Immunoreactivity of Iron-Repressible Cell Wall Proteins and Exoproteins of *Staphylococcus aureus*

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황색포도알균의 철억제성 세포벽 단백과 세포외 단백의 면역반응성

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저자들은 본 연구에서 황색포도알균에 의한 패혈증 환자의 회복기 혈청을 이용하여 황색포도알균의 철억제성 세포벽 단백들과 세포외 단백들의 면역반응성을 관찰하고자 하였다. 전기영동과 Western blot을 실시한 결과 철이 결핍된 배지에서 발현된 몇 가지 철억제성 세포벽 단백들은 회복기 환자혈청과 반응시켰을 때 강한 면역반응성을 보였으나 철이 풍부한 배지에서 발현된 대부분의 세포벽 단백들은 면역반응성을 보이지 않거나 상대적으로 약한 면역반응성을 보였다. 몇 가지 세포외 단백들은 철이 결핍된 배지에서만 발현되었으며 회복기 환자혈청과 반응시켰을 때 상대적으로 강한 면역반응성을 보였다.
이러한 결과를 통해 철억제성 세포벽 단백들과 세포외 단백들이 철이 상대적으로 결핍된 사람체내에서 발현될 뿐 아니라 면역원성을 가지고 있음을 알 수 있다. 따라서 포도알균에 의한 감염질환의 병인론 연구에 철결핍배지의 사용을 추천한다.

Key Words : 포도알균, 철, 철억제성 단백

We previously reported that the expression of iron-repressible high-affinity iron-uptake system of *Staphylococcus aureus* was stimulated by oxygen tension, and that several iron-repressible cell wall proteins could play an important role in the pathogenesis of staphylococcal infections as well as in uptake of iron in relatively iron-restricted conditions such as *in vivo* (1). Also in our other previous study (2), we observed that these iron-repressible cell wall proteins including proteins expressed only in iron-restricted condition showed strong immunoreactivity when reacted with human sera from the convalescent patient and the normal healthy volunteer. These results indicate that human body is an iron-restricted condition. Actually, iron availability is very low in vivo, especially in human body, despite large amounts being present. Most extracellular iron found in body fluids, such as plasma and mucosal secretions, is bound to the high-affinity iron-binding glycoproteins such as transferrin and lactoferrin, resulting in little free iron. Most bacteria respond to the environmental cue of restricted iron availability by de-repressing iron-uptake systems. In addition, this restricted iron availability also acts as an environmental signal to regulate expression of other genes (3).

Iron availability, however, is very high in conventional bacterial culture media commonly used in most laboratories, and most researchers prefer complex culture media because simple defined or deferrated media

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do not allow bacteria to grow well enough. We hereby had a question that laboratory common media could actually reflect the *in vivo* states, which is an iron-restricted condition. So, to make an answer for this question, we tried to compare the immunoreactivity of *S. aureus* cell wall proteins and exoproteins expressed in the same iron-sufficient and iron-deficient conditions as described in our previous reports because cell wall proteins and exoproteins were easily exposable immunogens to host immune system.

Details in other materials and methods were described in our previous studies (1, 2) except for the fact that the culture supernatants containing the secreted exoproteins were used.

EXPRESSION AND IMMUNOREACTIVITY OF CELL WALL PROTEINS

As described in our previous study (1), about 83, 64, 55, 38, 36, and 34 kDa of cell wall proteins were expressed more in the iron-deficient BHI than in the iron-sufficient BHI (Figure 1A-1). Most of these major proteins showed strong immunoreactivity and other minor proteins also showed relatively strong immunoreactivity (Figure 1B-1). In contrast, about 98, 79, 61, 45, 43 and 42 kDa of cell wall proteins were expressed more in the iron-sufficient BHI than in the iron-deficient BHI (Figure 1A-2). When these proteins were reacted with serum obtained from a convalescent patient after staphylococcal septicemia, only the protein of about 42 kDa showed relatively strong immunoreactivity, but other major proteins did not. Against our expectation, few other minor proteins on SDS-PAGE showed weak immunoreactivity (Figure 1B-2). Similar results were also shown when other complex media, such as nutrient and LB broths, were used and serum from normal healthy volunteer was applied (data not shown). These results indicated that iron-sufficient laboratory common media could not reflect iron-restricted in vivo states.

