Concentrations and Specific Antibodies to Staphylococcal Enterotoxin-C and Toxic Shock Syndrome Toxin-1 in Bovine Mammary Gland Secretions, and Inflammatory Response to the Intramammary Inoculation of These Toxins

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ABSTRACT. To investigate the pathological role of staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) in bovine mastitis, the production of SEs and TSST-1 was investigated in staphylococci isolated from 120 mammary gland secretions (MGS, 51 from no clinical sign-mammary glands and 69 from staphylococcal mastitic-mammary glands) collected from dairy farms where staphylococcal mastitis frequently occurred in Miyagi and Yamagata prefectures from 1997 to 1998. Concentrations of these toxins and specific antibody titers in each MGS were also measured. Furthermore, SEC and TSST-1 were inoculated into lactating mammary glands and inflammatory responses were analyzed. A high percentage of staphylococci including Staphylococcus aureus and coagulase-negative staphylococci isolated from both no clinical sign- and mastitic-MGS produced both SEC and/or TSST-1. The concentration of SEC increased with the severity of the mastitis, and was significantly higher (P<0.05) in acute mastitis than in no clinical signs-MGS. Titers of specific antibodies to TSST-1 in MGS were significantly higher (P<0.05) than those to SEC, regardless of whether or not the cows were lactating or mastitic. Specific antibodies purified from MGS neutralized each toxin in vitro. A significant increase (P<0.05) in somatic cell counts was induced by the intramammary inoculation of SEC but not TSST-1. These findings indicated that SEC rather than TSST-1 plays an important role in the pathology of staphylococcal bovine mastitis. The inflammatory activity of TSST-1 was probably neutralized by specific antibodies in MGS.

KEY WORDS: intramammary inoculation, specific antibody, staphylococcal bovine mastitis, staphylococcal enterotoxin-C, toxic shock syndrome toxin-1.

Staphylococci including Staphylococcus (S.) aureus and coagulase-negative staphylococci (CNS) are important causative agents for bovine mastitis [12, 26]. In many cases, staphylococci cause subclinical and/or chronic mastitis, which progresses to acute mastitis in the worst case. Staphylococci produce several virulent factors, and these factors are involved in a complex way in the pathogenesis of mastitis [31]. Among these, staphylococcal enterotoxins (SEs) are considered to be important virulent factors [31, 38]. SEs are the causative agents in staphylococcal food poisoning, which can lead to emesis and diarrhea in humans and monkeys [34]. Moreover, SEs exhibit a superantigenic action on T lymphocytes via specific Vβ molecules of the T cell receptor (TcR) and induce the production of various inflammatory cytokines [22, 38]. It has also been reported that toxic shock syndrome toxin-1 (TSST-1) is another extracellular toxin with similar superantigenic activity [22, 38]. The immunomodulating activity of superantigens causes toxic shock syndrome (TSS) characterized by fever, hypotension, congestion in a number of organs and lethal shock [22].

A correlation between these toxins and bovine mastitis has been discussed. S. aureus isolated from bovine mastitic mammary gland secretions (MGS) produced SEs, among which SEC was predominant, and TSST-1 [23, 30, 32]. In contrast, geographical variation in the proportion of isolates which carry genes encoding SEs and TSST-1 was reported [19, 21]. Therefore, the pathogenic roles of SEs and TSST-1 in bovine mastitis are controversial. On the other hand, these exotoxins and specific antibodies for them were also detected in MGS and commercial dairy products [33]. Nevertheless, possible changes in toxin concentrations in MGS accompanied by the severity of mastitis have not been clarified.

Niskanenn et al. reported that acute mastitis-like symptoms were induced when SEA was inoculated into bovine mammary gland [27]. Nevertheless, few reports are available on the inflammatory effects of intramammary inoculation of SEC which is predominantly produced by staphylococci isolated from MGS. Therefore, we conducted this study to evaluate the pathological roles of SEC and TSST-1 in staphylococcal bovine mastitis.

