Why is vaccinating against mastitis so difficult?

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Many different bacterial species have the ability to cause a repeated infection of the bovine mammary gland and the host response to these infections is what is generally described as mastitis. In this article I will try to explain why the development of vaccines against mastitis-causing pathogens is so difficult. However, I will also provide insight into new developments regarding vaccination against two main bacterial species causing bovine mastitis: *Escherichia coli* and *Staphylococcus aureus*. I will also describe that the host immune response differs significantly depending on the invading bacterial species, and that this may affect our ability to generate vaccines able to induce a long lasting memory. The relevance of fully understanding the bovine host response to intramammary infection is discussed, some major gaps in our knowledge are highlighted and directions for future research are indicated.

Our immune system consists of two arms: the innate immune system, the one we are born with, and the adaptive immune system, which develops over the years and shapes our fast response to re-occurring infections. The differences between these two arms are quite striking. Whereas the innate immune system is there from the beginning, and has as it's main aim to destroy invading pathogens by a process called phagocytosis (or in other words: eat and digest everything foreign), it will never develop a memory and will respond every time in exactly the same way. In contrast, the adaptive immune system is the one which becomes more and more specific every time we encounter the same pathogen, leading to a very specialised, fast and extremely specific response. To obtain a co-ordinated adaptive immune response, cells of the adaptive immune-system (T- and B-cells) need to be appropriately stimulated. This normally requires the uptake of a pathogen by cells of the innate immune system, the professional antigen presenting cells (APC), such as dendritic cells (DC) and macrophages (MØ). These cells are spread out through the tissue, and recognise invading pathogens using specific receptors, such as TOLL-like receptors (TLRs) via pathogen-associated molecular pattern (PAMP) being expressed by the pathogen. PAMPS are in most cases glycolipids and glycoproteins being present in the wall of pathogens, and include substances such as lipoteichoic acid (LTA), peptidoglycan (PGN) and lipopolysaccharides (LPS). Binding of PAMPs to TLRs stimulates a signal into the cells, resulting in the generation of three main signals necessary to subsequently stimulate the adaptive immune response: 1) Upregulation of MHC class II molecules (presenting peptides derived from the pathogen to the T-cell receptor); 2) Upregulation of co-stimulatory molecules helping to activate T-cells; and 3) secretion of pro-inflammatory cytokines (driving the maturation of T-cells, and thus subsequently B-cells) (Werling and Jungi, 2003). Upon their stimulation, APC present within the mammary gland tissue (Maxymiv et al., 2012) migrate into the regional draining lymphnodes where the majority of T- and B-cell stimulation occurs, resulting in the generation