EXPRESSION AND IMMUNOREACTIVITY OF EXOPROTEINS

About 50, 35, 32, 28 and 25 kDa exoproteins were expressed in the iron-deficient BHI (Figure 2A-1), but no proteins were observed in the culture supernatant obtained from the iron-sufficient BHI (Figure 2-A-2)

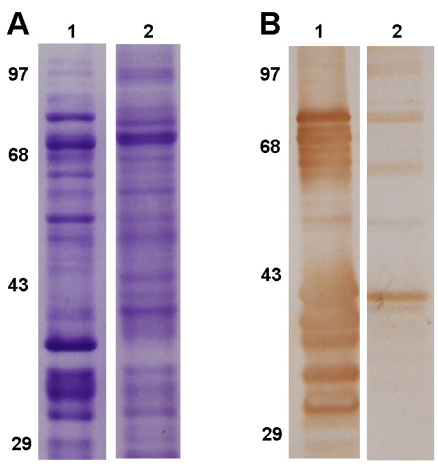

Figure 1. Expression **(A)** and immunoreactivity **(B)** of cell wall proteins of *Staphylococcus aureus* 6538 strain according to the iron concentration of media. A 100 μg of cell wall proteins was electrophoresed and transferred to nitrocellulose membrane for western blot analysis. **(A)** Coomassie staining of cell wall proteins in the bacteria grown in the iron-deficient (1) and iron-sufficient (2) media, respectively. **(B)** Immunoreactivity of cell wall proteins in the bacteria grown in the iron-deficient (1) and iron-sufficient (2) media, respectively, when the convalescent serum was applied. Standard protein sizes (kDa) are indicated on the left.

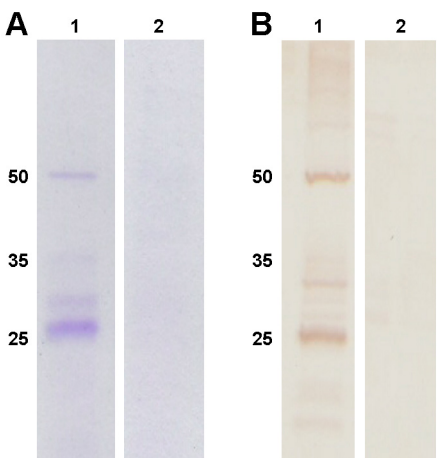

Figure 2. Expression **(A)** and immunoreactivity **(B)** of exoproteins of *Staphylococcus aureus* 6538 strain according to the iron concentrations of the media. 20 μL of the culture supernatant was electrophoresed and transferred to nitrocellulose membrane for western blot analysis. **(A)** Coomassie staining of exoproteins in the culture supernatant of the iron-deficient (1) and iron-sufficient (2) media, respectively. **(B)** Immunoreactivity of exoproteins in the culture supernatant of the iron-deficient (1) and iron-sufficient (2) media, respectively, when the convalescent serum was applied. Standard protein sizes (kDa) are indicated on the left.

at all. When these exoproteins were reacted with the patient's serum, about 50, 32 and 25 kDa protein showed relatively-strong immunoreactivity and about 35 and 28 kDa protein showed relatively-weak immunoreactivity (Figure 2B-1), but none of the proteins obtained in the iron-sufficient BHI did not show immunoreactivity (Figure 2B-2). Similar results were also shown when other complex media, such as nutrient and LB broths, were used and serum from normal healthy volunteer was applied (data not shown). These results certainly indicated that iron-sufficient laboratory common media could not reflect the iron-restricted *in vivo* states.