MATERIALS AND METHODS

Mammary gland secretions: Total 120 MGS were aseptically collected from no clinical signs-, staphylococcal chronic mastitic- and staphylococcal acute mastitic-mammary glands at dairy farms in Miyagi and Yamagata prefec-
tures where staphylococcal mastitis frequently occurred from 1997 to 1998 (Table 1). Some mastitic-glands were treated with antibiotics. No clinical signs-MGS were classified as being from the lactating period; MGS collected from lactating glands with no clinical signs of mastitis, the modified californian mastitis test (MCMT, P.L tester, Nippon Zenyaku Kogyo Co., Ltd., Koriyama, Japan) was negative, total bacterial counts in MGS were less than $1 \times 10^5$ colony formation units (CFU)/ml, somatic cell counts (SCC) in MGS were less than $3 \times 10^7$/ml, and the concentration of lactoferrin was less than 450 $\mu$g/ml [17]. From the dry period; MGS collected from dry glands had no clinical signs of mastitis and total bacterial counts in MGS were less than $1 \times 10^5$ CFU/ml. Total bacterial counts including staphylococci and other bacterial species were measured by cultivation on trypto-soya agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 37°C for 24 hr. Staphylococcal chronic mastitis was defined as follows; MCMT was positive, clinical signs of mastitis, such as swelling and induration of udders and clots in MGS, were detected, and repeatedly relapsed with staphylococci. Staphylococcal acute mastitis was defined as follows: staphylococci were causative agents, and more than two of acute systemic signs except for fever were seen in addition to clinical sings of mastitis. In this paper, mastitis means staphylococcal mastitis unless otherwise stated.

Measurement of SCC in MGS: SCC in MGS were measured by flow cytometer as described by Kai et al. [16].

Measurement and identification of staphylococci in MGS: Staphylococcal counts in MGS were measured by the method as described by Kai et al. [16]. Coagulase productions were determined by tube test of rabbit plasma (Eiken Chemical Co., Ltd., Tokyo, Japan). Identification of staphylococci was done with a commercial kit (Api staph system, bioMe‘riecix sa, Marcyl‘Etoile, France).

Measurement of SEs and TSST-1 by sandwich enzyme linked immunosorbent assay (ELISA): Isolated staphylococci were inoculated into trypto-soya broth (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and cultivated at 37°C for 24 hr. These cultures were centrifuged at 1,800 x g for 20 min, and the supernatants were collected. The supernatants and MGS were measured for concentrations of each SE (SEA, SEB, SEC, SED and SEE) and TSST-1 by the sandwich ELISA method originally described by Freed et al. [13]. Anti SE or TSST-1 sheep IgG and standard preparations of each toxin were purchased from Toxin Technology, Inc. (Sarasota, FL, U.S.A.) [18]. MGS was centrifuged at 4,000 x g for 5 min at 4°C, and the supernatant was used for the sandwich ELISA method. Ninety-six-well flat-bottomed module plates (Greiner Labortechnik GmbH, Kremsmünster, Bad Hallerstr, Austria) were coated with 80 $\mu$l of anti SE or TSST-1 sheep IgG (40 $\mu$g/ml) dissolved in sodium carbonate buffer (pH 9.6) overnight at 4°C. The plates were washed three times with phosphate buffered saline (PBS, pH 7.21) containing 0.05% Tween-20 (PBS-T) and blocked with 100 $\mu$l of PBS containing 0.1% human serum albumin (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan) at 37°C for 90 min. After three more washes with PBS-T, the plates were desiccated overnight and used for measurements. Eighty microliters of sample was added to each well and incubated at room temperature for 45 min. After washing three times with PBS-T, 100 $\mu$l of horseradish peroxidase (HRP)-conjugated anti SE or TSST-1 sheep IgG (Toxin Technology, Inc., Sarasota, FL, U.S.A.) was added. After incubation at room temperature for 45 min, the plates were washed three times with PBS-T. The ELISA was developed with o-phenylenediamine as substrate. Each sample was measured in duplicate. The detection range of each toxin was 0.5–10 ng/ml and the percentage of coefficient of variation was less than 5.0%. The specificity of the sandwich ELISA method was confirmed with commercial kit (SET-RPLA, TST-RPLA, Denka Seiken Co., Ltd., Tokyo, Japan). Staphylococci that were positive with both the sandwich ELISA method and the commercial kit were judged as toxin-producing staphylococci. Twenty MGS samples selected at random were tested with both the sandwich ELISA method and the commercial kit, and the same specificity was confirmed, but the toxin concentrations were hardly able to be measured with the commercial kit (based on reversed passive latex agglutination), and the sandwich ELISA method was used in this study.

