BPB Reports

Report

Identification of the Regions Responsible for Binding to Human Immunoglobulin G in Staphylococcal Superantigen-Like Protein 10

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Staphylococcal superantigen-like 10 (SSL10) is one of the immunoglobulin G (IgG) binding proteins produced by *Staphylococcus aureus* (*S. aureus*). SSL10 is reported to bind to Fc region of human IgG and interfere its effector functions. As SSL10 shows no homology with other staphylococcal IgG binding proteins, the mechanism of interaction between SSL10 and IgG remains to clear. In this study we attempted to identify the regions of SSL10 that are responsible for binding to human IgG (hIgG) by analyzing the binding ability of chimeras between SSL10 and its paralog, SSL7. The chimeras that retained either β1-β3 or β10-β12 of SSL10 bound to immobilized hIgG. On the other hand, chimeras that lacked both of these regions did not show binding activity to hIgG. In far western analysis, biotinylated hIgG interacted with SSL10 and chimera that retained β1-β3 and β10-β12 of SSL10. Collectively, SSL10 has two responsible regions for binding to hIgG, one is located in N-terminal half of oligonucleotide/oligosaccharide-binding (OB)-fold domain and the other is in C-terminal half of β-grasp domain. These findings would contribute to understand the mechanism of immune evasion of *S. aureus* and also to develop vaccines and drugs against *S. aureus*.

Key words staphylococcal superantigen-like protein 10, IgG, IgG binding protein

INTRODUCTION

Staphylococcus aureus (*S. aureus*) produces three immunoglobulin G (IgG) inhibitors to evade host immunity: Staphylococcal protein A (SpA), Staphylococcal binder of immunoglobulin (Sbi) and Staphylococcal superantigen like 10 (SSL10).[1–3] SSL10 is a member of SSL family that consists of 14 SSLs they are characterized by similar structures with staphylococcal superantigens (SAgs); they are composed of N-terminal half of oligonucleotide/oligosaccharide-binding (OB)-fold domain and C-terminal half of β-grasp domain. SSLs have no superantigenic activity despite their structural similarity with SAgs; several SSLs target host immune molecules.[4] SSL10 is reported to bind to CXCR4,[5] phosphatidylserine[6] and blood coagulation factors.[7] SSL10 is also reported to target on human IgG (hIgG) and interfere the effector functions of IgG, i.e. complement activation[3] and Fcγ receptor-meditated phagocytosis[2] via binding to the Fc portion of hIgG. SSL10 does not show homology with other staphylococcal IgG inhibitors, the molecular mechanism of interaction between SSL10 and IgG remains to clear.

We previously identified the regions of SSL10 responsible for binding to prothrombin by examining the binding activity of chimeras between SSL10 and its paralog SSL7.[8] We also identified the regions of SSL5 responsible for interaction with its target protein matrix metalloproteinase 9 (MMP-9) by using a series of chimeras between SSL5 and SSL7.[9] In this study, we attempted to identify the regions of SSL10 responsible for binding to hIgG by analyzing the binding activity of chimeras to hIgG.

MATERIALS AND METHODS

Reagents All chemical reagents and media unless otherwise stated were purchased from FUJIFILM Wako pure chemicals (Osaka, Japan), Nacalai tesque (Kyoto, Japan) and SIGMA (St. Louis, MO).

SSL7, SSL10 and Their Chimeras The gene of SSL7 and SSL10 were amplified from the genome of *S. aureus* (ATCC27733) as described previously.[3] Chimeras between SSL10 and SSL7, named SSL7x10, SSL10x7 and SSL7x10A-Q, were constructed by domain swapping.[8] SSL7x10R, the mutant of SSL10 replaced β1-β3 to that of SSL7, was constructed by megaprimer method as described previously.[8] The sites where swapped were shown in Fig. 1, and schematic representations of chimeras were depicted in Fig. 2. SSL7, SSL10 and their chimeras were produced in *E. coli* JM109 as N-terminal hexahistidine-tagged form and purified by Ni-conjugated Sepharose as described previously.[8] The recombinant SSLs were biotinylated using EZ-link NHS-LC-biotin (Thermo Fisher Scientific, Waltham, MA) in according to manufacturer's protocol.

Preparation of hIgG Normal hIgG was purified from human serum by ammonium sulfate precipitation and affinity purification using Protein G-Sepharose as described previously.[3] The trace amount of IgA in purified hIgG was removed