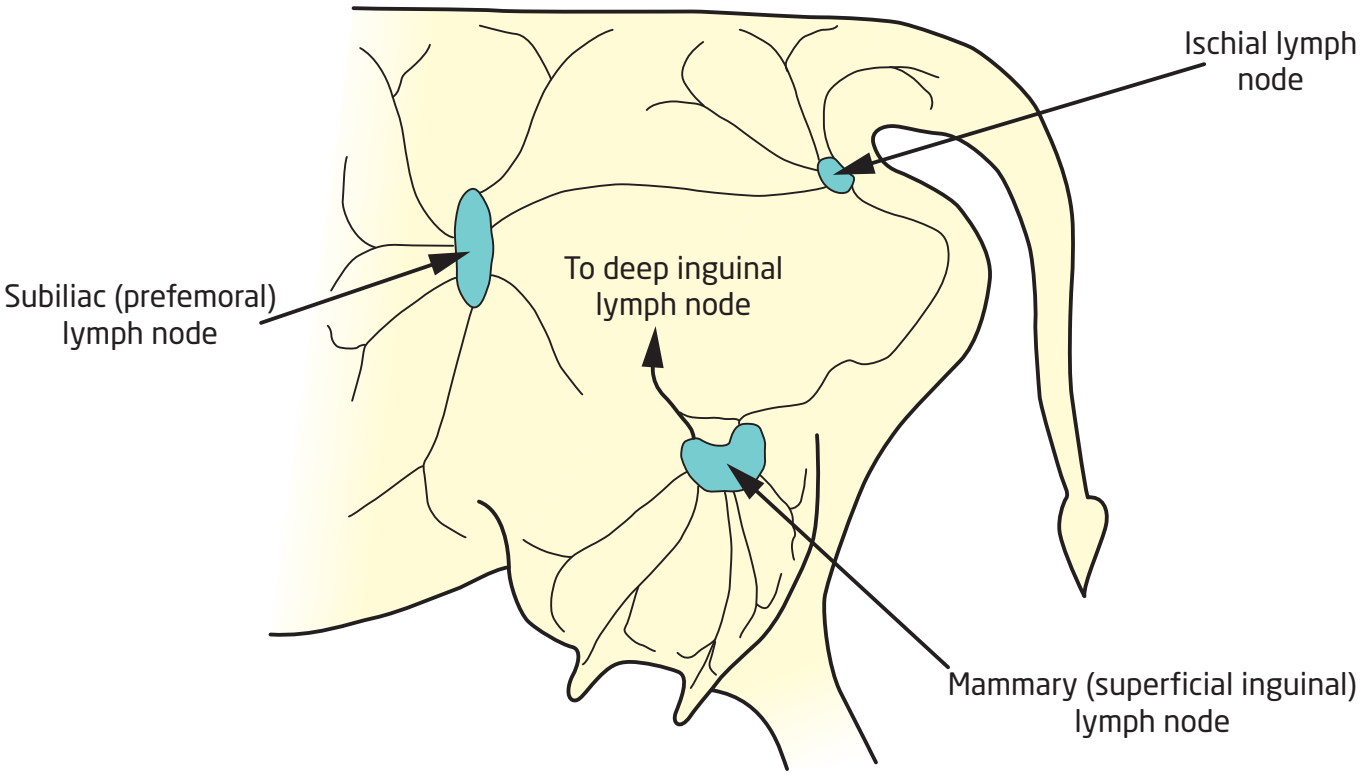


Figure 1.

of an adaptive immune response. In the case of the bovine udder, these lymphnodes are the mammary (superficial inguinal) lymphnode, the subiliac (prefemoral) lymphnode, and the ischial lymphnode (Figure 1). However, despite the fact that all necessary requirements for developing a long-lasting immunity to mastitis-causing pathogens are present, the host on its own does not seem to be able to stimulate the induction of a long-lasting adaptive immune response.

So, why does it not work?

Intramammary infections (IMI) in dairy cows are a major concern for the dairy industry. These infections lead to severe milk loss, are potentially fatal and are a major cost to dairy farmers. For this reason, there is an active research effort to understand the pathogenesis of mastitis, the inflammatory response to an intramammary infection, as well as the search for alternatives to antibiotic treatments. In the last decade our knowledge about the inflammatory response to infection has improved, both in terms of a better understanding of the mammalian immune response and the immune response of the bovine mammary gland (Rainard and Riollet, 2006). Similarly, biopsies of the mammary epithelium have revealed much about the regulation of genes involved in the host response to an IMI (Genini et al., 2011). The immune response pattern in the acute phase response was dominated by an up-regulation of chemokine and cytokine pathways, TLR signalling pathways and leukocyte transendothelial migration (Buitenhuis et al., 2011). The importance of the innate arm of the immune defence has been more fully appreciated with our increased understanding of the interaction of specific conserved PAMPs with TLRs.

However, important differences exist in the response to IMI caused by different bacterial species. Bacterial growth patterns and the associated innate immune response differ significantly between gram-negative bacteria such as *Escherichia coli (E. coli)* and gram-positive bacteria such as *Streptococcus uberis (S. uberis)*. Infections caused by *E. coli* are more typically, but not exclusively, associated with a fast and more dramatic immune response, whereas infections with *S. uberis* are characterized by a delayed and less dramatic response (Bannerman, 2009; Genini et al., 2011; Rambeaud et al., 2003). In contrast, *Staphylococcus aureus (S. aureus)* appears to mostly circumvent the host immune response and IMI typically result in a very moderate host response with minimal observable innate immune response (Petzl et al., 2008). These pathogen-specific responses can also be recognized in the somatic cell count patterns in milk relative to IMI, milk production losses and risks of culling and death.

A full understanding of the adaptive immunity in the context of mammary health provides challenges since the ruminant mammary gland is unique in that lymphocyte trafficking, which is essential to adaptive

immunity, is shared with the peripheral immune system rather than the common mucosal immune system (Figure 2). Protective immunity of the bovine mammary gland invoked by natural infection with bacterial organisms has shown to be relatively short-lived. A partial protection against subsequent natural infection disappeared within weeks (Schukken et al., 2009; Suojala et al., 2008). This relative inability to mount an adequate and long-lasting protective response to natural infection provides a major challenge for the development of effective vaccines to protect the bovine mammary gland from infection.

Pathogen - Evasion mechanisms

E. coli

Pathogenicity characteristics of gram-negative mastitis pathogens have been studied in recent years. It was shown that *E. coli* pathogens express a variety of virulence factors but no coherence between the severity of the disease and specific virulence factors could be defined (Bean et al., 2004; Suojala et al., 2008; Wenz et al., 2006). However, the ability to grow in mammary secretions and to liberate lipopolysaccharide (LPS) is crucial in the pathogenesis of mastitis caused by gram-negative bacteria. The faster bacterial numbers increase in the mammary gland, the more LPS is present in the mammary gland and the faster the inflammatory response and clinical disease may occur (Mehrzad et al., 2008). Gram-negative bacteria utilize milk nutrients to grow and multiply. A clear advantage for the gram-negative bacteria is the utilization of lactose as an energy source from milk, and a causal mechanism for the relationship between initial bacterial numbers and subsequent immune response was recently identified by demonstrating that the extent of induced cytokine synthesis (TNF-α, IL-8) in mammary epithelial cells (MEC) positively correlated with the concentration of *E. coli* particles (Gunther et al., 2010).