It is well known that iron is an essential nutrient as well as a global regulator of gene expression, and there is a big difference in the concentration of the freely available iron for bacterial growth between *in vitro* and *in vivo* states, especially in human body. Given the adaptive challenges, it is not surprising that pathogenic bacteria can evolve the ability to regulate expression of genes for survival and virulence (4, 5). Actually, the growth environment is known to exert a considerable effect on the structure of bacterial cell walls and the expression of virulence determinants, and there are also many reports indicating considerable differences between *in vivo*- and *in vitro*-grown organisms (6-10). In particular, the extremely low iron-availability in human body fluids constitutes a major environmental signal for infecting pathogens. This lack of freely available iron is due to the presence of transferrin, which are high-affinity iron-binding glycoproteins. Pathogenic bacteria compete for this transferrin-bound iron by de-repressing high-affinity iron-sequestering mechanisms usually based on low-molecular mass iron-chelators (siderophores) or their corresponding cell envelop protein receptors, which is still elusive, as shown in our previous report (1). Because *S. aureus* especially possesses both iron-sequestering mechanisms, the bacterium can grow well even in iron-restricted harsh environment, compared to other pathogenic bacteria that possess either.

Some researchers reported that there were marked differences in profiles of cell wall proteins between *ex vivo* human peritoneal dialysate- and *in vivo* chamber-grown staphylococci compared with the bacteria cultured in iron-sufficient laboratory common media. Growth *ex vivo* and *in vivo* resulted in the induction of several iron-repressible proteins which were also present in staphylococci grown in iron-restricted media (8, 9). These results suggested that *ex vivo* and *in vivo* states were relatively iron-restricted conditions and iron-sufficient laboratory common media could not reflect *in vivo* states. These results were supported by our present results that several iron-repressible cell wall proteins expressed in iron-restricted media showed relatively strong immunoreactivities when the convalescent serum was applied. There have been no reports concerning the expression and immunoreactivity of exoproteins in iron-restricted condition until now. In the present study, however, several iron-repressible exoproteins were expressed only in iron-deficient media, and moreover, these exoproteins showed immunoreactivities when the normal serum as well as the convalescent serum was applied. Similar results were also shown when other iron-sufficient laboratory common media, such as nutrient broth and LB broth, were used. Our results also indicated that most iron-repressible cell wall proteins or exoproteins were expressed and immunogenic *in vivo*, a relatively iron-restricted condition, and iron-sufficient laboratory common media could not reflect *in vivo* states. Furthermore, the findings that normal serum had antibodies against staphylococcal cell wall proteins and exoproteins, especially iron-repressible proteins, indicated that people had been infected at least once or twice by staphylococci, or exposed continuously by common antigens of commensal staphylococci (2, 11). Like staphylococci, other gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, isolated from both human and animal infections, were also shown to express iron-regulated outer membrane proteins that are indicative that growth *in vivo* occurs under iron restricted conditions (12, 13).

In summary, an in vivo state, especially human body, is a relatively iron-restricted condition and concentration of freely available iron exerts a considerable effect on the expression of cell wall proteins as well as exoproteins of *S. aureus*. Several iron-repressible cell wall proteins and exoproteins are expressed and are immunogenic in vivo. Moreover, *S. aureus* has abil-

ity to grow well in the iron-restricted conditions compared to other pathogenic bacteria.

Therefore, on the basis of our and other researchers' results, we recommend the use of iron-deficient media for studies concerning pathogenicthy of staphylococcus. In addition to the use of iron-deficient media, development of ex vivo systems, in which staphylococci can grow well and can mimic better *in vivo* states, is necessary.

ABSTRACT

In the present study, we tried to investigate immunoreactivity of iron-repressible cell wall proteins and exoproteins of *Staphylococcus aureus* when the convalescent serum obtained from the patient with septicemia was applied. On SDS-PAGE and western blot analysis, several iron-repressible cell wall proteins expressed in iron-deficient BHI showed strong immunoreactivity, whereas no or relatively weak immunoreactivity was shown in iron-sufficient BHI. Several exoproteins were expressed only in iron-deficient BHI and these exoproteins showed strong immunoreactivity. These results indicate that several iron-repressible cell wall proteins and exoproteins are expressed and are immunogenic *in vivo*. Since *in vivo* state is an relatively iron-restricted condition, we recommend the use of iron-deficient media for studies concerning pathogenicity of staphylococcal in human.

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