Invited review: Low milk somatic cell count and susceptibility to mastitis

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ABSTRACT

An enduring controversy exists about low milk cell counts and susceptibility to mastitis. The concentration of milk leukocytes, or somatic cell count (SCC), is a well-established direct indicator of mammary gland inflammation that is highly correlated with the presence of a mammary infection. The SCC is also used as a trait for the selection of dairy ruminants less prone to mastitis. As selection programs favor animals with less SCC, and as milk cells contribute to the defense of the mammary gland, the idea that susceptibility to mastitis could possibly be increased in the long term has been put forward and is still widely debated. Epidemiological and experimental studies aimed at relating SCC to susceptibility to mastitis have yielded results that seem contradictory at first sight. Nevertheless, by taking into account the immunobiology of milk and mammary tissue cells and their role in the defense against infection, along with recent studies on SCC-based divergent selection of animals, the issue can be settled. Apparent SCC-linked susceptibility to mastitis is a phenotypic trait that may be linked to immunomodulation but not to selection.

Key words: dairy ruminant, mastitis, genetic selection, somatic cell count

INTRODUCTION: ARE COWS WITH LOW MILK SOMATIC CELL COUNTS AT GREATER RISK OF MASTITIS?

The concentration of milk cells, or the SCC, is a very sensitive biomarker of mammary gland inflammation. Variations in SCC depend mainly on the recruitment of leukocytes from blood to tissue and finally to milk, most often in response to an inflammatory reaction elicited in the mammary tissue by the intrusion of bacteria into the mammary gland (MG). The SCC,

or parameters derived from this count, is often used to distinguish between infected and uninfected MG and to monitor udder health (Schukken et al., 2003). The SCC is thus used as an indirect indicator of MG infection. The recruited leukocytes play an important role in the defense of the MG, as shown by the very severe mastitis that develops when the leukocyte influx into milk is delayed or blocked (Craven and Williams, 1985; Vangroenweghe et al., 2005). The SCC, or often its log-derived SCS (Ali and Shook, 1980), is also widely used as a selection criterion for the genetic improvement of mastitis resistance (Odegard et al., 2003). In most countries, estimated breeding values for genetic selection on mastitis are usually based on linear models of log-transformed SCC, without a limit for low SCC. Average lactation SCC is generally used because its heritability is moderate (about 0.15) and the data are widely available (Rupp and Boichard, 2003; Rupp and Foucras, 2010). One limitation is that this trait is linked mainly to chronic subclinical infections and more loosely to short-lasting clinical infections that are typically induced by coliform bacteria. Nevertheless, a good genetic correlation (on average 0.6 to 0.7) is present between SCC and clinical mastitis (Rupp and Boichard, 1999; Heringstad et al., 2000; Carlen et al., 2004; Govignon-Gion et al., 2016), supporting the use of SCC as a surrogate for clinical mastitis, but it also shows that these 2 mastitis indicators are not the same trait (Mrode and Swanson, 1996).

The consensus is that high SCC should be selected against, but opinions diverge on the significance of low SCC. The question arises of the risk that could result from the selection of animals with very low concentrations of cells in their milk, which would predispose them to IMI and clinical mastitis. Some authors think that it is sensible to keep a reasonable concentration of cells in milk (Kehrli and Shuster, 1994; Schukken et al., 2001), whereas others have not seen a lower limit to the low SCC associated with reduced incidence of mastitis (Philipsson et al., 1995; Rupp and Boichard, 2000). It has also been put forward that the SCC has 2 distinct components, the baseline SCC under physiological and

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Figure 1. Relationship between some major determinants of the mammary gland (MG) health status. Genetic selection can affect the innate and adaptive immunity, for which the SCC is an important effector and marker, and important morphological traits of the MG. The innate/adaptive immunity is also determined by previous and current interactions with the microbial environment. The integration of these determinants will condition the MG susceptibility/resistance to mastitis. The MG will then respond to the infection pressure in more or less efficient ways, with the end result being clinical mastitis, transient or chronic subclinical mastitis, or no infection. Color version available online.

