Characterization of lymphocyte subpopulations and major histocompatibility complex haplotypes of mastitis-resistant and susceptible cows

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Bovine mastitis is an infectious disease with a major economic influence on the dairy industry worldwide. Many factors such as environment, pathogen, and host affect susceptibility or resistance of an individual cow to bovine mastitis. Recently, there has been considerable interest in defining genetic and immunological markers that could be used to select for improved disease resistance. In this study we have analyzed the lymphocyte subpopulations of mastitis-resistant and susceptible cows using monoclonal antibodies specific for bovine leukocyte differentiation antigens and flow cytometry. We have also used a microarray typing technique to define the bovine leukocyte antigen (BoLA) class I and class II haplotypes associated with resistance or susceptibility to bovine mastitis. A striking finding of the present study is that susceptibility to mastitis was associated with major histocompatibility complex (MHC) haplotypes that have only a single set of DQ genes. The study also revealed that susceptible cows had CD4:CD8 ratios of less than one in both their mammary gland secretions and peripheral blood. These results raise the possibility that the number of DQ genes that a cow has and/or a cow’s CD4:CD8 ratio could be used as indicators of susceptibility to bovine mastitis.

Key words: Cattle; Mastitis; Major histocompatibility complex; BoLA; Lymphocyte subpopulations; Genetics

Introduction

Bovine mastitis is an infectious disease with a major economic influence on dairy production. Prospects for the development of an effective vaccine are limited by the variety of microorganisms causing mastitis and a lack of information on the genetic factors that influence disease resistance. It is evident that resistance to infectious diseases is genetically determined. Consequently, there has been considerable interest in defining genetic and immunological markers that could be used to select for improved disease resistance.

Variations in leukocyte subpopulations at different stages of lactation and in mastitic cows suggest that the defense mechanisms of bovine mammary gland may be governed by cell-mediated immune responses. In a previous study we reported that the number of T lymphocytes in mammary gland secretions (MGS) was decreased during the periparturient period and that the average CD4:CD8 T lymphocyte ratio in MGS was less than 1.0 during the lactation period [30]. The CD4:CD8 ratio was even lower in cows with Staphylococcus aureus mastitis [31,46,53]. Several studies have suggested that the composition of T lymphocyte subpopulations in the MGS of cows might correlate with susceptibility to intramammary infection (IMI) [31,46,48]. Although these findings reveal that specific lymphocyte subpopulations may affect the defense of the bovine mammary gland, the functional significance of particular populations has not been completely defined [38,39].

Together with the lymphocyte subpopulations involved in bovine mammary defense against invading pathogens, the antigen presentation capability of antigen-presenting cells is critical for the establishment of effective immunity to IMI. Because of their important role in immune responses, major
histocompatibility complex (MHC) genes are candidate markers for disease resistance. The important role of MHC molecules in the regulation of immune response is attributable to the recognition by T lymphocytes of a complex of foreign peptide antigens and MHC class I or class II molecules. Studies have indicated that certain bovine MHC, also known as the bovine leukocyte antigen (BoLA) complex, class IIa haplotypes are associated with genetic resistance against mastitis [13,19,24,41,42,47]. However, the basis for this association has never been adequately explained. In this study we have analyzed the lymphocyte subpopulations from mastitis-resistant and susceptible cows using monoclonal antibodies specific to bovine leukocyte antigens and flow cytometry. We have also used a microarray typing technique to identify the BoLA class I and class IIa haplotypes associated with resistance or susceptibility to mastitis.

Materials and Methods

Experiment animals

Holstein cows used in this experiment were raised by the National Livestock Research Institute, Rural Development Administration, Korea. Two different groups of animals were selected based on mastitis infection frequency, the frequency of medical treatments and treatment conditions recorded over the past four years. One was termed the resistant group, with no history of medical treatment of mastitis. The other was referred to as the susceptible group with more than two treatments for bovine mastitis. Milk somatic cell counts (SCC) were determined using a Combitofoss™ 5000 milk analysis system (Foss Electric Co., Denmark). Over the four-year period, SCC of the resistant cows averaged below 200,000/ml while, with three exceptions, average somatic cell counts of the susceptible cows were higher than 200,000/ml (Table 1).

