MILK SOMATIC CELLS – WHAT DO THEY DO?

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How do animals get disease? The answer is through complex interactions among the host, the environment and the pathogen. It is these interactions that we need to understand in order to control and prevent mastitis in dairy cows. The phenotypic characteristics that a cow displays (P), are a combination of the genotypic value (G), and the environmental deviation (E) i.e. \( P = G + E \). Thus, identification of genes responsible for resistance or susceptibility to mastitis, and of important pathogen and management factors associated with mastitis, should be the aim of current and future research programmes.

Mastitis is not a single disease but is intra mammary infection caused by any of a large number of very different bacteria. For ease of understanding, bacterial pathogens have been subdivided into categories such as “contagious” and “environmental” pathogens and yet, it is becoming clear that the distinctions between the two are less definite than originally thought. Environmental pathogens such as *Escherichia coli* have been shown to persist in the mammary glands even in the dry period (2), contradicting the dogma held for many years that the dry gland did not support extended growth of *E. coli*. Similarly, *Streptococcus uberis* has been shown to persist within the udder and to transmit from cow to cow or quarter to quarter, rather like a contagious pathogen. There are a number of possible reasons for these recent results. First, new molecular and immunological techniques make isolation and identification of bacteria and bacterial strains more accurate; secondly, that bacteria are continually evolving to ensure their survival and persistence in the host or in the environment, and thirdly, that we may be breeding animals that are more or less susceptible to certain bacteria.

The udder is a major mucosal site in ruminant species both in terms of size and in importance for milk production and immunological protection of the neonate. The other major mucosal sites in the cow include the skin, the gastrointestinal tract, the respiratory tract and the reproductive tract. The udder differs from these other sites in a number of ways: the skin, gastrointestinal and respiratory tracts are in regular contact with foreign antigens or pathogens and the immune responses induced in these sites include protection against tissue invasion by pathogens, clearance of infections and, in the gut, tolerance to food antigens. The reproductive tract differs in that its immune system must be able to respond to infections introduced during service and at parturition, while not rejecting the conceptus.
which contains antigens foreign to the dam, that are derived from the sire. In the case of the udder, the mucosal surface is exposed to pathogen challenge less frequently than other mucosal sites, although it is potentially challenged twice daily by bacterial invasion through the teat duct during the milking process. Intramammary infection may also occur at times other than during milking, especially if the teat duct is open or damaged, allowing interaction between bacteria and the surface area of the mucosa which is particularly large, especially during lactation.

The immune response is important at mucosal sites and immune cells, in addition to soluble factors such as antibodies, provide some of the main protective mechanisms against infection in most species. Cells have been shown to be important in protection against other important infectious diseases of cattle including respiratory viruses, metritis and tuberculosis. Cells are, however, not all the same, and include subsets such as B cells, T helper (Th) cells, T cytotoxic/suppressor (Tc/s) cells, natural killer (NK) cells etc. A well-known example of the importance of different cell subtypes in disease include Human Immunodeficiency Virus where the number of Th cells is reduced (3). It is biologically likely, therefore, that cells will play a role in the defence of the udder, although it is difficult to extrapolate from other organs and species due to the wide variation in responses identified in previously published work.

The protection of mucosal sites comes from a complex system of interlinked defence mechanisms which can be classified into the ‘non-specific’ (innate) and ‘specific’ (acquired) immune systems: these systems have the combined ability to destroy or control most pathogens. Innate immune responses are triggered rapidly and focus on structural components associated with particular pathogens such as bacterial cell wall components (1), whereas, the acquired immune system requires a longer period of time to respond than the innate system, but focuses specifically on epitopes expressed by a pathogen. Different cell types are associated with the different arms of the immune response, with neutrophils, macrophages and NK cells considered part of the innate system and T and B cells part of the acquired system, although there is considerable interaction between the two systems.

Cells isolated from milk in normal, non-inflamed, mammary glands have been shown to comprise epithelial cells, B cells, T cells, macrophages and neutrophils. Macrophages form the greatest proportion of cells for most of lactation except in the period of coloostrogenesis or towards the end of lactation when neutrophils predominate (8). In the presence of infection, cells are recruited from the tissues surrounding the milk-secreting alveoli and from the systemic circulation and neutrophils form the greatest proportion of the cells in the mammary gland tissues and secretions. The relative
contribution of cells from the local tissues and from the systemic circulation to the mammary secretions is not clear. There is a tendency in some publications to consider all cells as a single population rather than identifying them as comprising different cell subtypes. This has lead to some immune responses being attributed in the literature to say, neutrophils - the cells present in greater numbers than other types during infection - when the measured response may actually be due to cells that are less numerous within the cell population but which may, nonetheless, be equally or more important than neutrophils. Interactions between bacteria and immune cells can happen at three levels in the udder: in the secretion collected in the gland cistern or in the ducts and alveoli; in the tissues surrounding the milk-secreting alveoli; or in the supramammary lymph node that drains lymph from the mammary glands. It is possible that induction of a variety of protective responses may occur at all of these three sites.

At the Symposium on Immunology of the Ruminant Mammary Gland in Stresa in 2000 (17) many of the presentations described laboratory-based studies on the role and function of immune cells. There are far fewer epidemiological or farm-based studies on the role of milk somatic cells in protection of the udder. It has been shown by many authors that high individual cow somatic cell counts (SCC) are positively correlated with clinical mastitis due to persistent infections especially with S. aureus (5). Fewer studies have examined low or very low SCC herds and cows but there is evidence that the risk of clinical mastitis is increased with low SCC compared to those with higher SCC (18). As the protective immune responses occur in individual mammary glands, more information on the role of SCC at the quarter level is required to increase understanding of local immunity against pathogens.