The implication of the body-udder barrier and the barrier between the two udder-halves:

1. **acquired immunity in the body is only partial and at a lower level in the udder**
2. **not all the immune responses in the mammary glands will be recognized by the body**

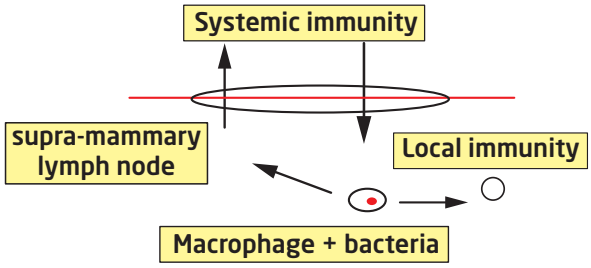


Figure 2.

S. aureus

S. aureus is an important cause of IMI in dairy cows. It is commonly assumed that most IMI are the result of cow-to-cow transmission where other infected animals in the herd are the source of the organism. However, other sources of *S. aureus* bacteria in the environment of a dairy cow have been described and in many herds a dominant, presumably contagious strain of *S. aureus* co-exists with a large collection of other, presumably non-contagious strains (Zadoks et al., 2002). Both in experimentally infected cows and in cows sampled longitudinally with a naturally occurring *S. aureus* IMI, a low and high shedding cycle were observed. Similar to *E. coli*, *S. aureus* has developed a variety of escape mechanisms to evade immune recognition (Garzoni and Kelley, 2009), and these occur on all levels of the host-cell, potentially leading to a persistent infection of the cell (Loffler et al., 2014). Indeed, persistent intramammary infections are an important component of the problem in bovine mastitis. Clinical mastitis with possible life-threatening severity is of importance to the cow and the dairy farmer, however the presence of persistent intramammary infections causing long-term increases in somatic cell counts and repeated clinical cases form another major concern to dairy producers. Persistent infections are very common for gram-positive organisms such as *Streptococcus agalactiae*, *S. aureus*, CNS and *Corynebacterium bovis*; are common for gram-positive pathogens such as *S. uberis* and *Streptococcus dysgalactiae* and are not uncommon in gram-negative bacteria such as *Serratia spp.*, *Klebsiella spp.* and have also been reported for *E. coli* IMI.

Vaccination against mastitis-causing bacteria

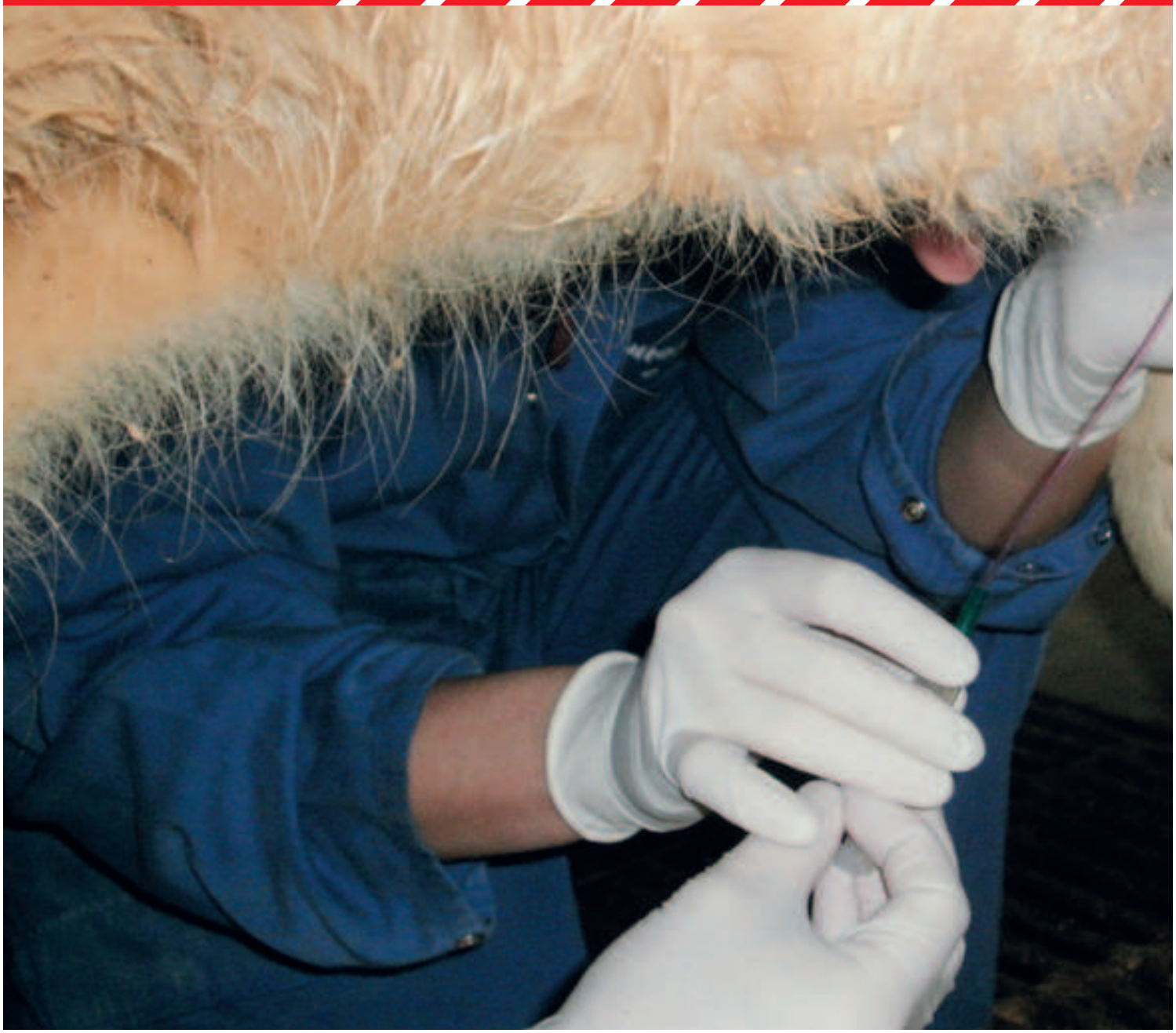
The immune response is often described as consisting of an innate and adaptive immune response arm. The adaptive immune system is the arm of the immune system that specifically responds to an antigen. Whereas the innate immune system uses either passive barriers or receptors that recognize conserved microbial molecules. The innate defence 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, eosinophils and natural killer (NK) cells The importance of the innate immune response in the udder, and the mechanisms involved, have been nicely discussed recently (Schukken et al., 2011).