environmental influence and the component related to the susceptibility to mastitis (Odegard et al., 2005), and that SCC is not the same trait in cows with and without mastitis (Heringstad et al., 2006). Several epidemiological surveys have tackled the question of low SCC and susceptibility to mastitis (Green et al., 1996; Barkema et al., 1998; Beaudeau et al., 1998; Rupp et al., 2000; Suriyasathaporn et al., 2000; Whist and Osteras, 2007; van den Borne et al., 2011). Their analyses provide seemingly contradictory results that do not resolve the issue, so that the question still lingers (Wellnitz et al., 2010). Other factors have to be considered to get over this apparent deadlock. By taking into account the different mechanisms that allow somatic cells and the mammary tissue to deal with IMI, it is possible to show that the apparently contradictory epidemiological studies are not irreconcilable. Moreover, recent experimentations on dairy ruminants selected on the basis of the SCC provide new information on key points of the issue, so that a convincing settlement can be put forward.

SEEMINGLY CONTRADICTORY EPIDEMIOLOGICAL STUDIES

Numerous epidemiological surveys have grappled with the issue of the relation between SCC levels and resistance to mastitis. It is difficult to work out the

resulting picture because the studies are based on recordings of clinical mastitis, bulk milk (herd) SCC, cow SCC, or quarter SCC. Moreover, the identification of the causing pathogens by bacteriological analyses have seldom been carried out. In most cases, clinical mastitis is monitored, but subclinical mastitis is often estimated through cow SCC values over the lactation period. Moreover, it is the integration of several major independent or interrelated factors that determines the MG health status (Figure 1). All this is somewhat confusing, but from this heterogeneous collection of data and concepts it is nevertheless possible to extract useful information.

The relationship between the SCC at the herd level (measured through the bulk milk SCC) and the incidence of clinical mastitis seems to depend on the type of infection prevalent in the herds. In a survey comparing herds with low (<150,000 cells/mL) and high (>750,000 cells/mL) bulk milk SCC, the incidence of clinical mastitis was higher in the latter (Erskine et al., 1988). Bacteriological analyses showed that most clinical MG infections were caused by streptococci and staphylococci in the high bulk milk SCC herds, and caused by coliform bacteria in the low bulk milk herds. This trend of a higher incidence of mastitis by coliform bacteria in a low bulk milk SCC herd has been reported by others (Hogan et al., 1989). Another study found that herds with bulk milk SCC less than 150,000 cells/

mL had more clinical mastitis cases than herds with bulk milk SCC more than 250,000 cells/mL (Milteneburg et al., 1996). Another study in the Netherlands found no association between bulk milk SCC and the incidence of clinical mastitis, but a wider variation in the incidence in low SCC (<150,000 cells/mL) herds (Barkema et al., 1998). Notably, the link between clinical mastitis incidence and low bulk milk SCC is nominal. In a study involving 1,771 herds with bulk milk SCC less than 100,000 cells/mL, the incidence of clinical mastitis varied from 0.6 to 147.1 cases per 100 cows per year (Peeler et al., 2000). This indicates that a high incidence of clinical mastitis in low SCC herds is not an inevitable fate. Moreover, in high bulk milk SCC herds the incidence of clinical mastitis can also be high because they have a high proportion of chronically infected cows, and these cows are prone to recurrent bouts of clinical mastitis. Thus, this confounding element has to be removed from the equation of the high SCC due to infections by contagious pathogens. Only in herds with low SCC, likely to have a low proportion of chronic subclinical mastitis, can the susceptibility of low SCC cows be evaluated (Bradley, 2002).

At the cow level, several studies have shown that the incidence of clinical mastitis usually increases as the SCC decreases. It has been reported that the risk of clinical mastitis increased in herds as the proportion of low SCC (<50,000 cells/mL) cows also increased (Beaudeau et al., 2002). In a herd monitored for infections for 12 yr, low SCC (<200,000 cells/mL) was associated with a higher risk of severe clinical mastitis (Suriyasathaporn et al., 2000). Several studies have suggested that clinical mastitis was more severe in low SCC herds or in cows with low SCC (Green et al., 1996; Barkema et al., 1998; Tadich et al., 1998). Although several factors may contribute to these observations, such as a higher proportion of infections by gram-negative bacteria, they could be interpreted as resulting in part from a decreased capacity of cows to control MG infections when their milk SCC is low.

