The Immune System of the Ruminant Mammary Gland
And Its Role in the Control of Mastitis

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ABSTRACT

Immunoglobulins in mammary secretion are derived from blood serum or are made locally by cells of the lymphocyte-plasma cell series situated close to the glandular epithelium. The major immunoglobulin in colostrum and milk of ruminants, IgG₁, is derived from the blood and is transferred into secretion selectively relative to IgG₂, probably by a mechanism requiring specific receptor sites on the basal or intercellular membrane of the glandular epithelium. Acute inflammation causes suppression of selective transfer of IgG₁, but there is a marked increase in the transfer of proteins, such as IgG₂ and serum albumin, which enter secretion nonselectively.

Infusion of antigen into the mammary gland of ruminants some weeks before parturition induces a persisting local production of antibody, most of which is associated with IgA and IgM. IgA cells in the mammary gland probably originate in the intestine, and prior antigenic stimulation of the gut may be required for maximal IgA antibody responses in the gland.

Local immunization with staphylococcal vaccines gives a measurable degree of protection against staphylococcal challenge. Systemic immunization with viable staphylococci leading to subcutaneous abscess formation elicits significant protection to subsequent mammary challenge which is attributable, at least in part, to specific antibody of the IgG₂ class cytophilic to polymorphs.

Immunoglobulins in mammary secretion are either of humoral origin or are made locally by cells of the lymphocyte plasma cell series located near the glandular epithelium. Radio-tracer and other studies indicate that most of the IgG in mammary secretion has a humoral derivation whereas IgA and IgM are synthesized locally. The concentrations of immunoglobulin in colostrum of all species are high compared with those detected during the copious milk production. The relative importance of the IgA and IgG secretory systems in the colostrum-forming gland varies greatly between species, Table 1. In ruminants and pigs IgG is the predominant immunoglobulin in colostrum whereas in rabbit and man IgA predominates.

Passage of substrates from the interstitial fluid compartment into secretion (Fig. 1) must occur either through the cells, presumably in vesicles, or between the cells. There is considerable evidence for the normal lactating and colostrum-forming mammary gland that large water-soluble molecules of the size of the serum proteins must pass into secretion through the cell rather than through tight junctions (17). Electron microscopic studies have drawn attention to small vesicles, thought to be transport vesicles, apparently originating at the basal border of the cell (2). More recently, Shoefl and Lascelles (35) reported that horseradish peroxidase or ferritin injected over the inguinal lobes of the mammary gland of mice lactating 3 to 7 days could be found in the alveolar lumen 1 to 3 h after injection. Electron opaque material was seen also in vesicles near the basal and intercellular borders of the cell and at various levels throughout the cell, including some apparently in the process of secretion into the alveolar lumen. There was no evidence of transfer of protein through the tight junctions although electron-dense material was relatively easy to find in the interalveolar spaces of the gland.

In ruminant animals the concentration ratio of IgG₁ : IgG₂ in colostrum far exceeds the comparable ratio in serum, and a selective mechanism for transfer of IgG₁ is operating. The simple hypothesis advanced several years
TABLE 1. Immunoglobulins (mg/100 ml) in colostrum of several species.

<table>
<thead>
<tr>
<th>Species</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
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<tbody>
<tr>
<td>Sheep</td>
<td>6000</td>
<td>200</td>
<td>410</td>
</tr>
<tr>
<td>Cow</td>
<td>5000–8000</td>
<td>450</td>
<td>600</td>
</tr>
<tr>
<td>Pig</td>
<td>5700</td>
<td>1000</td>
<td>270</td>
</tr>
<tr>
<td>Rabbit</td>
<td>240</td>
<td>450</td>
<td>10</td>
</tr>
<tr>
<td>Man</td>
<td>17</td>
<td>1800</td>
<td>80</td>
</tr>
</tbody>
</table>