In addition to all aspects of the innate response, milk in healthy cows has a resident population of immune cells. This population is generally dominated by macrophages but also contains neutrophils and lymphocytes (Sordillo, 2005). Lymphocytes are divided into two main groups: T and B lymphocytes. The T lymphocytes can be classified further into αβ T lymphocytes, which include CD4+ (T helper) and CD8+ (T cytotoxic) lymphocytes, and γδ T cells. In the lactating mammary glands, αβ T lymphocytes prevail and predominantly express the CD8+ phenotype (Shafer-Weaver et al., 1996; Shafer-Weaver and Sordillo, 1996). The function of activated cytotoxic T cells (CD8+) is to kill host cells infected with a pathogen, as detected by antigens expressed on the surface of infected cells. Helper T cells (CD4+) have a more indirect but equally important effect on the infection. When a T_H cell matures, it develops into one of four types of T_H cells. Stimulation of these mature T_H cells can cause the expression a large variety of cytokines that can direct the immune response toward a pro-inflammatory cytotoxic T cell-mediated (T_H1), B-cell-mediated (T_H2), neutrophil-mediated response (T_H17), or to counter-regulate the response (T_{reg}). During bacterial infection of the bovine mammary gland, large numbers of leukocytes migrate into the udder, resulting in the establishment of a host response against the pathogen. Currently, the specific leukocyte populations mediating this immune response are not well defined. Cell surface markers are used to identify the specific cell populations identified in the mammary immune response. There is an increasing range of well-characterized monoclonal antibodies (mAbs) available for, and raised against, bovine cell surface markers. A list of bovine specific antibodies against cell surface markers is maintained by the US veterinary immune reagent network and is accessible at http://www.umass.edu/vetimm/ruminants/index.html.

The adaptive immune system can not only specifically recognize a species of microbe, but also distinguish variants of a species. Antibodies generated by B cells recognize whole antigens, whereas the T-cell receptors recognize fragments of antigens presented by specialized molecules called major histo-compatibility complex (MHC) class I or class II molecules.

E. coli vaccine

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, although the efficacy of the vaccines to protect against IMI with these two organisms is still debated. Vaccination with a core J5 *E. coli* vaccine is commonly practiced on dairy farms in the USA and commercial J5 vaccines are now also available in Europe. The J5 vaccine is assumed to be effective in reducing the severity of clinical mastitis (Gonzalez et al., 1989). Higher J5-specific IgG1 and IgG2 antibody are typically observed in J5 vaccinates after vaccination. A distinguishing feature of immunological memory is the irreversible B cell genetic change from IgM production to production of other antibody isotypes, including IgG1 and IgG2 (Burton et al., 2005). In the bovine as well as in several other species, an immune response with more production of IgG2 antibody has been recognized as part of a Th1 or pro-inflammatory response, while a response with more IgG1 is part of a Th2 or anti-inflammatory response (Stevens et al., 1988). Because IgG2 is an important opsonizing antibody aiding in neutrophil phagocytosis of bacteria, and IgG2 has the ability to readily fix complement, it has been suggested that an IgG2 Th1-type response might be beneficial against bovine mastitis. Wilson et al (Wilson et al., 2007a, 2008; Wilson et al., 2007b, 2009) provided evidence that increased production of both J5-specific IgG1 and IgG2 antibodies are important mechanisms of J5 vaccine protection, including the production of a higher proportion of IgG2 than in non-vaccinates, a Th1 biased response. Serum ratio of J5-specific IgG1:IgG2 was reported to be less than one in vaccinates

