Cell Surface Antigen of Encapsulated *Staphylococcus epidermidis* ATCC 31432

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The cell surface antigen (CSA) from encapsulated *Staphylococcus epidermidis* ATCC 31432 was isolated and fractionated by DEAE-Sephadex ion-exchange chromatography. It yielded a single precipitin line against rabbit antiserum and was composed of galactose, glucosamine, and two (so far) unidentified carbohydrates linked to a protein backbone. Glycerol could not be detected. In an experimental mouse infection, CSA showed some protective activities, enhancing the stimulation of granulocytes as well as the immunoglobulin M response. Apparently, CSA is different from teichoic acid.

Coagulase-negative staphylococci are recognized as opportunistic pathogens (2, 3, 7, 14, 18, 20). Their cell surface substances (CSS) (such as slime or capsules) obviously are related to their pathogenicity (1, 4, 6, 10, 11, 18, 22). We previously reported that *Staphylococcus epidermidis* (an encapsulated coagulase-negative staphylococcal strain isolated from a clinical specimen) was lethal for mice, being resistant to phagocytosis (22). In experimental infections, a cell surface antigen (CSA) from encapsulated staphylococci could be positively correlated with the protective activity and capacity of neutralizing protective antibodies (11, 16, 22). However, the detailed biochemical and immunochemical properties of CSA still have to be elucidated. Therefore, this study was performed to determine the immunochemical characteristics of CSA from *S. epidermidis* ATCC 31432.

Female DD mice, 4 weeks old and weighing 15 to 17 g, and Japanese White rabbits (both from Nihon Clea Co. Ltd., Tokyo, Japan) and male BALB/c mice, 8 to 12 weeks old (from the Central Institute for Experimental Animals, Hanover, Federal Republic of Germany), were used for these studies. The preparation and analysis of rabbit antiserum were as previously described (11). For the preparation and partial purification of CSA, *S. epidermidis* ATCC 31432 was cultured in a dialyzed medium of brain heart infusion (Difco Laboratories, Detroit, Mich.) for 24 h at 37°C. CSA was obtained from the supernatant fraction of a sonicated cell suspension (phosphate-buffered saline, 0.1 M, pH 7.0). Partial purification of CSA was performed by DEAE-Sephadex A-25 ion-exchange chromatography (Pharmacia Fine Chemicals AB, Uppsala, Sweden), and its chemical characterization was carried out as previously described (15, 16). Immunoelectrophoresis was done on glass slides in 1.2% (wt/vol) Noble agar (Difco) containing 50 mM Veronal buffer (pH 8.6), and double-gel diffusion was performed as described by Ouchterlony (17).

To determine the capacity of absorbing passive protective activity, we added 30, 100, 300, and 1,000 μg of CSA to 1 ml of rabbit antiserum containing two units of protective activity (11). These mixtures were incubated at 37°C for 2 h and then centrifuged at 6,000 × g for 15 min at 4°C. The supernatant fraction (0.5 ml) was intraperitoneally (i.p.) injected into DD mice (n = 5), followed 30 min later by an i.p. challenge with 0.5 ml of 5% (wt/vol) mucin containing 107 CFU of *S. epidermidis* ATCC 31432. For active immunization, 1.0 and 3.0 μg of CSA were injected i.p. into DD mice (n = 5). *S. epidermidis* ATCC 31432 was administered i.p. 10 days later.

The induction of human granulocyte chemiluminescence and the measurement of the humoral immunoglobulin M response were carried out as previously described (5, 19). For control purposes we used zymosan (Sigma Chemical Co., St. Louis, Mo.) and *S. aureus* SG-511 Jena, which was grown overnight at 37°C in Mueller-Hinton broth (Difco), washed three times, suspended in phosphate-buffered saline, and adjusted to the turbidity of a McFarland no. 5 standard.

The binding of CSA from *S. epidermidis* ATCC 31432 to DEAE-Sephadex A-25 was different from that of the teichoic acid fraction. CSA was composed of galactose, glucosamine, and two unidentified sugars, as determined by gas-liquid chromatographic analysis. Concerning the amino acid composition of CSA, asparagine, glutamic acid, glycine, and alanine were detected as major components. Furthermore, CSA yielded a single precipitin line against rabbit antiserum and did not react with heterologous rabbit antiserum prepared from other encapsulated *S. epidermidis* strains, as described elsewhere (11).

