Recent Advances in Genetic Resistance to Mastitis in Cattle

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Introduction

Mastitis resistance is in focus from an animal welfare perspective as well as due to its impact on the economy of milk production. Consumer's acceptance of high performance dairy production systems is largely dependent on the health and well-being of the cows. Furthermore, mastitis is not only one on the major reasons for involuntary culling (Seegers *et al.*, 2003), but also for major productive losses. Recent economical estimations indicated an average loss per mastitis incident ranging from 140 to 570 € depending on the pathogen and the status of the cow (Bar *et al.*, 2008, Sorensen *et al.*, 2010). Selection experiments in cattle and sheep, however, demonstrated that within few generations substantial progress can be achieved by selection on mastitis resistance (Heringsstad *et al.*, 2003, Rupp *et al.*, 2009). Additionally, retrospective data on predicted transmitting ability for somatic cell score (SCS) clearly indicated that selection on sires` SCS resulted in favorable phenotypes of cows regarding productive live, percentage of cows culled for mastitis, and SCS (Miller *et al.*, 2009).

However, in spite of its relevance and the demonstrated achievable selection gain, the breeding values for mastitis resistance or somatic cell count in many dairy cattle populations do not show the desirable improvement (e.g., VIT 2010, www.vit.de). Reasons may be the unfavorable correlation of mastitis with milk performance traits, the difficulty in trait recording, the low heritability of the trait and lack of precise knowledge on the background of the existing, but not fully exploited genetic diversity regarding mastitis susceptibility. In recent years, the growing concern about functional traits in dairy production has fostered many studies on the background of genetically modulated variability in resistance to mastitis. Additionally, new tools became available for including respective outcome into dairy cattle breeding.

Physiological background of mastitis resistance

Mastitis resistance is the ability to either avoid contact to pathogens (e.g., due to favourable conformation or behaviour) or to successfully fight a pathogen after invasion (Figure 1). The latter can be impaired by a genuinely deficient immunological response or by factors decreasing immunological capacity (e.g., metabolic disequilibrium). Melendez *et al.* (2009) described mastitis being prominently at risk in cows with severely elevated plasma non-esterified fatty acids (NEFAs) level compared to mates with average to slightly increased

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NEFA levels. Nyman *et al.* (2008) agreed in NEFAs as an informative predictor of elevated SCS in primiparous heifers. This fits the observation of Morris *et al.* (2009) describing impaired splenic expression of genes relevant in immune response in individuals with severe negative energy balance. Furthermore, there is increasing evidence for a physiological link between fat metabolism and immune response in mammals providing a tie between the increased mastitis incidence and the lipomobilisation at the start of lactation (e.g., Moyes *et al.*, 2009.

Commonly, mastitis is recorded in a global definition without considering, e.g., the major host-driven differences affecting the course and the outcome of the disease. Some common protective mechanisms are in place that seem to act upon a variety of pathogens. Strandberg-Lutzow *et al.* (2008) identified upregulated mRNA and protein levels for S100 calcium-binding protein A12 (*S100A12*) and Pentraxin-3 (*PTX3*) in the mammary gland, when challenged with *S. aureus*, and demonstrated a growth inhibiting effect of S100A12 on *E. coli in vitro*. Correspondingly, there was a correlation between S100A12 and SCS in milk. However, there is growing evidence that frequently specific mechanisms are in action for fighting invading pathogens depending on the pathogen itself (Petzl *et al.*, 2008), because signaling pathways and speed of response to invasion can differ markedly. Jiang *et al.* (2008) provided evidence of the relevance of liver acute phase response to intra-mammary LPS challenge, which points to the divergent protecting mechanisms against *E. coli* (LPS) and e.g., *Streptococcus/Staphylococcus* (Lipoteichonic acid).

