Microbiology Education for Visually Impaired Students - Topics of Staphylococcal Infection -

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Abstract: We determined the morphological and immunological properties of encapsulated strains of *Staphylococcus* isolated from clinical specimens. Capsular polysaccharide antigens extracted from cell surface fractions induced resistance in mice against challenge with the homologous *Staphylococcus* strain. Hyperimmune rabbit serum prepared using these strains passively protected mice against challenge with only the homologous strain. The protective activity was absorbed with homologous capsular polysaccharide, but not with heterologous organisms, and the nature of the antibody was that of IgM. The capsular vaccine prepared by us was successful in preventing staphylococcal infections. In addition, because of the immunoglobulin treatment, mice were protected from infection with methicillin-resistant *Staphylococcus aureus* strains.

We detected encapsulation in these strains by electron microscopy although no capsules were detected by light microscopy with negative staining.

Understanding microbiology is often very challenging for visually impaired students, and there have not been significant improvements in the teaching aids used for educational purposes. However, the morphology of staphylococci can be identified using tactile graphics. Microbiology education is important for visually impaired students who aspire to become medical professionals.

Keywords: Capsule, Electron microscopy, Microbial morphology, Microbiology education, Slime, Staphylococcal infection, Tactile graphics, Visually impaired student

1. Introduction

Several studies describing the capsule and slime formation of *Staphylococcus aureus* were conducted during the early stages of investigation of staphylococci. Gilbert[1)] described the mucoid strain of *S. aureus* isolated from a patient with endocarditis. In 1934, the Smith strain was isolated from a patient with acute osteomyelitis[2)]. Rogers[3)] proposed that *S. aureus* produces a capsule containing the Smith surface antigen (SSA) in vivo and induces the production of a prospective antibody, but that capsule production would be easily be lost in vitro. Many investigators expressed much interest in his idea, and his hypothesis had a strong influence in the field of microbiological studies. In recent years, investigations have focused on other aspects on the basis of the different criteria used for studying *Staphylococcus* capsules. The Karakawa[4,5)] group investigated microcapsule and capsular substance-producing *S. aureus* strains. The Norcross[6, 7)] group regarded diffuse type growth of *S. aureus* strains in modified serum-soft agar as encapsulated. Ichiman[8)] has still regarded only highly capsule. The interpretation of the results of these experiments has caused some problems, depending upon the criteria selected for describing *Staphylococcus* capsules.

Coagulase-negative staphylococci (CNS) are important pathogens in opportunistic infections. Of these organisms, *Staphylococcus epidermidis* is clinically the most important organism[9)]. Numerous reports[10, 11)] have described the interaction

of host and parasite factors, which may result in the adhesion of the organisms onto implanted plastic devices or opportunistic infections in immunocompromised hosts. The significance of bacterial slime in the establishment of infection has been emphasized. On the other hand, the role of the capsule in causing infections has been postulated in *S. epidermidis*[12], *S. hyicus*[13], and *S. simulans*[8] strains, inducing the hypothesis obtained by encapsulated strains of *S. aureus.*

The present study has important implications for visually impaired students who aspire to become medical professionals.

2. Slime and Capsule

Slime and capsule are 2 different polysaccharides that vary in their biochemical and immunological properties, as noted by Wilkinson[14]. According to the description by Caputy and Costerton[15], slime that was immunologically and antigenically identical was produced by the Smith and Wiley staphylococcal strains. However, the glycocalyx of the Wiley strain was composed of a slime layer and that of the Smith strain had a tight, immunologically distinct integral capsule and a loose, peripheral slime layer. We have previously identified capsules in *S. aureus* (Figure 1), *S. epidermidis* (Figure 2), *S. hyicus* (Figure 3), and *S. simulans* (Figure 4) strains by electron microscopy[8, 12, 13], although no capsules were detected by light microscopy with negative staining.