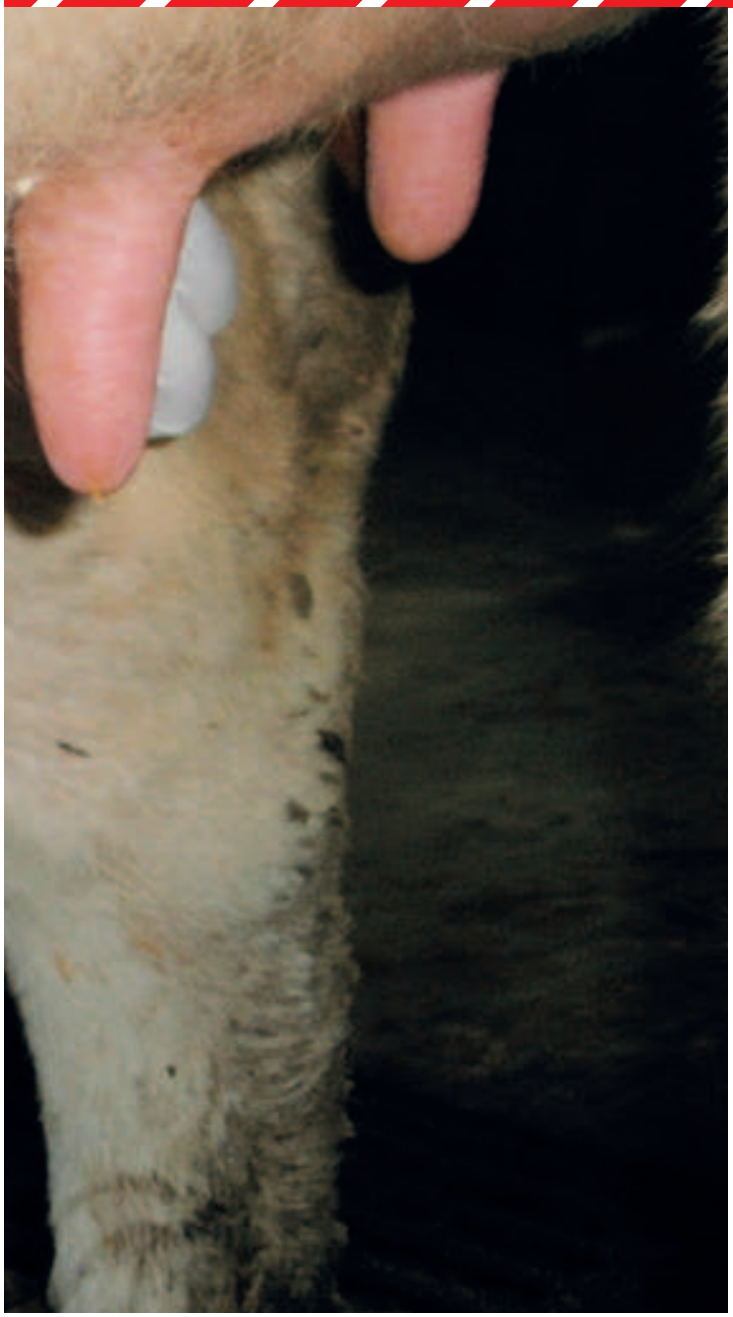


post-calving, thus demonstrating a Th1 biased response after calving.

Immunological memory stimulated by J5 vaccination is generally associated with lower bacterial growth after IMI, a reduced milk production loss and lower cull rates following clinical mastitis compared to unvaccinated controls. These benefits decrease on a continuous basis as the lactation progresses, with a waning of vaccine protection over time. This raises the question of the optimum J5 immunization schedule for producing long-lasting immunological memory associated with seroconversion to long-lasting high titers of anti-J5 antibody. Based on a study in steers, the authors suggested that a large number of doses of J5 bacterin may be needed to obtain a high concentration of IgG2 reactive against J5 (Chaiyotwittayakun et al., 2004).

S. aureus vaccines

A number of studies have been published on antibody-driven vaccination to prevent staphylococcal (predominantly *S. aureus*) IMI. Very extensive studies were performed by colleagues in Israel. Leitner et al described a field study of a *S. aureus* vaccine (Leitner et al., 2003a; Leitner et al., 2003b; Leitner et al., 2003c). A total of 452 Holstein heifers were included in the trial with 228 heifers being vaccinated and 224 serving as unvaccinated controls. Antibody response was detected in all vaccinated animals 4-5 weeks post-primary immunization and it was sustained for approximately 300 days. No significant difference in *S. aureus* infections was observed, in the vaccinated group 1.3% of heifers became infected and this was 2.7% in the control group. Middleton et al (Middleton et al., 2006) performed a challenge study in vaccinated and control heifers. All heifers were challenged with a heterologous strain of *S. aureus* by intramammary infusion on days 6-8 of lactation in a single infection-free mammary quarter. All cattle became infected with *S. aureus* after challenge and there were no differences in *S. aureus* clearance rates between groups. Vaccinated heifers did show a lower mean duration of clinical mastitis and a lower total mastitis score post-challenge than controls. More recently, Prenafeta et al evaluated a *S. aureus* vaccine based on an extracellular slime associated antigenic



complex from *S. aureus (Prenafeta et al., 2010)*. Twelve animals were vaccinated at 45 days before the expected parturition date and revaccinated 35 days later. All cows were challenged with a heterologous strain of *S. aureus* 23 days after calving. Immunization enhanced antibody titers against the slime-associated complex. However, there was no evidence of a difference between vaccinated and control groups with regard to IMI and clinical signs of mastitis following the challenge. Vaccinated cows showed a reduced S. *aureus* concentration in milk during the post-challenge period. More promising attempts involving PAMPs were recently made by Leitner et al who combined an *S. aureus* vaccine with an enhancer of phagocytosis, which enhanced clearance of bacteria (Leitner et al., 2013).