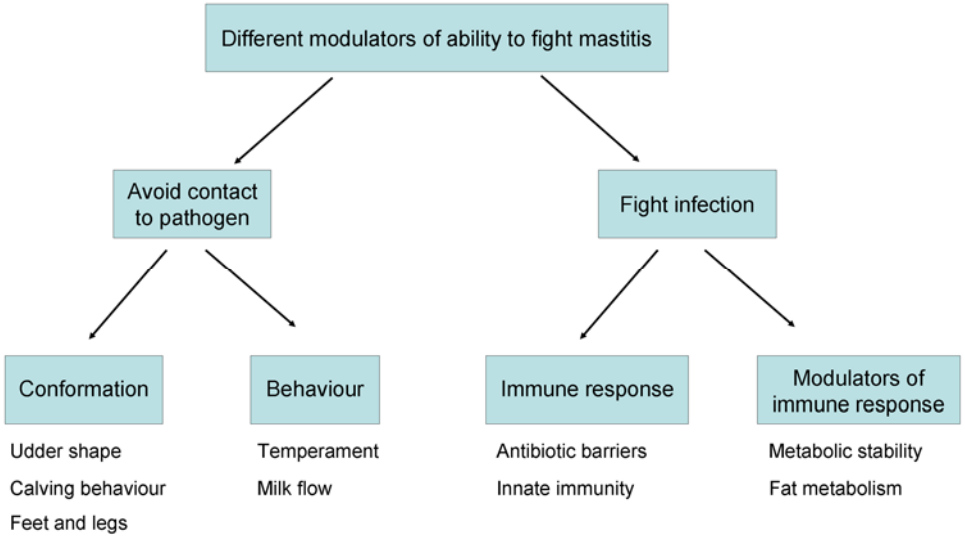

Figure 1: Potential strategies of the cow to fight mastitis

Genetic dissection of factors affecting mastitis resistance

Inevitable condition for successful selection schemes for improved udder health is a precise definition of the trait. Recently, several approaches to improve trait recording for monitoring and refining the mastitis phenotype via further complementary traits are described and

evaluated: milk flow pattern, milk conductivity, antimicrobial peptides including acute phase proteins (haptoglobulin, serum amyloid or lipopolysaccharide binding protein) or other biomarkers for mastitis or immune response assays. Additionally, new, yet unknown biomarkers are tested for using proteomic approaches (Boehmer *et al.*, 2008). Furthermore, flow cytometry of milk cells is suggested for diagnosis of subclinical mastitis (Koess and Hamann, 2008). Regarding mastitis recording itself, dissection of mastitis records respective to the time of incidence relative to calving (Luan *et al.*, 2010, Nilsen *et al.*, 2009) or number of mastitis cases during a lactation (Vallimont *et al*., 2009; Perez-Cabal *et al.*, 2009) is suggested. However, in spite of these scientific approaches towards refined mastitis measurements in many countries commonly still SCS is used as a surrogate trait for routine genetic evaluation of udder health due to lacking mastitis recording. But the divergent correlation between milk yield and mastitis or SCS (Rupp and Boichard, 2003) and the mutual genetic correlation of ≈ 0.7 (Sorensen *et al.*, 2009b) indicate that, although correlated, both traits surely do not share an identical genetic background. Even within reported cases of mastitis divergent heritabilities were estimated depending on the causative pathogen (Sorensen *et al.*, 2009a), and also a differential genetic correlation (0.45 – 0.77) between mastitis cases due to divergent pathogens was described. Even subtypes of pathogen species seem to trigger different host responses as demonstrated by divergent effects of different subtypes e.g., of *S. aureus* on somatic cell count (Graber *et al.*, 2009). The pathogen-dependence of susceptibility modulating genetic factors is further underlined by QTL for mastitis exhibiting pathogen specificity (Sorensen *et al.*, 2008). Potential drawbacks, however, for a pathogen-specifying mastitis monitoring seem to be problems with an unbiased phenotype recording (Sorensen *et al.*, 2009b).

In addition to the pathogen-specificity regarding genetically determined susceptibility to mastitis, there is growing evidence that mastitis is not the same trait over the course of lactation (Carlen, 2008). It can be speculated that e.g., different pathogens and different mechanisms affecting susceptibility (e.g., different ability to fight metabolic stress in the beginning of lactation vs. genuine differences in innate immunity or development of callous teat tips or divergent milk leakage) could be the reason for these differences.