More than 90% of DD mice (5 of 5 and 4 of 5; number of dead mice out of number of challenged mice) died after experimental infection with *S. epidermidis* ATCC 31432 when the protective activity of rabbit antiserum was absorbed with 1,000 and 300 μg of CSA, respectively, whereas most animals in the control group (1 of 10 [see above]) survived. A total of 100% of the actively immunized DD mice (0 of 5 and 0 of 5 [see above]) survived the staphylococcal infection, whereas 86% of the nonimmunized control mice (13 of 15) died. To determine the influence of CSA on the humoral immunoglobulin M response, we injected CSA i.p. into BALB/c mice. In comparison to a control group, this treatment led to an evident enhancement (127%) of the specific humoral immune response to sheep erythrocytes: (26 ± 5.8) × 103 PFU were formed per 106 spleen cells in the saline-treated controls, as opposed to (33 ± 5.9) × 103 PFU in the CSA-treated mice (1 mg of CSA per mouse). The same was true for the nonspecific cellular immune response, as luminol-dependent chemiluminescence.
of human granulocytes could be markedly increased by CSA (Fig. 1).

The presence of capsules or slime substances in coagulase-negative staphylococci has already been demonstrated (1, 4, 11, 18, 23). However, our knowledge concerning the biochemical and immunochemical properties of these substances is still very limited. We previously found that CSS from several encapsulated strains of S. epidermidis (ATCC 31432, SE-360, and SE-10) induced protective activity against those strains (11, 16, 22). Furthermore, active immunization of mice with CSS induced resistance against experimental infection with homologous staphylococci (11, 22). CSS could be isolated from S. epidermidis ATCC 31432 and fractionated by DEAE-Sephadex A-25 ion-exchange chromatography (16). Biochemical and serological analyses of CSS subfractions resulted in the detection of the following differences: (i) serologically inactive fractions (subfractions 1 and 2); (ii) serologically active fractions (so-called CSA fractions [subfractions 3 and 4]); (iii) teichoic acid-like fractions (subfractions 5 and 6, as described in reference 15); and (iv) nucleic acid fraction (subfraction 7). Immunologically active subfractions were separated from teichoic acid-like fractions by ion-exchange chromatography in a manner similar to that used for the preparation of CSA from S. aureus M (21). CSA was different from the S. epidermidis slime substance with respect to carbohydrate components and the above-mentioned items (i to iv) (13, 16).

According to our results, CSA seems to contain a type-specific antigen causing protective activity as well as absorbing activity of protective antibodies as well. Teichoic acid-like fractions of CSS and cell wall teichoic acid fractions displayed a much lower protective activity in mice than did CSA. Recent experiments demonstrated that experimental tumor growth in mice can be drastically reduced after the administration of certain CSS subfractions. The in vitro stimulation of human granulocytes and the increase in the immunoglobulin M response suggest a potent antifungal (antibacterial) CSA activity. Preliminary studies show that similar effects may be achieved in vivo after experimental infection of mice (unpublished data).

The application of certain CSS subfractions obviously leads to a potent stimulation of the specific humoral and nonspecific cellular immune systems. Protection-inducing antigens or CSAs of staphylococci are considered to be composed of polysaccharides (6, 8, 9). CSA of S. epidermidis ATCC 31432 seems to be composed of carbohydrates different from those in teichoic acid, because glycerol could not be detected and the phosphate content was very low (16). In addition, CSA was found to contain relatively high amounts of protein. Recent reports about the CSAs of S. aureus revealed that they are composed of unique amino sugars (9, 12, 21) similar to those detected in CSA of S. epidermidis ATCC 31432 (16).

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![Graph](https://via.placeholder.com/150)

FIG. 1. Effect of CSA on granulocyte activation. CSA (Δ) was obtained by DEAE-Sephadex A-25 column chromatography from S. epidermidis ATCC 31432. Zymosan (20 μg) (●) and S. aureus SG-511 (■) were used as controls.
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