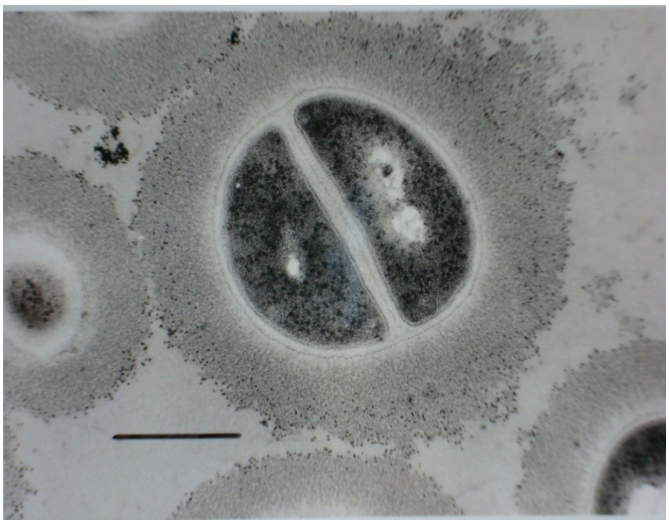

Figure 1. Ultra-thin section of an encapsulated strain of *Staphylococcus aureus*

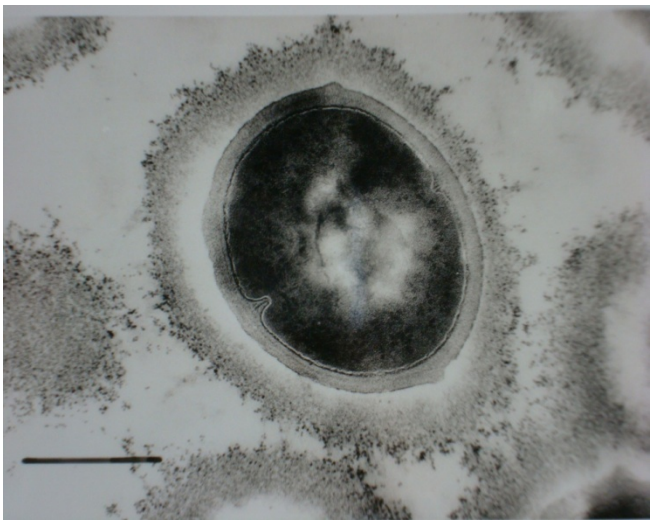

Figure 2. Ultra-thin section of an encapsulated strain of *Staphylococcus epidermidis*

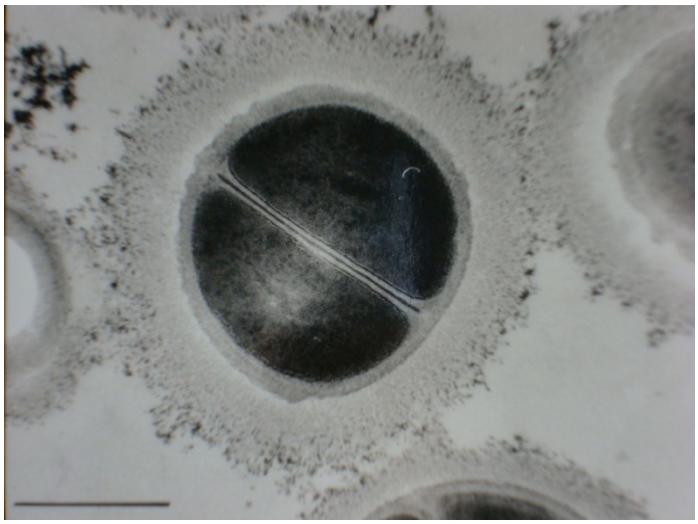

Figure 3. Ultra-thin section of an encapsulated strain of *Staphylococcus hyicus*

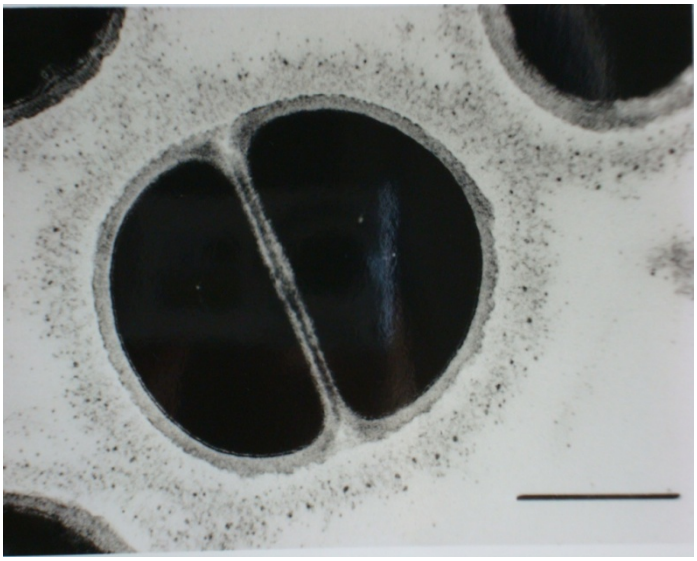

Figure 4. Ultra-thin section of an encapsulated strain of *Staphylococcus simulans*

Furthermore, a fluorescent antibody technique was developed for detecting capsular substances in *S. aureus*[16] and *S. epiderimidis*[17]. These findings indicate that most ordinary strains of staphylococci produce capsular type antigens although their capability varies according to the strain[8].