Table 1. Numbers and classification of MGS collected from lactating and dry glands

<table>
<thead>
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<th>No clinical signs-MGS</th>
<th>Chronic mastitic-MGS</th>
<th>Acute mastitic-MGS</th>
<th>Total</th>
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<tr>
<td>Lactating period</td>
<td>30</td>
<td>32</td>
<td>17</td>
<td>79</td>
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<tr>
<td>Dry period</td>
<td>21</td>
<td>17</td>
<td>3</td>
<td>41</td>
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<td>Total</td>
<td>51</td>
<td>49</td>
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<td>120</td>
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Purification of specific antibodies to SEC and TSST-1
from MGS: Bulk MGS were precipitated with 50% saturated ammonium sulfate. After centrifugation at 10,000 × g for 20 min, the precipitates were suspended in PBS and dialyzed against the same buffer. The dialysate was applied to a CNBr-activated Sepharose 4B (Amersham Biosciences, Corp., Piscataway, NJ, U.S.A.) column coupled with SEC or TSST-1. The SEC or TSST-1 affinity-column was previously equilibrated with PBS. After washing the column with equilibration buffer, elution was carried out with 4.5 M MgCl₂. To purify each immunoglobulin (Ig) isotype, the fractions eluted from the SEC or TSST-1 affinity-column were applied to a DEAE-cellulose (Whatman International, Ltd., Maidstone, England) column, equilibrated previously with 20 mM Tris-HCl (pH 7.4). Elution was carried out step-wise with 0.05 M, 0.1 M and 0.15 M NaCl, successively. The fraction eluted with 0.05 M NaCl, which predominantly contained IgG1, was used as the specific antibody fraction.

Cell culture and proliferative assay: Heparinized blood was obtained from clinically normal Holstein cows. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation across Lympholyte-H (Cedarlone Laboratories Ltd., Hornby, Canada) at 1,200 × g for 25 min. Cells at the interface were recovered, and washed three times with PBS, then suspended at a concentration of 1 × 10⁶/ml in RPMI-1640 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (Life Technologies, Rockville, MD, U.S.A.), 2 mM L-glutamine, 20 U/ml of penicillin (Meiji Seika Kai- sha, Ltd., Tokyo, Japan) and 100 µg/ml of streptomycin (Meiji Seika Kaisha, Ltd., Tokyo, Japan). Two hundred microliters of this cell suspension was added to each well of a 96-well round-bottomed microculture plate (Becton Dickinson Labware, Franklin Lakes, NJ, U.S.A.) and incubated with or without toxin (50 ng/ml at 37°C in 5% CO₂ for 96 hr. To test the inhibitory effect, a specific antibody fraction (50 µg/ml of total protein) purified from MGS was added to each well. Each well was pulsed with 0.5 µCi of [³H] thymidine during the final 6 hr of culture, and harvested onto a glass fiber filter. The counts of [³H] thymidine per minute (CPM) were measured in a liquid scintillation counter (Packard Instrument Company, Meriden, CT, U.S.A.). The data are presented as a stimulation index (S.I.) calculated by dividing the CPM of the culture stimulated with each stimulus by the CPM of unstimulated cultures (medium alone). Each stimulus was performed in triplicate.

Intramammary inoculation of staphylococcal toxins during the lactating period: Tested cows were bred in our laboratory, and 20 mammary glands of 9 Holstein cows were used. These cows were 3 to 5 years old, more than 6 months after parturition. Mammary glands used in this experiment met the following criteria: no clinical signs of mastitis were detected, SCC in MGS were less than 1 × 10¹² CFU/ml, S. aureus was not detected, SCC in MGS were less than 3 × 10¹²/ml and the concentration of lactoferrin was less than 450 µg/ml. SEC and TSST-1 were purchased from Toxin Technology, Inc. (Sarasota, FL, U.S.A.). Five micrograms of each toxin was dissolved in 10 ml of PBS, and sterilized by filtration through a membrane filter (pore size = 0.45 µm). After milking, teat ends were aseptically prepared and each toxin was inoculated into the teat sinus with a cannula. To ensure distribution of the toxin, the udders were massaged after the inoculation. As a negative-control, only 10 ml of PBS was used. In each cow, 1 or 2 mammary glands were inoculated with toxin, and 1 mammary gland was inoculated with PBS. The number of mammary glands inoculated with each substance was as follows: SEC-inoculated mammary glands = 9, TSST-1-inoculated mammary glands = 5, PBS-inoculated mammary glands = 6. MGS were collected at 0 (pre-inoculation), 0.1, 0.3, 1, 2, 3 and 7 days post-inoculation (dpi) and SCC and staphylococcal counts in MGS examined.