At variance with the studies cited above and the conclusion that can be drawn from them, other studies found a reduced risk of clinical mastitis associated with low SCC. The incidence of clinical mastitis can be lower in herds with low bulk milk SCC than in herds with moderate or high SCC (Berry, 1994). No association was observed between individual cow SCC and clinical mastitis for SCC up to 100,000 cells/mL, and an increased risk for higher SCC (Beaudeau et al., 1998). In a study involving 274 Dutch farms, cows with low SCC during early lactation were not more susceptible to clinical mastitis (de Haas et al., 2002). Another study involving 20,422 primiparous Holstein cows with SCC <400,000 cells/mL indicated that the cows with the lowest SCC

had the lowest risk of clinical mastitis (Rupp and Boichard, 2000). The same trend was observed in second lactation with a variety of SCC criteria (Rupp et al., 2000). A study involving 350 herds in Norway explored the association between early lactation, or drying-off SCC, and the occurrence of clinical mastitis during the ongoing or subsequent lactation: the results indicated that the cows with very low SCC (<20,000 cells/mL) were at the lowest risk of developing clinical mastitis (Whist and Osteras, 2007).

A DEBATE ABOUT THE SELECTION BASED ON MILK SOMATIC CELLS

From the observational studies mentioned above, and from the experimental data discussed below, a concern arose about the possible association of very low SCC with an increased susceptibility to mastitis. The idea emerged that a minimum concentration of somatic cells (leukocytes) would be necessary to protect the cow from MG infections. By positing a physiological optimal concentration of somatic cells in milk, the appropriateness of selecting cows for very low SCC can be questioned. Selection against cows with high SCC is supposed to favor resistance to mastitis, but the debate concerns very low SCC. Simply put, the question “Can somatic cell counts get too low?” can be asked. Diverse answers have been proposed to this question (Timms, 1990; Schukken et al., 2001).

The ongoing selection programs have no inferior limit for SCS, which was initially treated as a linear trait. Doubt was introduced about the pertinence of selecting for very low SCC, which would not be optimal for the resistance of the udder against infections (Heringstad et al., 2000). It has been suggested that a “physiological SCC,” associated with a physiological immune competence, can be opposed to a “very low SCC,” associated with an increased susceptibility to infection, which implicitly supposes that very low SCC is an abnormal condition (Wellnitz et al., 2010). It is thus important to know whether a risk is associated with the selection of cows with very few leukocytes in their milk. To answer this question, it is necessary to examine the biology behind the SCS trait. Also, invoking genetic selection as a driving force toward very low SCC supposes that there is a genetically determined SCC baseline, and that selection could favor higher or lower SCC baselines. This has not been documented, and as the physiological SCC baseline of Holstein cows about 1 mo in lactation is usually less than 20,000 cells/mL, it is difficult to imagine a lower SCC baseline. That infection/inflammation-free udder SCC is low is not a new consideration, as it had been claimed already in the early 1970s that any mid-lactation milk with higher

than 20,000 cells/mL was the result of inflammation accompanied by reduction in milk production (Schalm et al., 1971).

THE NATURE OF THE MILK SOMATIC CELLS

Different Cell Types Make Up the SCC, Which Undergoes an Inflammation Shift

Milk somatic cells are of several types. Leukocytes represent the bulk of the SCC, with a few epithelial cells that are shed from the epithelium (Schalm and Lasmanis, 1968; Concha, 1986). Milk somatic cells from healthy glands are mostly cells of the immune system, including lymphocytes, macrophages, and a small proportion of neutrophil granulocytes (Concha, 1986; Dosogne et al., 2003). The proportion of leukocytes is in the order of 99% in inflamed glands, but with most neutrophils (Riollet et al., 2000). Neutrophils play a major role in the control of bacterial proliferation in the lumen of the MG (Paape et al., 2002). Inflammation provokes a dramatic shift in the composition of the SCC, from mononuclear to polymorphonuclear cells (Sarıkaya et al., 2006). Consequently, the selection against high SCC is a selection against inflammation and neutrophil milk concentration, whereas the selection against low SCC would be a selection against milk concentration of macrophages and lymphocytes. This led some authors to consider that high and low SCC could be 2 different traits (Madsen et al., 2008). The concern that the selection for very low SCC would be unwise because it would lower the capacity of the MG to cope with infection has been put forward (Kehrli and Shuster, 1994). To evaluate the validity of this concern, a critical appraisal of the milk leukocytes is useful, by distinguishing the role of macrophages and lymphocytes from the role of neutrophils, and by discussing the notion of the “resident milk cells” that constitute the baseline SCC.