In CNS, slime production is markedly enhanced depending upon the culture condition, as in *S. aureus*. Christensen et al.[10] demonstrated slime production in *S. epidermidis* by using the following procedure: strains were cultured in a plastic tube, after which the culture broth was discarded, and the organisms adhered onto the surface of the tube were stained with Trypan blue. CNS had been considered as non-pathogenic organisms; however, they are now recognized as causing opportunistic infections associated with foreign bodies such as prosthetic valve endocarditis[11], cerebrospinal shunts[18], orthopedic devices[19], intravascular catheter[20], and peritoneal dialysis catheter[21]. They are also being increasingly recognized as infectious pathogens in neonates[22] and immunocompromised hosts. Christensen et al.[10] developed a technique for detecting slime-producing CNS strains on smooth surfaces. The presence of adherent growth was considered indicative of slime production. In addition, by using scanning electron microscopy, we found that a slime-producing staphylococcal strain was covered by a layer of material and that *S. epidermidis* attached to an acupuncture needle (Figure 5).

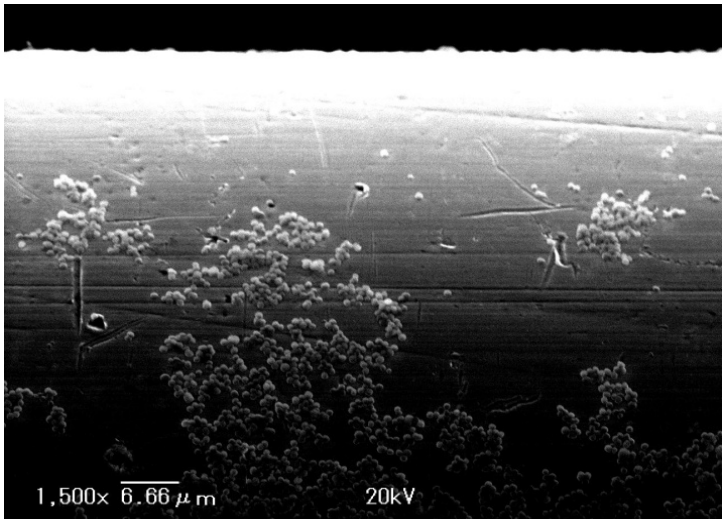

Figure 5. Scanning electron micrograph of *Staphylococcus epidermidis* attached to an acupuncture needle

3. Common Biological Properties

A high level of virulence in mice of the Smith diffuse strain compared to that of its compact variant has been previously noted[23]. This high virulence results in a significant lethal effect of the strain when intraperitoneally injected to mice; however, the effect was not observed on intravenous injection. An intraperitoneal injection of Smith strain (10^2 to 10^4 CFU with mucin) killed mice[24]. As mentioned above, one of the biologically unique features of the Smith diffuse strain is the formation of an antiphagocytic capsule. For normal phagocytosis, phagocytes must first recognize the bacterium. In non-encapsulated strains, phagocytosis occurs due to an association of the serum complement and IgG, both of which are present in the sera of normal humans without a history of staphylococcal infection. However, high numbers of protective IgM antibodies against encapsulated *S. aureus* have been detected even in so-called normal human sera[8]. Phagocytosis is facilitated by the pronase-sensitive membrane receptor of polymorphonuclear leucocytes, and the presence of immune antibodies increases complement fixation. With immune antibodies, however, complement binding was restricted to the cell wall. Capsules with phagocytosis-retarding substances mask the cell surface peptidoglycan, thus blocking the opsonization process. These facts indicate not only production of a unique substance such as antiphagocytic substance in encapsulated strains but also impede the production of a number of metabolites or inhibition of secretion of the substance by the presence of a capsule.