Statistical analysis: Data on concentrations of toxins, specific antibody titers, S.I. of cell proliferative assay, and SCC in MGS were shown as the mean ± SD, and analyzed with Student’s t-test.

RESULTS

Production of SEC and TSST-1 by staphylococci isolated from MGS: Staphylococci (S. aureus and CNS) isolated from MGS in both lactating and dry periods were examined for the production of SEs and TSST-1. CNS species isolated in this study were S. xylosus (45%, 54 isolates/total 120 isolates), S. sciuri (13%, 15/120) and S. lentus (7.5%, 9/120) in order of frequency. As shown in Table 2, a high percentage of S. aureus isolated from either no clinical signs- or mastitic (chronic and acute) MGS produced SEC, and the percentages of SEC-producing S. aureus from no clinical signs-, chronic mastitic- and acute mastitic-MGS were 42, 54 and 50%, respectively. The percentage of TSST-1-producing S. aureus was 25% for no clinical signs-MGS, 46% for chronic mastitic-MGS and 40% for acute mastitic-MGS. Twenty-five percent of isolates from no clinical signs-MGS produced both SEC and TSST-1. The percentage of both toxins-producing S. aureus was 39% for chronic mastitic-MGS and 30% for acute mastitic MGS. The percentages of SED-producing S. aureus from no clinical signs-, chronic mastitic- and acute mastitic-MGS were 33, 39 and 30%, respectively.

A high percentage of CNS isolated from no clinical signs- and chronic mastitic-MGS produced SEC and/or TSST-1. The percentages of SEC-producing CNS were 60% for no clinical signs-MGS and 72% for chronic mastitic-MGS. The percentages of TSST-1-producing CNS were 62% for no clinical signs-MGS and 67% for chronic mastitic-MGS. Fifty-two percent of isolates from no clinical signs-MGS and 54% from chronic mastitic-MGS produced both toxins, but the percentage of toxin-producing CNS isolated from acute mastitic MGS was less than that from no clinical signs- and chronic mastitic MGS. The percentage of SEC- and TSST-1-producing CNS was 21% and 6.9%, respectively, and both toxins-producing CNS was not detected.

Concentrations of SEC and TSST-1 in MGS: Concentra-
tions of SEC and TSST-1 in MGS were measured. As shown in Fig. 1, the concentration of SEC increased with the severity of mastitis in both lactating and dry periods. In acute mastitic-MGS, the concentration of SEC was 2.4 × 10^5 CFU/ml in the lactating period and 2.3 × 10^5 CFU/ml in the dry period, significantly (P<0.05) higher than in no clinical signs-MGS (0.66 × 0.09 ng/ml in the lactating period, 0.65 ± 1.7 ng/ml in the dry period). The concentration of TSST-1 was significantly (P<0.05) higher than that of SEC in no clinical signs-MGS, but no clear correlation with the severity of mastitis was detected in either period.

Specific antibodies to SEC and TSST-1 in MGS: We also measured the antibody titers specifically reactive to SEC and TSST-1 in no clinical signs-, chronic mastitic- and acute mastitic-MGS during both lactating and dry periods. The results (Fig. 2) clearly indicate that the titers were significantly (P<0.05) higher for antibodies specifically reactive to TSST-1 than to SEC in no clinical signs- and chronic mastitic-MGS during both periods. In acute mastitic-MGS during the dry period, specific antibody titers were 48 times higher (P<0.06) for TSST-1 (130 units/ml) than SEC (2.7 units/ml).

To determine whether the specific antibodies detected in MGS neutralize toxin, we tested the inhibitory effect of each specific antibody on the mitogenic activity of the toxin. The S.I. of cultures with SEC alone and SEC + specific antibody was 44 and 1.7, respectively, 96% of the inhibition measured. In the case of TSST-1, the S.I. of cultures with toxin alone and toxin + specific antibody was 49 and 14, respectively, 72% of the inhibition measured.

Intramammary inoculation of SEC and TSST-1 into lactating mammary glands: As shown in Fig. 4, SCC in SEC-inoculated mammary glands reached a mastitic level (more than 3 × 10^6/ml) from 0.3 dpi and a maximum (6.2 × 10^6/ml) at 2 dpi. SCC in SEC-inoculated mammary glands were still higher than in PBS-inoculated mammary glands at 7 dpi, with 5.9 × 10^6/ml measured, and significantly (P<0.05) higher than PBS-inoculated mammary glands at 1–7 dpi. In the case of TSST-1-inoculated mammary glands, SCC was reached maximum at 1 dpi, but levels were lower than 2 × 10^5/ml throughout the period of the experiment. In each group, staphylococcal counts in MGS were less than 2 × 10^5 CFU/ml, and S. aureus was not detected during the experiment (data not shown).