Combination vaccines

A very promising study was published this year using the Startvac vaccine (Hipra, Spain) (Schukken et al., 2014), which was introduced within the last few years in Europe. This vaccine combines the *E. coli* J5 strain and several *S. aureus* components (Harro et al., 2010; Prenafeta et al., 2010). Here, vaccination resulted in a moderate reduction of new IMI as well as a reduction in the duration of IMI caused by staphylococci.

Discussion

Although much progress has been made in understanding the pathobiology of mastitis, there are still important areas that remain poorly understood. Among these important gaps in our knowledge are relationship between intramammary infection, TLR-based immune response and the resulting cytokine profiles. The compromised up-regulation of inflammatory cytokines in *S. aureus* infected glands may, at least partially, contribute to the persistent course of infection caused by this pathogen. Further research on identifying factors responsible for the differentially expressed cytokine profiles may be fundamental to developing strategies that mitigate the outcome of bovine mastitis. There are circumstances where an apparent disconnect exists between IMI and an up-regulation of TLRs and subsequent cytokine production. The molecular causes for this delay are currently unknown. Once they are unravelled, then these might possibly offer new molecular targets for improved therapy of persistent *S. aureus* infections. A better understanding of differences in host immune response to different bacterial pathogens may provide opportunities for up or down regulating of the immune responsiveness.

There is currently a very shallow insight in the cell-mediated immunity as it pertains to mastitis. It is unclear how the cell-mediated immunity cascades after an IMI and whether pathogen specific and lactation stage specific patterns exist. Preliminary evidence would suggest that during late gestation, the cell-mediated immunity is biased toward a T_H2 dominance changing the dominant direction of protection against invading bacteria. Furthermore, the pathogen-specific importance of the cell-mediated immunity is suspected, with a suggested role for lymphocytes in the acquired protection against *S. uberis* IMI (Hill, 1988) To better understand the pathogenesis of mastitis and increase our ability to modify immune responsiveness, future research into cell-mediated immunity in mastitis is warranted.

An ability to modify the immune response to an IMI will likely provide therapeutic opportunities to either up or down-regulate the immune response depending on the clinical condition of the patient. Further genomic and proteomic research on the impact of calving and the start of lactation on transcription of the host genome will provide insight in the underlying reasons for immuno-suppression in the peri-parturient period. These findings support a holistic approach to the study of the bovine immune response. These studies would include genetics but also physiological status of the animal. The recent completion and release into the scientific community of the bovine genome (http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/), provides a unique opportunity to better understand the underlying biological reasons for improved udder health.

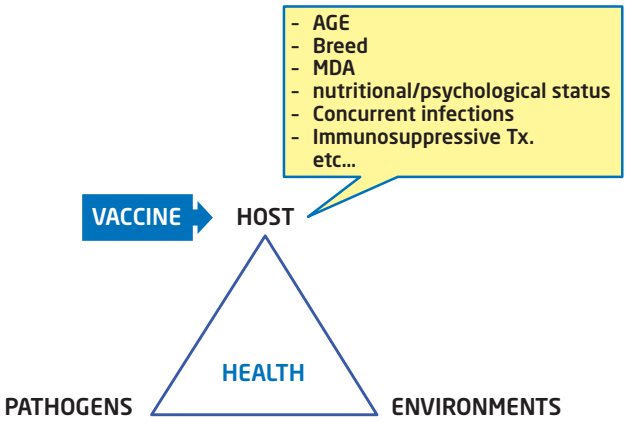


Figure 3.

Finally, our lack of highly efficacious intervention tools to support the immune response of mastitis affected cows, contributes to pain and suffering in these animals. Further research into the value of immune-altering, symptomatic and antimicrobial therapy is warranted, certainly with regard to newly recognized elements such as pain control and milk production losses. Similarly, prevention of IMI and subsequent clinical mastitis through a next generation of vaccines will provide more long-term solutions to the increasing problem of mastitis in dairy cows.

Outlook

Whereas the studies described in this article clearly indicate that vaccination against mastitis-causing pathogens will become more and more advanced within the next years, it also raised several semantic issues, which may impact on our understanding on an efficient vaccine. Indeed, different groups within the process of developing new vaccines may have completely different definitions when thinking about mastitis and the term "efficient vaccine". These definitions may vary from "free of any inflammation markers" in terms of mastitis to "SCC is back in normal range", or "induction of sterile immunity" to "reduction of severity of clinical signs by XY per cent" in the case of vaccines. It will become more and more important over the next years, that all parties involved, farmers – practitioners – pharma-companies and veterinary scientists will become more aware of the different definitions each group is working with, and keep these in mind when talking to each other. And lastly, one has to keep in mind that vaccination may represent only one side of dealing with mastitis – other factors have to be taken into account as well and should not be neglected (Figure 3). M²

References

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

Bean, A., Williamson, J., Cursons, R.T., 2004, Virulence genes of Escherichia coli strains isolated from mastitic milk. Journal of veterinary medicine. B, Infectious diseases and veterinary public health 51, 285-287.