A fluorescent antibody technique was developed for the determination of encapsulated strains. Encapsulated strains exhibiting 4+ and 3+ intensities were phage non-typable and virulent to mice, while 2+ strains were phage typable and nonvirulent to mice[17]. When the cell volume indices of virulent and nonvirulent strains were compared, all virulent strains showed significantly higher indices than nonvirulent strains[16]. When these strains were intraperitoneally injected into mice, the strains with high cell volume indices resisted ingestion by peritoneal cells, whereas strains with low cell volume indices were sensitive to phagocytosis. In ultra-thin sections of mouse-virulent strains stained with ferritin-conjugated rabbit antiserum, well-defined capsules were detected around cell walls; however, no capsule was detected around the walls of nonvirulent strains[8].

4. Infection and Immunity

A specific characteristic of mice immunized with the heat-killed Smith strain is high resistance against homologous strains along with rapid phagocytosis of invading microorganisms by intraperitoneal leukocytes[25]. This resistance is assumed to be mostly humoral and could be passively transferred to other animals[24]. Koenig et al. [25] have suggested the presence of a phagocytosis-retarding antigen that is present around the cell surface of Smith and Smith-like strains. Morse[26] recognized the induction of a protective effect in mice against challenge with a Smith diffuse strain, and polysaccharide substances extracted from the culture supernatant of the Smith diffuse strain were designated as SSA. Haskell and Hanessian[27] identified this

substance as 2-amino-2 deoxy-D-glucuronic acid polymer, which was present on the cell surface, including the capsule.

In small animals immunized with a single injection of a suitable dose of encapsulated strains, protective activity against the homologous strain usually appears 10–15 days after the injection, but resistance remarkably decreases in the third week and disappears at 4 weeks after the injection[24]. This resistance could be maintained by administration of a booster dose, but such a resistance is assumed to be essentially transient. The resistance factor yielded by immunization with the encapsulated strain is humoral. In rabbits immunized with encapsulated strain, IgM was found to be the major resistance factor. The complement-fixing ability of IgM is considerably higher than that of IgG. Therefore, it would be reasonable that IgM possesses much higher activity in opsonization than does IgG. Moreover, the IgM antibody has been shown to be associated with protective activity in human serum against encapsulated strains of *S. epidermidis* [28], which is similar to the actions of encapsulated strains of *S. aureus* [29] and group B streptococci[30] in normal human serum. The protective activity in normal human serum is sensitive to 2-mercaptoethanol and is removed by absorption with rabbit anti-human IgM serum.

Furthermore, passive protective activities of 3 different classes of monoclonal antibodies were found in mice challenged with encapsulated strains of *S. epidermidis*. Monoclonal IgM antibody passively protected mice against challenge with a homologous strain, but no other monoclonal antibodies (IgG1 or IgG2b) were detected [31].

5. Enzyme-Linked Immunosorbent Assay

A specific and rapid enzyme-linked immunosorbent assay (ELISA) was previously used to detect immunoglobulins attached to capsular polysaccharides in human serum[32]. Positive IgG, IgM, and IgA titers of more than 1:6400, 1:1600, and 1:400, respectively, were observed against passive protective human serum with this assay. However, IgG, IgM, and IgA titers of less than 1:400, 1:100, and 1:50, respectively, were detected in serum without any protective activity. When inhibition ELISA was performed to examine the cross-reactivity of passive protective human serum to homologous and heterologous capsular polysaccharides, remarkable inhibition was observed with homologous capsular polysaccharide, whereas no inhibition was observed with heterologous substances[33].

In protective human serum, however, the activity was inhibited by 2-mercaptoethanol and was removed by absorption with anti-IgM serum[34]. Moreover, IgA may block the antiphagocytic action of IgG and IgM antibodies[35]. The major passive protection in the immune system of mice is provided by IgM antibodies against capsular polysaccharide antigens.

According to these results, ELISA was a sensitive and specific method for the detection of normal human serum antibodies, and this method could replace the challenge method in mice that is used for the detection of antibodies to encapsulated strains[36].

6. Vaccine

We have previously prepared the following vaccine: 25 mg each of heat-killed Smith diffuse strain (capsular type A) and ATCC-21734 (capsular type B) of *S. aureus*, expecting an adjuvant effect of cell wall component, plus 3 mg of capsular polysaccharide extracted from strain ATCC-31432 (capsular type I) of *S. epidermidis*[37]. These were intramuscularly injected into dairy cows twice, at intervals of 2 weeks, in 2 herds in Georgia, USA. In one herd, the vaccine was used in 196 dairy cows, and in the other herd, the vaccine was used in 250 dairy cows. Similar numbers of untreated dairy cows served as controls. Subsequent losses in milk production were compared between the treated and control groups. In a herd, the main pathogen of bovine mastitis was found to be group B streptococci[38]; however, milk production loss was significantly reduced 2 weeks after vaccination, and the lowest milk production loss was 35% less than that of the control group. This effect was observed for about 3 months. In the second herd, the main causative pathogen for mastitis was assumed to be staphylococci. In the 250 dairy cows that were administered the prepared vaccine, milk production loss was 32% less than that of the control group, and the effect was observed for about 6 months.