Isolation of bacteria

Isolation and identification of pathogens from milk of mastitis-susceptible cows was performed by the method of Joo and colleagues [18]. In brief, milk samples from individual quarters of mastitis-susceptible cows were cultured on 5% sheep blood agar (KOMED, Sungnam, Korea) and incubated at 37°C for 48 h. Bacterial colonies presumptively identified by colony characteristics, catalase reaction, hemolytic patterns, coagulate test and biochemical tests were speciated following the National Mastitis Council protocols [17]. Isolates were further analyzed using the VITEK® system (bioMérieux, Inc., Marcy-Etoile, France).

Preparation of mononuclear leukocytes from mammary gland secretions and peripheral blood

MGS and peripheral blood were collected in acid citrate dextrose (ACD). Peripheral blood mononuclear leukocytes were separated from erythrocytes and most granulocytes by density gradient centrifugation using Lymphopaque™ (density = 1.086, Nyegaard, Oslo, Norway). Platelets and residual erythrocytes were removed by treatment with Tris-NH₄Cl (0.83% w/v, pH 7.3) followed by two or three washes in phosphate-buffered saline (PBS; pH 7.2) containing 20% ACD. Two hundred ml of MGS were obtained aseptically from each quarter of lactating cows and then pooled. MGS were mixed with an equal volume of PBS-ACD-EDTA solution (PAE; PBS pH 7.2, 20% ACD, 20 mM EDTA) and centrifuged at x 400 g for 30 min at 10°C. Cell pellets were diluted with PAE in 50 ml conical tubes and separated by density gradient centrifugation over Lymphopaque as described above. After several washes in PAE, fluorescence flow cytometry was used to examine the relative proportion of lymphocytes.

Monoclonal antibodies

The panel of monoclonal antibodies (mAb; VMRD, Inc., Pullman, WA) used to examine leukocyte subpopulations is shown in Table 2.

Flow cytometric analysis

Cells were resuspended to 10⁷ cells per ml in PBS containing 10 mM EDTA, 0.1% sodium azide, 10% ACD and 2% gamma-globulin free horse serum (first wash buffer; PBS-FB), then distributed in 50 µl aliquots (5 × 10⁶ cells) to wells of V-bottomed, 96 well microtiter plates (Costar®, Corning Inc., Corning, NY) to which 50 µl of PBS-FB or mAb (0.7 µg per 50 µl) had been previously added. Cells were washed in PBS-FB or mAb (0.7 µg per 50 µl) had been previously added. Cells were incubated for 30 min at 4°C, then washed three times in PBS-FB. Cells were then mixed with 100 µl of a 1:200 dilution of fluorescein-conjugated goat anti-mouse Ig (heavy and light chain specific; Caltag Laboratories, Burlingame, CA). Following incubation for 30 min at 4°C, cells were washed in PBS containing 0.1% sodium azide.

Table 1. Average somatic cell counts of bovine mastitis-resistant and susceptible cows (1,000 cells/ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cows</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>15</td>
<td>732</td>
<td>446</td>
<td>578</td>
<td>162</td>
<td>219</td>
<td>571</td>
<td>703</td>
<td>444</td>
<td>511</td>
<td>557</td>
<td>138</td>
<td>261</td>
<td>877</td>
<td>117</td>
<td>327</td>
<td>442±234</td>
</tr>
<tr>
<td>Resistant</td>
<td>15</td>
<td>95</td>
<td>116</td>
<td>131</td>
<td>41</td>
<td>126</td>
<td>117</td>
<td>129</td>
<td>76</td>
<td>57</td>
<td>103</td>
<td>71</td>
<td>68</td>
<td>79</td>
<td>61</td>
<td>95</td>
<td>91±25</td>
</tr>
</tbody>
</table>

*Groups are statistically different with a probability of P<0.001.