The Arguable Notion of “Milk Resident Cells”

In the past, milk cells of healthy uninflamed MG were attributed the role of sentinels of the MG, and they were considered as important contributors to MG alertness until recently (Concha, 1986; Sordillo and Streicher, 2002; Oviedo-Boyso et al., 2007). Nowadays, this position is arguable. The prominent cells in the milk of healthy MG, macrophages and lymphocytes, are often referred to as milk resident cells or immune resident cells, explicitly or implicitly (Dosogne et al., 2001; Kehrli and Harp, 2001). This may be misleading, as these cells are shed at each milking, and consequently do not reside very long in the MG lumen. This is partic-

ularly true of the lumen of the gland cisterns and ducts, which are flushed by the milk flow during milking. The composition of the “resident” cell population that is analyzed in milk results from the continuous migration of leukocytes from the blood and mammary tissue compartments into the MG lumen. It is important to keep in mind that this population is not necessarily representative of the leukocyte population that truly dwells in the mammary tissue. It is implicitly admitted that these “resident” cells are the first to detect intruding bacteria, to ingest and kill them, and to emit signals that trigger the inflammatory response. The point to consider here is the role these migrating cells can play in the defense of the MG against infections. An important parameter is their concentration. This is because these cells cannot swim and head for their targets, and consequently depend on encounters whose probability is a function of concentration and convection currents. The frequency of contact with bacteria will determine both cell activation and phagocytosis efficiency. It has been documented with in vitro experiments that a threshold concentration of phagocytes of about 5×10^5 cells/mL is necessary to get a net decrease of bacterial numbers (Leijh et al., 1980, 1981; Li et al., 2002) when both prey and predators are maintained in suspension under constant agitation, which is not the case in the MG. Are macrophages adherent to the epithelial lining? As MG infection is first detected at the level of cisterns and large ducts (Petzl et al., 2016), it is at these sites that such cells would be useful. In fact, histological and immunohistochemical examinations of the epithelium lining the cisterns and large ducts have not evidenced macrophage-like cells adhering to the epithelium, so that the existence of a sizeable population of adhered macrophages remains to be established (Nickerson and Pankey, 1984; Nickerson et al., 1984; Maxymiv et al., 2012; Rainard et al., 2013).

If particulate encounters are not efficient in triggering the MG response to infection, it could be suggested that soluble or micellar bacterial products (metabolites, exotoxins, exosomes, membrane vesicles, and so on) emitted by bacteria that grow and multiply in milk can diffuse and reach milk leukocytes. Then the activity of these cells is an important point to consider. Milk macrophages have a much lower phagocytic capacity than blood monocytes or neutrophils (Sandgren et al., 1991; Dosogne et al., 2001; Denis et al., 2006). Milk macrophages are also poorly responsive to IFN- γ and poor producers of IL-1 β , tumor necrosis factor- α , and nitric oxide compared with blood monocytes (Politis et al., 1991; Denis et al., 2006). They are also poorly efficient at presenting antigens to lymphocytes (Politis et al., 1992). Milk lymphocytes functions are not well defined. Most milk lymphocytes display the phenotype

of memory T cells, twice as many express the $\alpha\beta$ receptor than the $\gamma\delta$ receptor, and they are predominantly CD8+ T cells (Park et al., 1992; Taylor et al., 1994; Soltys and Quinn, 1999). Some of these CD8+ T cells have an immunosuppressive activity on CD4+ T cells (Park et al., 1993). Again, interactions of lymphocytes with other effector cells will depend on the frequency of haphazard collisions, and thus on milk cell concentration. From the above considerations, it seems unlikely that the low concentrations of leukocytes found in milk from a healthy MG play a significant role in its defense.