DISCUSSION

In this study, we investigated the production of SEs and TSST-1 by staphylococci isolated from 120 MGS collected from dairy farms in Miyagi and Yamagata prefectures, where staphylococcal mastitis frequently occurs from 1997 to 1998. We demonstrated a high percentage of SEC- and/or TSST-1-producing staphylococci including CNS as well as S. aureus isolated from MGS (Table 2). Many workers have isolated SE- and TSST-1-producing S. aureus from bovine mastitis by using genetical and immunological methods [19, 21, 23, 30, 32]. On the other hand, geographical variation in the presence of genes encoding SEs and TSST-1 was reported [19, 21]. In Japan, although the percentage of enterotoxigenic S. aureus reported by Matsunaga et al. was lower than that shown in this study, Takeuchi et al. reported a high percentage of TSST-1-producing isolates similar to ours [23, 32]. The predominant SE detected in this study was SEC consistent with earlier reports [23, 30, 32], but SED is the major type of SE in Brazil [7]. These observations also suggest geographical variation in production even among subtypes of SEs and TSST-1.

CNS is among the bacteria most commonly isolated from dairy cows, S. simulans and S. chromogenes are most frequently isolated [15, 24, 26]. In this study, the most frequent isolate was S. xylosus. Generally, CNS has been considered less pathogenic than S. aureus, but we detected a high percentage of SEC-producing CNS isolated from no clinical signs- and chronic mastitic-MGS. It was reported that SEC-producing CNS species, such as S. simulans, S. chromogenes and S. xylosus were isolated from healthy and mastitic ruminants [28, 35]. Moreover, CNS isolated from bovine and goat MGS produced other virulence factors, such as hemolysin and cytotoxic factors [4, 39]. These observations and the SEC productivity shown in this study suggest a more important role for CNS in the pathogenesis of bovine mastitis.

The percentage of toxin producing-CNS shown in this study was higher than those in other reports.Orden et al. reported that the percentages of SEC and TSST-1 produc-
SEC is a one of the causative agents of mastitis

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Valle et al. detected 17% (4/23) of SEC producing-CNS isolated from healthy goats [35]. The specificity of the sandwich ELISA method was confirmed with a commercial kit (data not shown). In this study, we analyzed MGS collected from dairy farms where frequently occur staphylococcal mastitis in a limited region and period. These factors may influence the percentage of toxin producing-CNS. Therefore, we considered that further investigations of toxin producing-CNS by means of genetical as well as immunological methods are needed in extensive regions and over long periods.

In acute mastitic-MGS during both periods, the percentage of toxin-producing staphylococci was less than in chronic mastitic-MGS (Table 2). Moreover, staphylococcal counts in acute mastitic-MGS during the dry period were less than in chronic mastitic-MGS (data not shown). Since medical treatments with antibiotics were prior to the collection of MGS, especially in acute mastitis, the decrease in the percentage of toxin-producing staphylococci was probably caused by the low viability of the staphylococci, but the concentration of SEC in acute mastitic-MGS was higher than that in chronic mastitic-MGS (Fig. 1). These results indicate that SEC was produced in MGS before the crisis of mastitis, and had reached a high concentration by the day when medical treatments were done.

The concentration of SEC increased with the severity of mastitis, and was significantly higher in acute mastitic-MGS than in no clinical signs-MGS (Fig. 1). Although the concentration of TSST-1 in no clinical signs-MGS was significantly higher than that of SEC, no clear correlation with the disease status was detected. These results suggest that SEC rather than TSST-1 plays an important role in the pathogenesis of bovine mastitis. Specific antibody titers were significantly higher for TSST-1 antibodies than SEC antibodies in commercial milk and dairy products [33]. The specific antibodies purified from MGS neutralized each toxin in vitro (Fig. 3). Moreover, it was reported that specific antibodies neutralized the lethal effects of SEs and
TSST-1 *in vivo* [3]. Considering these observations, it is suggested that the inflammatory activity of TSST-1 is neutralized by specific antibodies in MGS.