Buitenhuis, B., Rontved, C.M., Edwards, S.M., Ingvartsen, K.L., Sorensen, P., 2011, In depth analysis of genes and pathways of the mammary gland involved in the pathogenesis of bovine Escherichia coli-mastitis. BMC genomics 12, 130.

Burton, J.L., Madsen, S.A., Yao, J., Sipkovsky, S.S., Coussens, P.M., 2005, An immunogenomics approach to understanding periparturient immunosuppression and mastitis susceptibility in dairy cows. Acta Vet. Scand., 42, 407-424.

Chaiyotwittayakun, A., Burton, J.L., Weber, P.S., Kizilkaya, K., Cardoso, F.F., Erskine, R.J., 2004, Hyperimmunization of steers with J5 Escherichia coli bacterin: effects on isotype-specific serum antibody responses and cross reactivity with heterogeneous gram-negative bacteria. Journal of dairy science 87, 3375-3385.

Garzoni, C., Kelley, W.L., 2009, Staphylococcus aureus: new evidence for intracellular persistence. Trends in microbiology 17, 59-65.

Genini, S., Badaoui, B., Sclep, G., Bishop, S.C., Waddington, D., Pinard van der Laan, M.H., Klopp, C., Cabau, C., Seyfert, H.M., Petzl, W., Jensen, K., Glass, E.J., de Greeff, A., Smith, H.E., Smits, M.A., Olsaker, I., Boman, G.M., Pisoni, G., Moroni, P., Castiglioni, B., Cremonesi, P., Del Corvo, M., Foulon, E., Foucras, G., Rupp, R., Giuffra, E., 2011, Strengthening insights into host responses to mastitis infection in ruminants by combining heterogeneous microarray data sources. BMC genomics 12, 225.

Gonzalez, R.N., Cullor, J.S., Jasper, D.E., Farver, T.B., Bushnell, R.B., Oliver, M.N., 1989, Prevention of clinical coliform mastitis in dairy cows by a mutant Escherichia coli vaccine. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 53, 301-305.

Gunther, J., Liu, S., Esch, K., Schuberth, H.J., Seyfert, H.M., 2010, Stimulated expression of TNF-alpha and IL-8, but not of lingual antimicrobial peptide reflects the concentration of pathogens contacting bovine mammary epithelial cells. Veterinary immunology and immunopathology 135, 152-157.

Harro, J.M., Peters, B.M., O'May, G.A., Archer, N., Kerns, P., Prabhakara, R., Shirtliff, M.E., 2010, Vaccine development in Staphylococcus aureus: taking the biofilm phenotype into consideration. FEMS immunology and medical microbiology 59, 306-323.

Hill, A.W., 1988, Pathogenicity of two strains of Streptococcus uberis infused into lactating and non-lactating bovine mammary glands. Research in veterinary science 45, 400-404.

Leitner, G., Lubashevsky, E., Glickman, A., Winkler, M., Saran, A., Trainin, Z., 2003a, Development of a Staphylococcus aureus vaccine against mastitis in dairy cows. I. Challenge trials. Veterinary immunology and immunopathology 93, 31-38.

Leitner, G., Lubashevsky, E., Trainin, Z., 2003b, Staphylococcus aureus vaccine against mastitis in dairy cows, composition and evaluation of its

immunogenicity in a mouse model. Veterinary immunology and immunopathology 93, 159-167.

Leitner, G., Pinchasov, Y., Morag, E., Spanier, Y., Jacoby, S., Eliau, D., Pitcovski, J., 2013, Immunotherapy of mastitis. Veterinary immunology and immunopathology 153, 209-216.

Leitner, G., Yadlin, N., Lubashevsy, E., Ezra, E., Glickman, A., Chaffer, M., Winkler, M., Saran, A., Trainin, Z., 2003c, Development of a Staphylococcus aureus vaccine against mastitis in dairy cows. II. Field trial. Veterinary immunology and immunopathology 93, 153-158.

Loffler, B., Tuchscherr, L., Niemann, S., Peters, G., 2014, Staphylococcus aureus persistence in non-professional phagocytes. International journal of medical microbiology : IJMM 304, 170-176.

Maxymiv, N.G., Bharathan, M., Mullarky, I.K., 2012, Bovine mammary dendritic cells: a heterogeneous population, distinct from macrophages and similar in phenotype to afferent lymph veiled cells. Comparative immunology, microbiology and infectious diseases 35, 31-38.

Mehrzad, J., Janssen, D., Duchateau, L., Burvenich, C., 2008, Increase in Escherichia coli inoculum dose accelerates CD8+ T-cell trafficking in the primiparous bovine mammary gland. Journal of dairy science 91, 193-201.

Middleton, J.R., Ma, J., Rinehart, C.L., Taylor, V.N., Luby, C.D., Steevens, B.J., 2006, Efficacy of different Lysigin formulations in the prevention of Staphylococcus aureus intramammary infection in dairy heifers. The Journal of dairy research 73, 10-19.