ago to account for selective transfer (15, 16) is essentially correct. It was suggested that selective transfer of IgG₁ required specific receptors on the basal or intercellular membranes (Fig. 2); transport vesicles forming at these sites would contain relatively more IgG₁ than IgG₂ or other interstitial fluid protein. It was suggested further that protein is ferried across the cell in vesicles without degradation and secreted into the alveolus without loss of cytoplasmic or membranous material. This hypothesis is different from the one advanced by Brambell (4) to account for selective transfer of IgG across the yolk sac endoderm of rabbits and guinea pigs, the gut of rats, and the placenta of the human. Brambell suggested that the IgG incorporated in vesicles became attached to receptors on the interior surface of the vesicle and, in contrast to other unattached proteins, is protected from proteolytic digestion when these transport vesicles fuse with lysosomes during passage across the cell. Thus, in Brambell’s degradation system, IgG is ferried across the cell, whereas other interstitial fluid proteins initially incorporated into the vesicle along with IgG are degraded. Clearcut evidence against a mechanism involving degradation and in favor of the conservative mechanism (Fig. 2) is in the publications of Brandon, Watson, and Lascelles (5), Cripps, Fulkerson, Griffiths, McDowell, and Lascelles (7), and Sasaki, Davis, and Larson (33).

Selective transfer of IgG₁ continues into lactation reduced (20). However, in the context of immunity to mastitis, concentrations of IgG₁ and IgG₂ in milk are only a fraction of those in blood (Table 2), and in this restricted immunological sense, the milk in the mammary gland is isolated from the rest of the body.

Various factors affect transport of immunoglobulin into secretion, and in a discussion on mastitis it is important to discuss briefly...
Table 2. Immunoglobulin (mg/100 ml) in mammary secretion of cow.

<table>
<thead>
<tr>
<th></th>
<th>IgG₁</th>
<th>IgG₂</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood serum</td>
<td>1400</td>
<td>1300</td>
<td>39</td>
<td>380</td>
</tr>
<tr>
<td>Colostral whey</td>
<td>4000–9000</td>
<td>250</td>
<td>470</td>
<td>540</td>
</tr>
<tr>
<td>Milk whey</td>
<td>40</td>
<td>6</td>
<td>11</td>
<td>9</td>
</tr>
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</table>

one of those aspects, the effects of inflammation. During the first few hours after an acute inflammatory insult, such as the infusion of endotoxin or staphylococcal alpha hemolysin, there is a marked increase in concentration of serum proteins, including the immunoglobulins, but selective transfer of IgG₁ is inhibited (20). In other words, the glandular epithelium has lost its power to restrict and discriminate in the transfer of protein from interstitial fluid into milk. Preliminary observations suggest that 1 to 2 days after the infusion, when the symptoms of acute inflammation have subsided, but when milk production still is depressed profoundly, selective transfer of IgG₁ actually increased to exceed those prior to application of the inflammatory insult (15). Essentially similar conclusions were reached by Harmon, Schanbacher, Ferguson, and Smith (11) who observed that IgG and serum albumin increased dramatically during acute inflammation induced experimentally with Escherichia coli but that IgG remained elevated after serum albumin had returned to normal.

Local Immunity

Earlier studies by my colleagues and me (14, 18, 22, 23, 27, 29) showed that infusion of particulate or soluble antigens into ruminant mammary glands of sheep during the dry period a few weeks before parturition induces the local production of antibody which persists throughout the ensuing lactation (Fig. 3). Workers in other laboratories have confirmed these findings with a variety of antigens in cows and sheep (32, 33, 40, 41). Most of the locally produced antibody was associated with IgA in particular and IgM, but not to any extent with IgG (17). In the sheep a moderate local antibody response follows antigenic stimulation during the dry period, and in the cow the response appears to be less pronounced; whereas in the guinea pig, the mammary gland of which has a highly developed IgA system, the response is striking and persistent (21). During lactation, lymphoid cells are not often seen in mammary tissue sections of sheep and cattle, but their concentration increases progressively during involution, and in the completely involuted gland of sheep, lymphoid cells appear to outnumber other cell types (19). Local antigenic stimulation of sheep and cows some weeks before parturition results in the appearance of IgA-containing cells and plasmablasts in significant numbers in the parturient gland, and some of these cells persist into the following lactation.

