recent studies, the distinction between the immune response in lactation compared to the immune response in late gestation has been emphasized. For this reason the two immune response will be discussed separately.

Immune response after intramammary infections in lactation

Invasion of the mammary gland by pathogenic bacteria triggers a battery of immune responses through interactions between a diverse array of pathogen-borne virulence factors and the immune surveillance mechanisms of the host. The adaptive immune response is initiated by Antigen presenting cells (APC). Dendritic cells (DC) are the major APC in the mammary gland and their function is crucial in the regulation of both the early innate immune responses and the subsequent adaptive immunity. Recognition and uptake of antigen at the site of infection induces maturation of the DC (Della Chiesa et al. 2005) and homing to the supramammary lymph nodes (Maxymiv et al. 2012) where they present the antigen to naïve T cells. Activated dendritic cells express high levels of antigen-MHC complexes, secrete cytokines, and upregulate co-stimulatory surface molecules such as CD40 and B-7 molecules, all which as necessary signals to induce and influence T cell activation and differentiation; in lactating cows, high levels of IL-12 secretion has been suggested to promote Th1 differentiation. IL-12 produced by DC also induces IFNγ production by other innate immune cells include NK cells thereby contributing to immediate pro-inflammatory responses. A predominant Th1 response results in a pro-inflammatory response, where production of pro-inflammatory cytokines results in a massive influx of polymorphnuclear cells that aim to kill the invading organisms.

Immune response after intramammary infection in late gestation

Pregnancy presents a major challenge to the maternal immune system, both in normal and pathologic states (Denney et al., 2011). The immune response is modulated to allow establishment and maintenance of a viable pregnancy without rejection. Progesterone in concentrations present during pregnancy is a potent inducer of the TH-2 cytokines IL-4. A TH-2 shift in pregnancy is also characterized by reduction in IFN-γ and IL-2 producing CD4+ and CD8+ T cells (Raghupathy, 2001). Dendritic cells play a major role in immune regulation observed in pregnancy. Circulating pregnancy factors including progesterone and estrogens impact DC activation by impairing cytokine production and surface marker expression found necessary for T cell activation and Th1 differentiation, and induction of IFNγ production in other innate immune cell populations. The dominant Th2 response results in a limited inflammatory response and in most cases no signs of clinical mastitis will be the result of an intramammary infection.

Discussion

As we showed earlier (Quesnell et al., 2012), late-gestation inoculation with 100 cfu of *E. coli* C1 results in a predictable establishment of an IMI during the last weeks of gestation. A high proportion of the initially established IMI (65%) were still present in early lactation and generally presented as mild clinical mastitis. Late-gestation IMI often result in clinical mastitis in early lactation and form one of the most important challenges to reduce clinical mastitis on well-managed dairy farms. Further research in the pathobiology of IMI in late gestation and the potential interventions that would prevent or reduce such infections is important. Vaccination before late gestation, either parenteral or local may be valuable as an aid in reducing these IMI. The particular immune response dynamics as it changes across lactation and gestation will proof to be essential to design adequate interventions.

References

Akira, S., Takeda, K. 2004. Toll-like receptor signalling. Nat Rev Immunol. 4, 499-511.

Bannerman, D.D. 2009. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J. Anim Sci. 87, 10-25.

Burton, J.L., Erskine, R.J. 2003. Immunity and mastitis. Some new ideas for an old disease. Vet. Clin. North Am. Food Anim. Pract. 19, 1-25.

Della Chiesa M, Sivori S, Castriconi R, Marcenaro E, Moretta A. 2005. Pathogen-induced private conversations between natural killer and dendritic cells. Trends Microbiol. 13:128-136.

Denney JM, Nelson EL, Wadhwa PD, Waters PD, Waters TP, Mathew L, Chung EK, Goldberg RL, Culhane JF. 2011. Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine 53:170-177.

Günther J., Esch K., Poschadel, N., Petzl, W., Zerbe, H., Mitterhuemer, S., Blum, H., Seyfert, H.M. 2011. Comparative kinetics of *E. coli* vs. *S. aureus* specific activation of key- immune pathways in Mammary Epithelial Cell: *S. aureus* elicits a delayed response dominated by IL-6, but not by IL-1A or TNF-{alpha}. Infect Immun. 79, 695-707.

Gurjar AA, Klaessig S, Salmon SA, Yancey RJ Jr, Schukken YH. Evaluation of an alternative dosing regimen of a J-5 mastitis vaccine against intramammary Escherichia coli challenge in nonlactating late-gestation dairy cows. J Dairy Sci. 2013 96:5053-63.

Maxymiv NG, Bharathan M, Mullarky IK. 2012. Bovine mammary dendritic cells: a heterogeneous population, distinct from macrophages and similar in phenotype to afferent lymph veiled cells. Comp Immunol Microbiol Infect Dis. 35:31-38.

Quesnell RR, Klaessig S, Watts JL, Schukken YH. 2012. Bovine intramammary Escherichia coli challenge infections in late gestation demonstrate a dominant antiinflammatory immunological response. J Dairy Sci. 2012 Jan;95(1):117-26.

Raghupathy R. 2001. Pregnancy: success and failure within the Th1/Th2/Th3 paradigm. Semin Immunol. 13: 219-27.

Saito, S., Nakashima, A., Shima, T., Ito, M. 2010. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 63, 601-610.

Schukken YH, Günther J, Fitzpatrick J, Fontaine MC, Goetze L, Holst O, Leigh J, Petzl W, Schuberth HJ, Sipka A, Smith DG, Quesnell R, Watts J, Yancey R, Zerbe H, Gurjar A, Zadoks RN, Seyfert HM; members of the Pfizer mastitis research consortium. Host-response patterns of intramammary infections in dairy cows. Vet Immunol Immunopathol. 2011 Dec 15;144(3-4):270-89.

Schukken YH, Bronzo V, Locatelli C, Pollera C, Rota N, Casula A, Testa F, Scaccabarozzi L, March R, Zalduendo D, Guix R, Moroni P. Efficacy of vaccination on Staphylococcus aureus and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. J Dairy Sci. 2014 May 29.

Werling, D., Piercy, J., Coffey, T.J., 2006. Expression of TOLL-like receptors (TLR) by bovine antigen-presenting cells—potential role in pathogen discrimination? Vet. Immunol. Immunopathol. 112, 2–11.

Zadoks, R.N. 2007. Sources and epidemiology of *Streptococcus uberis*, with special emphasis on mastitis in dairy cattle. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2.