Petzl, W., Zerbe, H., Gunther, J., Yang, W., Seyfert, H.M., Nurnberg, G., Schuberth, H.J., 2008, Escherichia coli, but not Staphylococcus aureus triggers an early increased expression of factors contributing to the innate immune defense in the udder of the cow. Veterinary research 39, 18.

Prenafeta, A., March, R., Foix, A., Casals, I., Costa, L., 2010, Study of the humoral immunological response after vaccination with a Staphylococcus aureus biofilm-embedded bacterin in dairy cows: possible role of the exopolysaccharide specific antibody production in the protection from Staphylococcus aureus induced mastitis. Veterinary immunology and immunopathology 134, 208-217.

Rainard, P., Riollet, C., 2006, Innate immunity of the bovine mammary gland. Veterinary research 37, 369-400.

Rambeaud, M., Almeida, R.A., Pighetti, G.M., Oliver, S.P., 2003, Dynamics of leukocytes and cytokines during experimentally induced Streptococcus uberis mastitis. Veterinary immunology and immunopathology 96, 193-205.

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

Schukken, Y.H., Günther, J., Fitzpatrick, J., Fontaine, M.C., Goetze, L., Holst, O., Leigh, J., Petzl, W., Schuberth, H.J., Sipka, A., Smith, D.G.E., Quesnell, R., Watts, J., Yancey, R., Zerbe, H., Gurjar, A., Zadoks, R.N., Seyfert, H.M., 2011, Host-response patterns of intramammary infections in dairy cows. Veterinary immunology and immunopathology 144, 270-289.

Schukken, Y.H., Hertl, J., Bar, D., Bennett, G.J., Gonzalez, R.N., Rauch, B.J., Santisteban, C., Schulte, H.F., Tauer, L., Welcome, F.L., Grohn, Y.T., 2009, Effects of repeated gram-positive and gram-negative clinical mastitis episodes on milk yield loss in Holstein dairy cows. Journal of dairy science 92, 3091-3105.

Shafer-Weaver, K.A., Pighetti, G.M., Sordillo, L.M., 1996, Diminished mammary gland lymphocyte functions parallel shifts in trafficking patterns during the postpartum period. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine 212, 271-280.

Shafer-Weaver, K.A., Sordillo, L.M., 1996, Enhancing bactericidal activity of bovine lymphoid cells during the periparturient period. Journal of dairy science 79, 1347-1352.

Sordillo, L.M., 2005, Factors affecting mammary gland immunity and mastitis suceptibility. Livestock Production Science 98, 89-99.

Stevens, T.L., Bossie, A., Sanders, V.M., Fernandez-Botran, R., Coffman, R.L., Mosmann, T.R., Vitetta, E.S., 1988, Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. Nature 334, 255-258.

Suojala, L., Orro, T., Jarvinen, H., Saatsi, J., Pyorala, S., 2008, Acute phase response in two consecutive experimentally induced E. coli intramammary infections in dairy cows. Acta veterinaria Scandinavica 50, 18.

Wenz, J.R., Barrington, G.M., Garry, F.B., Ellis, R.P., Magnuson, R.J., 2006, Escherichia coli isolates' serotypes, genotypes, and virulence genes and clinical coliform mastitis severity. Journal of dairy science 89, 3408-3412.

Werling, D., Jungi, T.W., 2003, TOLL-like receptors linking innate and adaptive immune response. Veterinary immunology and immunopathology 91, 1-12.

Wilson, D.J., Grohn, Y.T., Bennett, G.J., Gonzalez, R.N., Schukken, Y.H., Spatz, J., 2007a, Comparison of J5 vaccinates and controls for incidence, etiologic agent, clinical severity, and survival in the herd following naturally occurring cases of clinical mastitis. Journal of dairy science 90, 4282-4288.

Wilson, D.J., Grohn, Y.T., Bennett, G.J., Gonzalez, R.N., Schukken, Y.H., Spatz, J., 2008, Milk production change following clinical mastitis and reproductive performance compared among J5 vaccinated and control dairy cattle. Journal of dairy science 91, 3869-3879.

Wilson, D.J., Mallard, B.A., Burton, J.L., Schukken, Y.H., Grohn, Y.T., 2007b, Milk and serum J5-specific antibody responses, milk production change, and clinical effects following intramammary Escherichia coli challenge for J5 vaccinate and control cows. Clinical and vaccine immunology : CVI 14, 693-699.

Wilson, D.J., Mallard, B.A., Burton, J.L., Schukken, Y.H., Grohn, Y.T., 2009, Association of Escherichia coli J5-specific serum antibody responses with clinical mastitis outcome for J5 vaccinate and control dairy cattle. Clinical and vaccine immunology : CVI 16, 209-217.

Zadoks, R.N., van Leeuwen, W.B., Kreft, D., Fox, L.K., Barkema, H.W., Schukken, Y.H., van Belkum, A., 2002, Comparison of Staphylococcus aureus isolates from bovine and human skin, milking equipment, and bovine milk by phage typing, pulsed-field gel electrophoresis, and binary typing. Journal of clinical microbiology 40, 3894-3902.