Origin of Antibody-containing Cells in the Mammary Gland

In the laboratory rodent and human there is a growing body of evidence suggesting that IgA precursor cells which localize in the mammary gland arise in the Peyer's patches (1, 6, 12, 27, 31). Current evidence seems to suggest that antigens in the gut are transported...
across the epithelium covering Peyer's patches into the lymphoid tissue, resulting in the release into the mesenteric lymph of specifically sensitized cells of the IgA type. These cells appear to enter the bloodstream and subsequently to leave it to enter or lodge in interstitial areas of mucus sites in the body, especially the intestinal lamina propria, but also the mammary gland. Specific antigen in isolated segments of intestine of rats appears to have the added effect of fixing the cells in the lamina propria and also may cause their multiplication (13). These results suggest that maximal IgA antibody responses in the ruminant mammary gland may require initial sensitization of the gut prior to local antigenic stimulation. On the other hand, larger IgA/IgM antibody responses in the mammary secretion of bovine are obtained if the local infusion of antigen some weeks prior to parturition is preceded by an intramuscular injection of antigen in adjuvant (39). Notwithstanding, it is suggested that to achieve maximal IgA antibody responses of the mammary gland, it also may be necessary to sensitize the intestine prior to local antigenic stimulation.

**Immunization against Staphylococcal Mastitis**

Systemic immunization of ruminant animals with killed staphylococcal vaccines has not proven to be a successful measure in protection against staphylococcal mastitis (8, 25). On the other hand, significant protection has been obtained in sheep and goat models by local immunization with staphylococcal vaccines (9, 24, 30). In our studies, vaccines were infused into one udder half 3 to 4 wk before parturition while the other half served as control. Both glands were challenged during the subsequent lactation with a large dose of staphylococci (usually 10^6 washed cells) from an acute case of bovine mastitis. The various vaccine preparations tested in our experiments are in Table 3. The relative protection by local immunization was assessed by comparing the following indicators of mastitis in immunized and control udder halves: a) visible and palpable evidence of inflammation, b) persistence of leucocytes in milk, c) persistence of staphylococci in milk, and d) effects on milk production.

Following vaccination with toxoid vaccines, there was a degree of protection of immunized glands, since the challenge of the immunized gland resulted in a less intense inflammatory response and a smaller reduction in milk production than in nonimmunized glands. However, bacterial numbers were the same in immunized and nonimmunized glands and, probably as a consequence, the clinical differences between immunized and nonimmunized glands became obscured with time.

The protection conferred by polyvalent cell-toxoid vaccines was more than with toxoid, particularly in the decrease in bacterial numbers in milk from immunized glands. Even so, protection was not complete.

Additional experiments were designed to assess the value of systemic immunization during lactation and during the dry period by adjuvant compared with local immunization using aqueous vaccines. While immunization during lactation gave no protection at all as compared with nonimmunized controls, systemic immunization during the dry period did provide a measurable degree of protection, as assessed by milk production performance, but the protection was less than that achieved.

**TABLE 3. Staphylococcal vaccines.**

<table>
<thead>
<tr>
<th>1)</th>
<th>Toxoided α-Haemolysin.</th>
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<tr>
<td>2)</td>
<td>Staphylococcal toxoid—Commonwealth Serum Laboratories, Melbourne, Australia (C.S.L.)—contain toxoided α-, β-, δ-Haemolysin and leucocidin.</td>
</tr>
<tr>
<td>3)</td>
<td>Polyvalent cell-toxoid—C.S.L. seven strains of staphylococci of different phage types—most common field isolates. Vaccine contains killed bacterial cells, toxoided α-Haemolysin and leucocidin.</td>
</tr>
<tr>
<td>4)</td>
<td>Monovalent cell-toxoid vaccine. Strain of <em>Staphylococcus aureus</em> displaying typical β- and weak α-Haemolysis recently isolated from a case of acute mastitis in cow.</td>
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</table>
by local immunization (25). Thus, at this point the best immunization approach available is an imperfect procedure of local immunization.

By analogy with some infections of other mucosal surfaces, e.g., *Vibrio cholerae* and *Escherichia coli* infection of the gut, where it has been suggested that adhesion to mucosal cells is the first stage in the pathogenesis, Frost (10) has presented evidence that organisms showing ability to adhere to mammary ductular epithelium, such as *Staphylococcus aureus* and *Strept.agalactiae*, are frequently those giving rise to mastitis. The weight of our evidence suggests that IgA antibody generated by local immunization may afford the limited protection it does by covering bacterial adhesion sites and inducing agglutination of bacteria which then are flushed more readily from the gland at subsequent milkings. The IgA system in the ruminant mammary gland compared with species like human, rabbit, and guinea pig is poorly developed. It may be possible to enhance local protection if we can devise ways of stimulating the IgA/IgM system with appropriate antigens.