Concurrently to improving conventional selection schemes by specific trait recording and refined statistical methods, the development of strategies for decreasing mastitis susceptibility has been a major field of research for marker assisted selection, either by QTL studies or, recently, by approaches of Genomic selection. Improvement of low heritability traits is expected to be especially rewarding for approaches applying genetic markers due to problems with conventional selection schemes.

From the substantial number of QTL modulating mastitis incidence and/or somatic cell score described in the literature (summarized by Ogorevc *et al.*, 2009), several loci have been further refined by fine mapping approaches to improve knowledge of the molecular nature of the QTL and enhance the implementation of marker information into practical selection schemes (BTA6: Nilsen *et al.*, 2009; BTA9: Sahana *et al.* 2008; BTA11: Schulman *et al.* 2009, BTA18: Baes *et al.* 2009, Brand *et al.* 2009). Nilsen et al (2009) resolved a QTL for mastitis resistance and SCS in the close vicinity of the casein cluster on BTA6. They provide an example how the import of a favorable haplotype for milk production traits that is

unfavorable for mastitis resistance into a population spread out and gained a substantial frequency in the population due to increased selection pressure on production traits. The opposite phenomenon is highlighted by the obviously declining frequency of a putatively causal advantageous allele in the *FEZL* gene (Sugimoto *et al.*, 2006, Sonstegard *et al.*, 2005). It demonstrates that identification of causal mutations enables frequency control of valuable or deleterious alleles with large effects, a genetic model currently under discussion as background for disease liability in humans (Bochukova *et al.*, 2010). The aspect of population specific QTL was highlighted by Schulman *et al.* (2009), who identified a QTL for mastitis in Finnish Ayrshire cattle, which was essentially absent in two other Nordic breeds. Another feature of population-specificity of QTL affecting health traits is demonstrated by Bennewitz *et al.* (2003), who reported differences regarding QTL detection in a granddaughter design from two Holstein populations sharing common grandsires depending on the country, in which the sons were tested.

Regarding genetic variants associated with udder health, a number of trait-associated gene variants have been reported (for review see Ogorevc *et al.*, 2009, Verschoor *et al.*, 2009, Duangjinda *et al*., 2009, Alain *et al*., 2009). However, up to now, all variants more or less lack convincing confirmation studies regarding their causal role in modulating mastitis incidence, although for some of the genes, successful in-vitro-assays suggesting a potential functional role in mastitis have been described (e.g., Sugimoto *et al.*, 2006).

Interestingly, Daetwyler *et al.* (2008) described a number of significant associations with SCS from a first whole genome association study with a 10k Affymetrix SNP chip, but the authors did not find an overlap with the results obtained from a variance component QTL analysis in the same data set. Analogous lack of congruence between results exploiting within-family linkage or across-population linkage disequilibrium was obtained from some LALD analyses (e.g., Sahana *et al.*, 2008, Brand *et al.*, 2009). Whether this is due to the experimental design (e.g., selection and density of markers) or true characteristics of the QTL (clusters of QTL in the same chromosomal region, rare alleles with large effects) remains to be established. The wealth of data that will be generated in the course of applying Genomic selection in dairy cattle populations might help resolving this question.

Up to now, Genomic selection approaches in different countries and different settings with several thousand proven sires in the training population achieved remarkably similar reliabilities for genomic breeding values of 61 - 66 % for the genomic SCS-EBV/PTA (VIT 2009, www.vit.de, 2009, AIPL, 2010, http://www.aipl.arsusda.gov/). This value was smaller than for milk production traits, however, still the most accurate compared to EBV for other functional trait complexes.

Novel insights into mechanisms affecting genetic background of variable mastitis resistance by merging functional and genetic analyses

Ogorevc *et al.* (2009) assembled a comprehensive database collecting information from QTL analyses, association studies, mouse knock-out models, gene expression studies related to

milk production and/or mastitis or microRNAs expressed in mammary gland. However, most recent studies focussed on one of these aspects, which makes limited use of the available resources. Combining the physiological and the genetic approach should provide new opportunities to unravel the genomic variation underlying divergent capability to fight mastitis infection.