Neutrophils and the Concept of the Leukocyte Barrier

At the onset of MG inflammation, the milk cell profile changes dramatically. A feature of the MG is its capacity to mount swift and massive neutrophilic inflammatory reactions, bringing to milk hordes of neutrophils that can reach concentrations of more than a hundred million cells per milliliter (Schalm and Lasmanis, 1968). Even in long-lasting *Staphylococcus aureus* chronic mastitis, neutrophils predominate in milk (Riollet et al., 2001). Neutrophils are indispensable to keep IMI by most mastitis-causing pathogens under control (Paape et al., 2002). As discussed above, a critical concentration of neutrophils is necessary for effective bacterial killing in suspension. This concentration (about 3 to 5×10^5 cells/mL) is easily reached and maintained in the milk of inflamed MG. Long ago, experimenters realized that a pre-existing milk leukocytosis could prevent the establishment of infection upon instillation of bacteria into the MG lumen (Schalm et al., 1964, 1967). They coined the concept of the leukocyte barrier to infection, effected by the neutrophils present in milk as a result of local inflammation (Schalm and Lasmanis, 1968). This inflammation may be caused by a pre-existing infection. It is more difficult to elicit experimentally a new infection in glands already infected and shedding a few hundred thousand cells per milliliter than in healthy glands secreting low SCC milk. Low-grade inflammations such as those caused by the so-called minor pathogens (CNS or *Corynebacterium bovis*) are enough to reduce the rate of success of experimental infections (Postle et al., 1978; Linde et al., 1980; Poutrel and Lerondelle, 1980; Schukken et al., 1999). Field experiments have sometimes documented some protection afforded by infections by minor pathogens against new infections by major pathogens such as *S. aureus*, streptococci, or coliform bacteria, although with moderate efficacy (Black et al., 1972; Bramley, 1975; Brooks et al., 1983; Bradley, 2002; Piepers et al., 2010; Piepers et al., 2013), and overall less convincingly than did experimental challenge studies (Reyher et al.,

2012). The leukocyte barrier concept was implemented by intentionally inducing a chronic inflammation in the udder. This was achieved by inserting a plastic loop (the intramammary device) into the gland through the teat canal (Corlett et al., 1984). Unfortunately, the protective effect was uneven and correlated with the magnitude of local inflammation and particularly to the SCC, efficiency requiring SCC >500,000 cells/mL (Paape et al., 1981; Poutrel et al., 1983). The development of the device was abandoned, as the elicited inflammation also induced tissue alteration and reduced milk production (Nickerson et al., 1991).

According to the leukocyte or immune barrier concept, a healthy uninflamed MG is more receptive to new IMI than an already infected and inflamed gland. A logical consequence of the leukocyte barrier effect is that herds with low levels of IMI, when exposed to the same infection pressure, are in principle more prone to new infections than herds with high levels of chronic IMI, where a high proportion of glands consistently secrete moderate to high SCC milk. The herds with a low proportion of chronic IMI have typically low bulk tank milk SCC. They run the risk of new infections by coliform bacteria, as sometimes observed in epidemiological studies.

THE MAMMARY EPITHELIUM: A SENTINEL AND A LINE OF DEFENSE

As inflammation and neutrophils are necessary to protect the MG from infection, and as somatic cells in healthy glands are probably not sufficient to trigger inflammation, what are the cells that ensure MG alertness and capacity to respond to bacterial intrusion? The answer lies in the second line of defense of the MG (i.e., the MG epithelium and associated cells).

Several studies have established that mammary epithelial cells (MEC) are equipped to sense the presence of bacteria and bacterial products (Strandberg et al., 2005; Lahouassa et al., 2007; Yang et al., 2008). They express several of the pattern recognition receptors that detect the bacterial ligands named microbe-associated molecular patterns (Akira et al., 2006; Jungi et al., 2011). This is true at least of the toll-like receptors (TLR) 2, TLR4, and TLR9 and nucleotide-binding oligomerization domain (NOD)-like receptors NOD1 and NOD2 (Goldammer et al., 2004; Porcherie et al., 2012). The MEC react to the microbe-associated molecular patterns they have sensed by producing several self-defense reagents (antimicrobial peptides and reactive oxygen species) and mediators of inflammation (cytokines and chemokines; Strandberg et al., 2005; Lahouassa et al., 2007; Günther et al., 2010; Bougarn et al., 2011).