In another vaccine experiment performed in Hokkaido, Japan, an improvement in bovine mastitis was observed in a number of dairy cows treated with the vaccine, indicating the therapeutic effect of this vaccine[39]. Experimental studies performed using a mouse model of staphylococcal mastitis demonstrated that capsular polysaccharide expression results in an

increased capacity for persistence in the host[40]. This finding also outlines important considerations in developing a vaccine for the prevention of *S. aureus* disease in healthcare settings[41].

7. Specific Immunoglobulins

A high population of protective antibodies against encapsulated strains was detected in normal human sera. In our previous study, we developed a protective immunoglobulin preparation by eluting the complex of heat-killed Smith diffuse strain and its antibody in pooled human sera by using propionic acid in the presence of sucrose[42]. With this procedure, specific immunoglobulins protected mice from lethal infection. In this preparation, 54% of the protein was immunoglobulin and 20% was IgM[43]. These findings indicate that serum opsonins against encapsulated strains of *S. aureus* play an important role as a barrier to invasion by these organisms[44].

After treatment with this immunoglobulin preparation, mice were protected from death due to infection with a homologous capsular type methicillin-resistant *Staphylococcus aureus* (MRSA) strain, but no protection was observed even with a high dose of methicillin[45].

8. Tactile graphics of morphology

Visually impaired students have been able to study microbiology remarkably well with the aid of tactile graphics that provide an understanding of microbial morphology and Gram staining[46]. Furthermore, the students could use scanning electron microscopy for a study of staphylococci by using tactile graphics[47]. A transmission electron microscope was used to create these tactile graphics, and the effects of disinfectants on bacterial morphology were evaluated[48]. In an ultra-thin section of an encapsulated strain stained with ferritin-conjugated rabbit antiserum, well-defined capsules were detected around cell walls (Figure 6). However, no capsule was detected around the walls of non-encapsulated strain of *S. aureus* (Figure 7).

The use of tactile graphics is important for microbiology education for visually impaired students who aspire to become medical professionals.

Figure 6. Tactile graphics of an encapsulated strain of *Staphylococcus aureus*

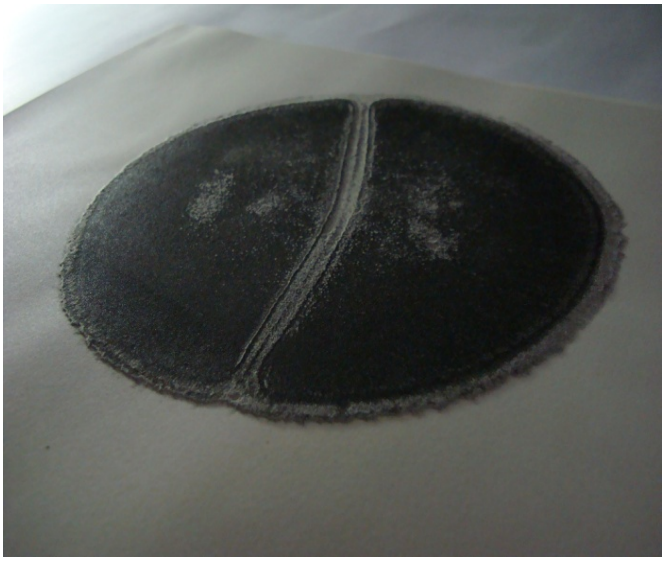

Figure 7. Tactile graphics of a non-encapsulated strain of *Staphylococcus aureus*

9. Conclusion

Capsular polysaccharides expressed by *S. aureus* are clearly important in the pathogenesis of staphylococcal infections. They enhance staphylococcal virulence by impeding phagocytosis, resulting in bacterial persistence in the bloodstream of infected hosts. With the emergence of MRSA strains, new strategies are needed to combat staphylococcal infections. The capsular vaccine is successful in preventing staphylococcal infections. In addition, immunoglobulin treatment protected the mice from MRSA infections.

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