The isotype of the specific antibodies measured in this study is mainly IgG1, because a HRPOD-conjugated anti bovine IgG1 heavy chain was used as secondary antibody in the sandwich ELISA. IgG1 is the predominant isotype in bovine MGS, and mainly transferred from the bloodstream [20]. Therefore, it was speculated that specific antibodies detected in MGS originated in the bloodstream. In dairy cows, it was reported that TSST-1-specific antibody titers in serum increased along with the parturient history [14]. In humans, TSS occurs most frequently in young women during menstruation and is caused by an infection of TSST-1-producing *S. aureus* in the vagina [6]. These observations suggest that infection with TSST-1-producing staphylococci occurred through parturition, and specific antibodies to TSST-1 were developed and accumulated in the dairy cows. Moreover, it was reported that TSST-1 is capable of crossing epithelial cell barriers and subsequently transferred to the bloodstream [29]. On the other hand, SEC specifically remains in the mucosal epithelium [29]. These observations suggest that the specific antibody to TSST-1 is easily induced in humoral immunoreactions, although that of SEC is hardly induced in mucosal epithelium.

SCC in SEC-inoculated mammary glands was significantly higher than that in PBS-inoculated mammary glands. In contrast, SCC in TSST-1-inoculated mammary glands was equal to or less than in PBS-inoculated mammary glands (Fig. 4). These results indicate that SEC rather than TSST-1 is a more potent inducer of inflammatory responses in mammary glands. Ebling *et al.* reported that no significant differences were detected in SCC after intramammary infection (IMI) with *S. aureus* containing a gene for SEC or not [10]. These observations are inconsistent with our results. This discrepancy is probably caused by the difference in the experimental design. Since they inoculated the intact live-*S. aureus*, it is suggested that the effects of SEC were masked by the effects of *S. aureus* IMI. On the other hand, Ebling *et al.* detected significant differences in γδ T lymphocyte subpopulations in mammary glands infected with *S. aureus* containing the SEC-gene [10]. But in this study, we did not analyze the subpopulations of SCC increased by the intramammary inoculation of SEC. Therefore, to elucidate the pathological roles of SCC in mastitis, further investigations are needed concerning this point.

SEs stimulate bovine T lymphocytes and macrophages via specific Vβ molecules of TcR and the major histocompatibility complex (MHC) class II molecule, and induce the various inflammatory mediators [22, 25, 38]. Superantigen-reactive cells such as T lymphocytes and macrophages are known to exist in the mammary gland [1, 36]. These observations suggest that SEC stimulates MGS-cells and induces the production of various inflammatory mediators in the mammary gland. Neutrophils do not normally express MHC class II molecule, but these cells expressed MHC class II molecules on stimulation with T lymphocyte-derived cytokines and directly interacted with superantigens [11]. Therefore, MHC class II-positive neutrophils are probably stimulated by SEC in the mammary gland. Consequently, we speculate that superantigen-reactive cells, such as T lymphocytes and MHC class II-positive cells, are targets of SEC in the pathogenesis of staphylococcal bovine mastitis.

In addition, other pathological activities of SEs have been suggested. SEs but not TSST-1 are known to be a causative agent of staphylococcal food poisoning, and have emetic activity in humans and monkeys [29, 34]. It was reported that antibodies which neutralize emetic activity do not inhibit mitogenic activity [5]. Moreover, studies with chemical modifications and mutagenesis suggest that food poi-
soning is not caused by the superantigenic activity of SEs [5]. These observations indicate that SEs have specific activity against gastric and intestinal mucosal epithelium. We earlier reported that mammary gland epithelium is similar to intestinal epithelium in terms of intraepithelial lymphocytes [1, 2, 36, 37]. Therefore, SEC may have a specific action on the mammary gland epithelium similar to that on intestinal epithelium.

The existence of unidentified receptors for SEs other than TcR and MHC class II molecules is also suggested. It was reported that SEB binds to human proximal tubular cells via glycosphingolipid [8]. Moreover, Diener et al. reported that acute inflammation was induced by SEs independently of T lymphocytes and MHC class II molecules [9]. These observations suggest that additional SE-reactive cells exist in the mammary gland, other than T lymphocytes and MHC class II-positive cells, such as macrophages and activated neutrophils. These additional cells may also play pathogenic roles in bovine mastitis. For these reasons, to elucidate the mechanism by which mastitis is induced by SEC, it is important to investigate the pathological effects of SEC not only on superantigenic activity via TcR and MHC class II molecules but also on additional unidentified activities.

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