It is accepted that leucocytes play an important role in protection of the mammary gland against bacterial infection including staphylococcal infection. The question arises, what can be done to increase the polymorph's phagocytic ability?

Systemic immunization gives relatively high protection against staphylococcal mastitis only when live vaccines are administered (8). Watson (36) recently confirmed the original observation of Derbyshire and, in addition, has drawn attention to the correlation between the degree of abscessation at the site of vaccination and protection on challenge. Furthermore, Watson (36, 38) in in vitro studies on polymorphs collected from ewes immunized with live staphylococci, demonstrated a significantly enhanced capacity for phagocytosis of staphylococci compared with polymorphs from ewes which were immunized with a killed vaccine. These results suggested that polymorphs may be activated similarly to activated macrophages in classical cell-mediated immunity. However, that polymorphs from infected ewes had no more ability to phagocytose *Pseudomonas* than polymorphs from ewes immunized with killed vaccine did not support this view (37).

The possible significance of antibody with specialized affinity for the cell surface (cytophilic) of polymorphs was investigated. Watson (37) reported that approximately 25% of polymorphs collected from dry mammary glands of ewes shortly after infusing 5 μg of bacterial lipopolysaccharide (26) have IgG2 on their surface with a virtual absence of other immunoglobulin classes (Table 4). The IgG2 could be removed by incubation at 37°C for 1 h and washing in cold Hanks buffered salt

<table>
<thead>
<tr>
<th>Table 4*</th>
<th>The percentages of polymorphs which specifically stained with FITC-conjugated antisera values are means ± standard errors for 8 ewes.</th>
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<tbody>
<tr>
<td><strong>Antisera</strong></td>
<td><strong>Washed untreated polymorphs</strong></td>
</tr>
<tr>
<td>RAS-FITC</td>
<td>23.6 ± 2.8</td>
</tr>
<tr>
<td>RA1-FITC</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>RA2-FITC</td>
<td>26.4 ± 2.9</td>
</tr>
<tr>
<td>RAa-FITC</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>RAa-FITC</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>RALC-FITC</td>
<td>15.7 ± 2.1</td>
</tr>
<tr>
<td>RAFb-FITC</td>
<td>24.3 ± 3.0</td>
</tr>
</tbody>
</table>

solution, and restoration of surface IgG2 was brought about by exposure of the immunoglobulin-denuded cells to blood serum at 4°C. When polymorphs from ewes immunized with live staphylococci were treated as above to remove surface immunoglobulin, their enhanced phagocytic activity also was removed but could be restored by incubation in blood serum from similarly immunized ewes (38).

There is agreement that the polymorph plays an important role as the final effector in elimination of pathogens from the mammary gland. It is also clear that the ability of polymorphs to phagocytose and kill staphylococci is enhanced by antibody, e.g., cytophilic antibody of the IgG2 class. It is not yet clear against which antigenic determinants the phagocytosis-promoting antibody is directed. Antibody directed against cell wall components or possibly against leucocyte-inhibiting, extracellular toxins such as leucocidin, may be playing a role. Therefore, it seems important in future studies to determine the relative phagocytosis-promoting activities of anti-staphylococcal antibodies of various specificities by, in the first instance, in vitro techniques.

There is also tenuous evidence that locally-produced antibody of the IgA or IgM classes directed against staphylococcal cell wall antigens may inhibit attachment of staphylococci to the glandular epithelium and, thus, interfere with the establishment of staphylococcal infection in the gland. In these circumstances, organisms would be flushed out of the gland at milking. The IgA system in the ruminant animal, especially in its mammary gland, is developed relatively poorly, and in future research consideration might be given to the possibility of enhancing the activity of this local immune system by combinations of local gut and local mammary gland antigenic stimulation. The work of Derbyshire (8) and Watson (36, 38) on live staphylococcal immunization suggests that conventional killed vaccines provide either insufficient antigenic mass or, alternatively, that key immunogens are not produced in adequate concentrations in in vitro culture. Considerably more attention must be given to various aspects of vaccine formulation.

Finally, it seems unrealistic to expect any immunization technique to have much effect on new infection rate, but there is reason to hope that vaccine preparations and procedures can be devised to limit the establishment of infection in the glandular epithelium.

REFERENCES