Figure 2. The mammary gland (MG) epithelium is equipped to sense bacteria and trigger inflammatory responses. This is a schematic representation of the mammary epithelium at the level of cisterns and large ducts. Dendritic cells/macrophages and cluster of differentiation (CD) 8+ T lymphocytes are closely associated with the bi-layered epithelium, and other macrophages and lymphocytes in the sub-epithelial tissue. The mammary epithelium is well equipped to sense bacteria and trigger inflammation on its own, and luminal cells are not required. MEC = mammary epithelial cells. Color version available online.

Besides epithelial cells, the mammary epithelium that lines mammary cisterns and large ducts comprises leukocytes closely associated with the epithelial lining, such as dendritic cells, macrophages and lymphocytes (Tatarczuch et al., 2000; Leitner et al., 2003; Maxymiv et al., 2012; Figure 2). It is likely that the response of MEC to bacteria is amplified by the cooperation with these epithelium-associated leukocytes, as in vivo stimulation of the MG or in vitro stimulation of mammary tissue are of a greater magnitude and a wider nature than would be expected on the basis of the in vitro response of MEC (Günther et al., 2009; Bougarn et al., 2010). It is of interest to note that the response of MEC to *Escherichia coli* and *S. aureus* correlates well with the initial response of the MG, and better than the response of macrophages to *Streptococcus uberis* (Bannerman et al., 2004a,b; Gilbert et al., 2013; Günther et al., 2016a,b). This is in accordance with the idea that milk leukocytes are not the initiators of MG inflammation, and that MEC stimulation is necessary to set in motion the mammary tissue response. The cooperation between MEC and tissue leukocytes is likely to be particularly useful and efficient near the portal of entry of pathogens, that is, the Furstenberg's rosette at the proximal end of the teat canal, and the

teat sinus. In response to infection, these sentinels of the MG have been shown to produce chemokines, cytokines, and antimicrobial proteins (Rinaldi et al., 2010; Lind et al., 2015).

From the preeminent role of the mammary epithelium and the likely low contribution of milk leukocytes to the initiation of inflammation, one could infer that the low SCC MG are not more susceptible to IMI than high SCC glands. This goes counter to common observation. In fact, the SCC could be an indicator of the reactivity of the MG to bacterial stimuli, not because milk cells trigger the reaction, but because higher concentrations of migrated cells reflect higher concentrations of tissue-associated cells. Besides, beyond a critical concentration, inflammatory milk cells (different from the "resident cells" in uninflamed glands) could contribute to inflammation initiation. Some observations tend to support these considerations (Wellnitz et al., 2010). This point would deserve further research, but is beyond the scope of our current subject.

The point at issue is the influence of SCC-based genetic selection on the SCC of the progeny and their susceptibility to IMI. In other words, does the SCC selection, without the lowest SCC limit, interfere with the MG reactivity and capacity to defend itself against

infection? Thanks to recent studies of the effect of SCC-based divergent selection on the susceptibility to mastitis, we are now able to answer this question.

DAIRY RUMINANTS SELECTED FOR LOW SCS HAVE NO INCREASED SUSCEPTIBILITY TO MASTITIS

Experiments have been conducted on dairy ewes, goats, and cows with a view to testing the effect of SCS selection on the incidence of IMI and clinical mastitis under field or experimental conditions. The background SCC of ewes and particularly of goats are notably higher than cow SCC, so that the issue of very low SCC has not yet pervaded the small ruminant dairy farming context, although constant progress to lower values can be observed (Bergonier et al., 2003; Contreras et al., 2007; Souza et al., 2012). Nevertheless, the data obtained with small dairy ruminants are relevant to the issue of the influence of the SCS selection on the reactivity of the MG and its susceptibility to infection. A divergent selection of dairy sheep was carried out on the basis of estimated breeding values for lactation mean SCS of dams and progeny-tested rams. This yielded 2 groups of ewes with a marked divergence in SCS of about 3 genetic standard deviations, high and low SCS ewes (Rupp et al., 2009). Under conventional herd conditions, the high SCS ewes had higher lactation mean SCC than the low SCS ewes, as expected. Importantly, the difference in SCC was the result of a higher prevalence of IMI, not of higher baseline milk SCC: at the initial SCC recording both SCS groups had similar milk cell counts and few IMI, although a slight difference was already present at lambing as shown by more bacteriological isolates from colostrum. The difference in SCC between the 2 groups evolved in the course of lactation (Rupp et al., 2009). Most IMI were caused by CNS, and clinical mastitis occurred mostly in the high SCS group. Interestingly, the infected MG of high SCS ewes tended to have higher SCC than the glands of low SCS ewes infected by the same pathogen. This could indicate either a more efficient defense as suggested by the low persistence of IMI in the low SCS ewes, a better regulation of the inflammatory response, or a lesser reactivity to infection stimulus by low SCS ewes. This latter possibility was explored using experimental infections. Ewes of the 2 groups were inoculated with *Staphylococcus epidermidis* in one gland, then with *S. aureus* in the other gland during the subsequent lactation (Bonnefont et al., 2011). The low SCS ewes controlled the infection slightly better than did the high SCS ewes, as shown by a reduced milk bacterial shedding, which suggests a more efficient bactericidal activity of the cells recruited in milk. Importantly, no