One strategy to fight mastitis is a decreased contact to the pathogen achieved e.g., by a shallow and tightly attached udder. A concurrent analysis of udder health and conformation traits on BTA 18 revealed a strong concordance between a QTL for SCS and QTL affecting udder conformation traits (Brand *et al.*, in press). However, the authors demonstrated that this concordance is restricted to single, however powerful grandsire families, while other large families lack respective concordance. These data support the hypothesis of several QTL affecting udder health on BTA18, one of them acting via udder morphology. The targeted genomic region on BTA18 also seems to be a major contributor to other fitness traits, at least in Holsteins as demonstrated by Cole *et al.* (2009). Due to its impact on dairy cattle efficiency, further studies to dissect the background of trait association seem warranted.

Merging data from physiologically and from genetically driven research projects initiated targeted approaches to unravel the background for genomic loci affecting udder health. Taking advantage of previous QTL mapping data in the commercial population, a pilot proof-of-principle selection experiment (MAS) had been performed applying information on marker assisted EBV of sires and established marker-QTL haplotypes for BTA18 (Kühn *et al.*, 2008). Compared to individuals selected by conventional selection (CON), first lactation heifers marker-selected prior to first calving for low and high susceptibility to mastitis showed a uniform highly divergent somatic cell count between groups already early in lactation. This contrast between groups was the more remarkable as the heifers from the MAS groups comprised halfsibs in contrast to a very divergent genetic background of the CON heifers displaying less difference between selection groups.

Consecutive targeted and holistic transcriptome profiling experiments in primary mammary gland epithelial (MEC) cell cultures of the selected heifers contributed information on potential mechanisms underlying the targeted BTA18 QTL. Griesbeck *et al.* (2009) demonstrated an increased mRNA expression for a number of genes relevant in innate immune response after challenging the MEC with a *S. aureus* or *E. coli* strain, both relevant mastitis pathogens. Whereas at early time points (1h) after challenge, only insignificant differences between cells from lowly and highly susceptible heifers according to MAS were detected, these differences increased in size over time and were significant after 24 h for *Toll like receptor 2 (TLR2)*, *Tumor necrosis factor alpha (TNFα*), *Interleukin 1β (IL1β)*, *Interleukin 8 (IL8), chemokine (C-C motif) ligand 5 (CCL5), Complement factor 3 (C3)* and *lactoferrin (LTF)* (Figure 2). These results point towards a specific variation in *TLR2* signaling and consecutive innate immune response as potential background of the QTL. In contrast to the substantial, significant differences in targeted gene expression between groups selected for low and high susceptibility by means of marker assisted selection, groups that were conventionally selected displayed only small, mostly insignificant differences. As selected on polygenic effects comprising the entire genome, the background of mastitis

susceptibility within the conventionally selected groups is presumably very divergent comprising also non-immunological factors e.g., metabolic stability.