Many experimental studies have shown that high, or even moderate, numbers of somatic cells in milk protect against infection with both major and minor pathogens (3). The cell numbers were increased either by mechanical stimuli (11) or by the introduction of bacteria. What remains uncertain is whether protection is conferred by activated innate immune cells and/or antigen primed immune cells, or whether competition between bacterial species for essential nutrients prevents establishment of infection with the “challenge” bacteria. Similarly, the presence of certain bacterial species of low pathogenicity, sometimes termed “commensals”, has been shown to confer protection against other, more pathogenic bacteria. As it is likely that cells, and other soluble factors derived from cells or elsewhere, increase in the presence of bacteria, it is difficult to ascertain the precise mechanisms involved in udder protection.

There are also likely to be differences in the type of immune responses that are important in clearance of infection caused by different
pathogens. Th cells were shown to be important in protection of the udder in the early stages of infection with S. aureus (13), while in later stages, Tc/s cells may inhibit these Th responses (12). In mice it has been shown that responses against intracellular pathogens (e.g. mycobacteria) may result from Th1-type responses where certain soluble factors (cytokines such as interferon-γ and interleukin-2) are released, whereas responses against extracellular factors such as toxins may result from Th2-type responses where the cytokines interleukin-4 and interleukin-10 predominate (9). There is, to date, limited work on this area in relation to bovine mastitis, however, there are potential comparisons that could be made between S. aureus as a pathogen that has been shown to persist intracellularly within neutrophils and macrophages, and E. coli that produces extracellular endotoxin as an important virulence factor. For some pathogens, cells of the acquired immune response with specific memory of that pathogen may be important in protection, either by directly killing cells or by attracting other immunologically competent cells to the site of infection, while for other pathogens, cells of the innate immune response may be more important. These cells may be involved in phagocytosis and bacterial killing, or by activating and directing cells involved in specific immunity. The role of Toll-like receptors and pathogen-associated molecular patterns in bovine mastitis may be of future interest with the recognition that one form of the receptor regulates endotoxin that is produced by Gram-negative bacteria, while another form of the receptor regulates Gram-positive responses (4).

If it is accepted generally that cells are important in defence of the udder, then the argument remains as to whether it is important to have cells actually present in the mammary secretion ready to interact immediately with bacteria when they penetrate the teat duct or, whether it is reasonable to rely on the recruitment of effector cells from the general circulation into the already infected gland. It is possible that the answer may vary with pathogen type e.g. E. coli are known to produce large amounts of endotoxin within 4-6 hours of intramammary invasion and that the systemic effects of the toxin can be very rapid and severe. Cell recruitment has been shown to start around 4 hours and to peak around 12 hours after infection. Other pathogens may produce toxins that act on local tissues and may take longer to have a pathological affect on the host and to induce an immune response. Some of the factors of importance in recruitment of cells into mucosal sites have been identified in other species as intercellular adhesion molecules on blood vessels and immunological and inflammatory cells. Neutrophils express β2 integrin which interacts with intercellular adhesion molecules on blood vessels and permits movement of cells into tissues. The importance of cell recruitment is seen in an extreme example with leucocyte adhesion deficiency in cattle caused by mutations in the β2 integrin molecule (16), where affected individuals are unable to recruit cells into distant sites and develop multiple infections (7).
It is accepted widely that high SCC are undesirable for economic (19) and milk quality reasons. Many genetic studies have shown a positive linear association between SCC and clinical mastitis in that as SCC reduces, then clinical mastitis also reduces (10), suggesting that selection for low SCC also provides benefits to dairy cow welfare. However, the studies on the relationship between SCC and clinical mastitis have often not included pathogen-specific diagnoses of mastitis. Rogers and colleagues (14) showed that sires with the lowest predicted transmitting ability (PTA) for somatic cell scores had daughters that had the lowest clinical mastitis rates for all pathogen types. It is possible, that with cows being culled increasingly for high cell counts and bulls being selected for low PTA for SCC, that the potential increased risk of infection in low SCC quarters may soon become apparent.

Breeding for a certain trait that may be associated with immune function, such as low SCC, may potentially and inadvertently, lead to altered or reduced immunity by other mechanisms, as has been shown to occur in number of species. It is possible that cattle may be selected for breeding that have either poor innate responses, poor specific responses or poor cell recruitment ability. There are numerous genes that may be involved in mastitis resistance or susceptibility but the Major Histocompatibility Complex that is involved in recognition of antigens (15), the Toll genes, and genes coding for adhesion molecules, may be of particular importance. The rate of loss of genetic variation is proportional to the rate of inbreeding and the major influences on this rate are the number of parents used of each sex and how much selection is taking place in the population (20). The number of cattle breeding companies and breeding bulls is declining and hence breed diversity is reducing rapidly worldwide. The effect of this on disease susceptibility, and on mastitis susceptibility in particular, should be watched carefully.

In conclusion, it is likely that somatic cells are very important in mammary gland defence. It may not be total numbers that are important but the cell type, subtype and the products and interactions of those cells that may affect the outcome of infection – cure, persistence of infection, or even death of the cow. It is also likely that the important immune cells will differ for the widely different bacterial species and strains. It remains to be seen if cells must be present within the mammary secretions or the mammary tissues or whether cell recruitment can be relied upon for a speedy and successful response to intramammary infection. It is likely that the answer will come from continued field and laboratory studies with both naturally-occurring and experimentally-induced mastitis.
REFERENCES