difference was observed between groups in terms of the promptitude, magnitude, and composition of the leukocyte recruitment in milk at the onset of infection. This is not in favor of a lesser reactivity of the MG of animals selected for a favorable SCS.

The response to infection of different cell types likely to take part in the defense of the MG was investigated through gene expression profiling. The first and more numerous cells that come into contact with bacteria are the MEC. Epithelial cells from high and low SCS ewes were isolated and stimulated in vitro with *S. aureus* (Bonnefont et al., 2012). Transcriptome profiling differences between the 2 groups did not suggest leads that could explain the difference in susceptibility to infection under field conditions. Similarly, another study carried out with cows selected on the basis of a QTL related to SCS did not show differences among the expression of the 10 immunity-associated genes monitored in the study (Griesbeck-Zilch et al., 2009). Nevertheless, a more thorough study of MEC stimulated with *E. coli* or *S. aureus* clearly indicated that MEC from the low SCS QTL cows were more reactive to the bacterial stimuli (Brand et al., 2011). Specifically, the cells overexpressed genes encoding several cytokines and chemokines, and genes of the noncanonical NF- κ B pathway. These studies suggest that MEC may contribute to the mechanisms behind the SCS-linked susceptibility to mastitis. Besides MEC, the cells recruited in milk during infection (mainly neutrophils) were examined in a microarray-based gene expression study (Bonnefont et al., 2011). Several of the differently expressed genes between the 2 groups involved immune cell trafficking, pathogen recognition, and immune regulation. The response of bone marrow-derived dendritic cells stimulated with killed *S. aureus* was also investigated (Toufeer et al., 2011). Distinct transcriptional profiles were obtained from the cells of the high and low SCS groups. Some genes of the IL-1 β signaling pathway were overexpressed in the cells from high SCS ewes, whereas a few genes linked to the TLR2 pathway were overexpressed in the cells from low SCS ewes. This is in keeping with a better controlled and more efficient inflammatory response by the low SCS animals.

A divergent selection based on the same principle as that used for ewes was implemented with Alpine goats. An observational study conducted under field conditions in a conventional herd yielded results similar to those of the sheep study (Huau et al., 2015). Over lactations, the mean SCC were higher in the milk of high SCS goats than in the milk of low SCS goats, in relation with a higher prevalence of chronic IMI, mainly by CNS. As for ewes, the initial SCC at first sampling in uninfected glands were similar in both groups. Upon experimental infection with *S. aureus*, there was no in-

dication of different initial recruitment of leukocytes in milk and shedding of bacteria (Cremonesi et al., 2012). A divergent selection on SCS involving Holstein and Normande cows confirmed that the baseline SCC in uninfected glands is not affected by the selection (Lefebvre et al., 2016).

Overall, it appears that differences in the response of the SCS lines to infection are multiple and complex, involving the immune system but also the metabolic stress response (Bouvier-Muller et al., 2016). Nevertheless, one important point emerges from these studies: the low SCS animals are not less reactive than high SCS animals, but seem to manage MG infections more efficiently by mobilizing their immune system more appropriately.