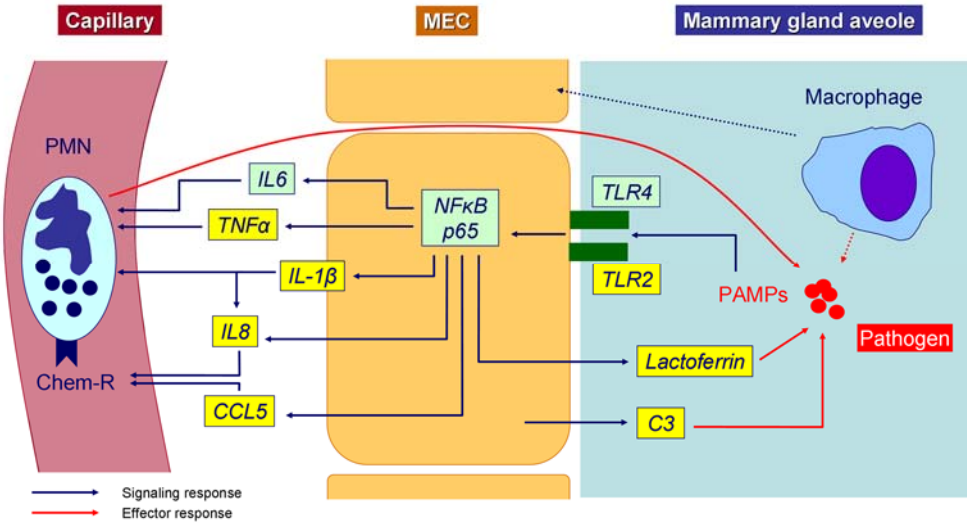

Figure 2: Differences in the expression of genes involved in innate immunity from MEC of heifers divergently susceptible to mastitis 24 h after challenge with *S. aureus*. MEC: Mammary gland epithelial cell, Chem-R: chemokine receptor, PMN: neutrophil granulocyte, PAMPS: pathogen associated molecular pattern (data from Griesbeck-Zilch *et al.*, 2009).

The results about the divergent coordinated expression of innate immunity genes point towards self-regulatory mechanisms in the MEC as source of divergent mastitis susceptibility. A central role of NFκB regulated effects on pathogen response is demonstrated, although NFκB, specifically NFκBp65, is not divergently expressed. However, due to its crucial role as regulating transcription factor, differences in NFκBp65 are presumably under much stricter constrains compared to effector molecules like lactoferrin. The results from the in-vitro studies clearly elicit functional pathways affected by genetic modulators, which have to be scrutinized for potential genetic variants associated with udder heath. Furthermore, data merging genetics and physiology suggest that divergent results from functional studies monitoring udder health on in-vivo or in-vitro samples (e.g., Swanson *et al.*, 2009) should consider the genetic make-up of the individuals included in the studies, because this could contribute a major source of hidden variation. Additionally, the results confirm the previous evidence for an essential role of the MEC not only for milk production, but also in activation of early immune response.

Conclusion

Although significant progress has recently been achieved regarding the background of genetic variability in the resistance to mastitis and the best strategies for its implementation in dairy cattle, there is no immediate remedy for the currently high incidence of mastitis.

Successful future breeding strategies will require not only a refined analysis of genetic variants modulating susceptibility of the disease, but also an enhanced understanding of the respective physiological mechanisms.

References

Alain, K., Karrow, N., Thibault, C. *et al.* (2009). *BMC Genomics* 10: 18.

Baes, C., Brand, B., Mayer, M. *et al.* (2009). *J. Dairy Sci.* 92: 4046-4054.

Bar, D., Tauer, L. W., Bennett, G. *et al.* (2008). *J. Dairy Sci.* 91: 2205-2214.

Bennewitz, J., Reinsch, N., Grohs, C. *et al.* (2003). *Genet. Sel. Evol.* 35: 319-338.

Bochukova, E. G., Huang, N., Keogh, J. *et al.* (2010). *Nature* 463: 666-670.

Boehmer, J. L., Bannerman, D. D., Shefcheck, K. *et al.* (2008). *J. Dairy Sci.* 91: 4206-4218.

Brand, B., Baes, C., Mayer, M. *et al.* (2009). *BMC Genetics* 10.

Carlen, E. 2008. Genetic evaluation of clinical mastitis in dairy cattle. Swedish University of Agricultural Science.

Cole, J. B., Vanraden, P. M., O'Connell, J. R. *et al.* (2009). *J. Dairy Sci.* 92: 2931-2946.

Daetwyler, H. D., Schenkel, F. S., Sargolzaei, M. *et al.* (2008). *J. Dairy Sci.* 91: 3225-3236.

Duangjinda, M., Buayai, D., Pattarajinda, V. *et al.* (2009). *J. Anim. Sci.* 87: 469-476.

Graber, H. U., Naskova, J., Studer, E. *et al.* (2009). *J. Dairy Sci.* 92: 1442-1451.

Griesbeck-Zilch, B., Osman, M., Kuhn, C. *et al.* (2009). *J. Dairy Sci.* 92: 4621-4633.

Heringstad, B., Klemetsdal, G., and Steine, T. (2003). *J. Dairy Sci.* 86: 2990-2999.

Jiang, L., Sorensen, P., Rontved, C. *et al.* (2008). *BMC Genomics* 9.

Koess, C., and Hamann, J. (2008). *J. Dairy Res.* 75: 225-232.

Kühn, C., Reinhardt, F., and Schwerin, M. (2008). *Arch. Anim. Breed.* 51: 23-32.

Luan, T., Woolliams, J. A., Lien, S. *et al.* (2009). *Genetics* 183: 1119-1126.

Melendez, P., Marin, M. P., Robles, J. *et al.* (2009). *Theriogenology* 72: 826-833.

Miller, R., Norman, H., Wright, J. *et al.* (2009). *J. Dairy Sci.* 92: 2224-2228.

Morris, D. G., Waters, S. M., McCarthy, S. D. *et al.* (2009). *Physiol. Genomics* 39: 28-37.

Moyes, K., Drackley, J., Morin, D. *et al.* (2009). *BMC Genomics* 10: 19.

Nilsen, H., Olsen, H. G., Hayes, B. *et al.* (2009). *Anim. Genet.* 40: 701-712.

Nyman, A. K., Emanuelson, U., Holtenius, K. *et al.* (2008). *J. Dairy Sci.* 91: 2996-3009.

Ogorevc, J., Kunej, T., Razpet, A. *et al.* (2009). *Anim. Genet.* 40: 832-851.

Perez-Cabal, M. A., de los Campos, G., Vazquez, A. I. *et al.* (2009). *J. Dairy Sci.* 92: 3472-3480.

Petzl, W., Zerbe, H., Gunther, J. *et al.* (2008). *Veterinary Res.* 39: 1-23.

Rupp, R., Bergonier, D., Dion, S. *et al.* (2009). *J. Dairy Sci.* 92: 1203-1219.

Rupp, R., and Boichard, D. (2003). *Veterinary Res.* 34: 671-688.

Sahana, G., Lund, M. S., Andersson-Eklund, L. *et al.* (2008). *Anim. Genet.* 39: 354-362.

Schulman, N., Sahana, G., Iso-Touru, T. *et al.* (2009). *Anim. Genet.* 40: 509-515.

Seegers, H., Fourichon, C., and Beaudeau, F. (2003). *Veterinary Res.* 34: 475-491.

Sonstegard, T. S., Van Tassell, C. P., and Li, R. (2005). *J. Anim. Sci.* 83: 201.

Sorensen, L. P., Guldbrandtsen, B., Thomasen, J. R. *et al.* (2008). *J. Dairy Sci.* 91: 2493-2500.

Sorensen, L. P., Madsen, P., Mark, T. *et al.* (2009a). *Animal* 3: 647-656.

Sorensen, L. P., Mark, T., Madsen, P. *et al.* (2009b). *J. Dairy Sci.* 92: 3457-3471.

Sorensen, L. P., Mark, T., Sorensen, M. K. *et al.* (2010). *J. Dairy Sci.* 93: 358-369.

Strandberg-Lutzow, Y., Donaldson, L., Gray, C. *et al.* (2008). *BMC Veterinary Research* 4: 02.

Sugimoto, M., Fujikawa, A., Womack, J. E. *et al.* (2006). *Proc. Natl. Acad. Sci. USA* 103: 6454-6459.

Swanson, K., Stelwagen, K., Dobson, J. *et al.* (2009). *J. Dairy Sci.* 92: 117-129.

Vallimont, J. E., Dechow, C. D., Sattler, C. G. *et al.* (2009). *J. Dairy Sci.* 92: 3402-3410.

Verschoor, C., Pant, S., Schenkel, F. *et al.* (2009). *Mamm. Genome* 20: 447-454.