RECONCILED CONCEPTS OF RESISTANCE TO MASTITIS AND LOW SCC

The question we intend to answer is, “Are cows or herds with low cell counts at greater risk of mastitis infections due to lower immunity in the mammary gland?” (Timms, 1990). Since the time when the question was aptly announced, new knowledge has been gained on the contribution of milk and tissue cells to MG immunity, and on the effect of genetic selection on the milk SCC. By taking into account this new information to interpret field observations, it is possible to offer an answer that settles the issue.

Epidemiological surveys that dealt with the relationship between low SCC and susceptibility to mastitis seem to yield conflicting results. In fact, if the interpretations of study results seem contradictory, this is because different mechanisms underlying the occurrence of IMI are operating:

- The immune barrier created by the milk leukocytosis associated with pre-existing infections.
- The infection pressure generated by exposure to different pathogens, of contagious or environmental sources.
- The degree of genetic susceptibility of the host to MG infections.
- The MG reactivity conditioned by current or previous environmental events.

Resistance or susceptibility to infection is a phenotypic trait of the udder that is valid for only a limited period because it is mainly determined by the physiological status of the animal and at the same time by its genetic background. A major factor that underlies the MG susceptibility trait is the existence of previous or concomitant udder infections that induce persistence

of innate immunity known as trained immunity (Netea et al., 2011), or concomitant immunity such as that resulting from the leukocyte barrier. This immunity expresses itself essentially at the level of the individual gland (mammary quarter for cows or half udder for small ruminants), even though some effects pervade the whole udder and others implicate the whole organism. An important consequence of the local expression of refractoriness to infection is that the higher the proportion of glands chronically infected in a herd, the lower the incidence of new infections, and reciprocally, the higher the proportion of healthy glands, the higher the risk of new infections. Obviously, the infection pressure will determine if a herd with a low level of IMI, usually characterized by a low bulk milk SCC, will be afflicted by a high incidence of clinical mastitis or not. As by definition few cows in these herds have chronic IMI, and this results from an efficient control of, or low exposure to, infections by contagious pathogens, the occurring clinical mastitis would be caused by environmental pathogens, usually coliform bacteria. This phenomenon can explain why low SCC quarters and low SCC herds can be found to be more susceptible to mastitis in epidemiological surveys. This does not necessarily translate to a higher genetic susceptibility to mastitis of low SCC animals. That is why the studies that examined the susceptibility of cows with a favorable SCS index did not find a higher incidence of mastitis even with very low SCC.

The objective of the genetic selection against mastitis is to increase the proportion of animals with low levels of IMI and less clinical mastitis, with the promotion of herds with a high proportion of phenotypically “receptive” glands as a collateral consequence. The notion of receptive or susceptible MG is tricky. The selection against mastitis could affect 2 tiers of resistance: (1) in regard to the MG morphology and the efficiency of the teat canal, the effect is a reduction of penetration of bacteria into the MG; (2) in regard to the MG immune system, the effect could be on an improved efficiency of the immune response and bacteriological cure, or a reduction of the severity of the disease, both effects resulting in low clinical mastitis incidence. Thus an MG of a low SCS animal will be more phenotypically receptive because it is uninflamed than an MG of an infected high SCS cow, and less susceptible because of genetic resistance. The net effect should be to the advantage of low SCS cows under similar infection pressures.

CONCLUSIONS

In conclusion, to answer the question “Can SCC get too low?” we have 2 answers, because the question can be construed in 2 ways:

(1) Are very low SCC an indicator of a high susceptibility to mastitis? Several experiments tend to show that MG reactivity is increased when the SCC increases, in relation to the basal inflammation that is already in place. This may be linked to a state of alertness of the MG in relation to the local innate immunity. This could be advantageous under certain conditions, but in any case this is inflammation, with its associated drawbacks. The very low SCC apparent susceptibility may be a phenotypic trait linked to immunomodulation, a question that deserves further research, but is definitely not a consequence of selection.

(2) Do very low SCC resulting from selection of low SCS animals have a negative effect on the susceptibility of the MG to infection? To this question, the answer is clearly NO, as evidenced by experiments conducted with divergently selected animals. The SCS-based selection does not affect the SCC of healthy, uninflamed glands, and it does not reduce the reactivity of the healthy glands to infection. Moreover, opposing “very low SCC” to “physiological SCC” is not justified because the normal baseline SCC is already very low.

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