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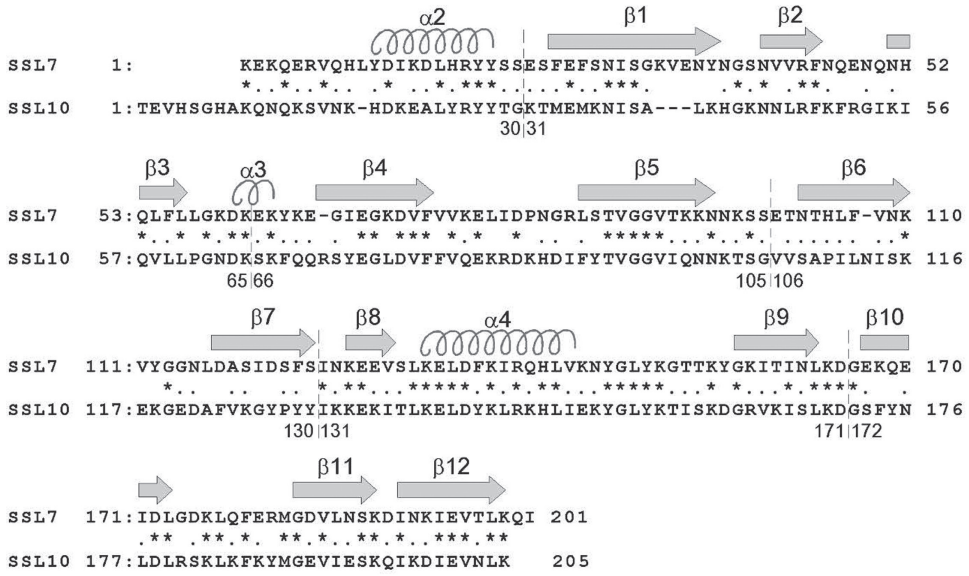

Fig. 1. The Primary Sequences and Secondary Structures of SSL7 and SSL10

The homology of amino acids between SSL7 and SSL10. Identical amino acids are marked with asterisk, similar amino acids are marked with dot. Secondary structure showed by helix (α-helices) and arrow (β-stands) were reported by Arcus *et al*. The positions domain swapped were indicated dotted lines and the amino acid positions of SSL10.

by incubation purified hIgG fraction with SSL7-conjugated Sepharose at 4°C for 16 h.

Solid Phase Binding Assay Interaction between SSLs and IgG was determined using solid phase binding assay as described previously.[8,9] Briefly, hIgG (0-1.0 μg/mL) was adsorbed on MaxiSorp™ microtiter plate (Nalge Nunc, Naperville, IL). After blocking with heat denatured 1% BSA, the wells were treated with biotinylated SSLs (1 μg/mL, 50 μL in PBS-0.05% Tween 20) at room temperature for 1 h, then treated with horse radish peroxidase (HRP)-conjugated Streptavidin (Pharmingen, SanDiego, CA). The peroxidase activity bound to each well was determined using 3, 3', 5, 5'-tetramethybenzidine as substrate. The absorbance in each well was read at 450 nm/750 nm by using iMark Microplate Absorbance Reader (Bio-Rad Laboratories, Hercules, CA).

Far Western Blot Analysis SSL7, SSL10 and their chimeras (1 μg/lane) were separated on SDS-polyacrylamide gel (12.5%) under non-reducing conditions, and transferred onto a polyvinylidene fluoride (PVDF) membrane (FluoroTrans W; Pall, Port Washington, NY). The membrane was blocked with BlockAce (Dainippon Pharmaceutical, Osaka, Japan) and incubated with biotinylated hIgG (20 μg/mL) for 1 h, then with NeutrAvidin-HRP (Thermo Scientific) for 30 min. The band was visualized with the ECL detection system (GE Healthcare).

Statistical Analysis Statistical evaluation was performed using one-way ANOVA.

RESULTS

The secondary structure of SSLs is consist of α1 signal peptide that removed during secretion, α2, β1, β2, β3, α3, β4, β5, β6, β7, β8, α4, β9, β10, β11 and β12. OB-fold domain of SSLs is from α2 to β5, and β-grasp domain is from β6 to β12 (Fig. 1).[10] To identify the responsible region of SSL10 for binding to hIgG, a series of chimeras between SSL10 and SSL7 were examined their binding ability to immobilized hIgG. SSL10 but not SSL7 bound to immobilized IgG as previously reported (Fig. 2A).[3] SSL7x10, the chimera consisted of OB-fold of SSL7 and β-grasp of SSL10, and its complementary chimera SSL10x7 that consisted of OB-fold of SSL10 and β-grasp of SSL7, significantly bound to hIgG (Fig. 2A). SSL7x10A that held β1-β5 of SSL10 significantly bound to hIgG, on the other hand SSL7x10B that held α2 of SSL10 lost the binding to hIgG (Fig. 2B). SSL7x10E that retained β1-β3 of SSL10 but not SSL7x10F that retained β4-β5 of SSL10 significantly bound to hIgG (Fig. 2C). SSL7x10R, the mutant of SSL10 replaced β1-β3 to that of SSL7 significantly bound to hIgG (Fig. 2D). Above results suggests that β1-β3 of SSL10 is responsible for binding to hIgG but it is not the only region of SSL10 to bind to hIgG. SSL7x10G that held α2 and β6-β7 of SSL10 and SSL7x10K that held α2 and β8-α4-β9 of SSL10 did not significantly bind to hIgG (Fig. 2E). On the other hand SSL7x10L and SSL7x10M those retained β10-β12 of SSL10 significantly bound to hIgG (Fig. 2F). SSL7x10Q, the mutant of SSL10 substituted β1-β3 and β10-β12 to those of SSL7 lost binding ability to hIgG, whereas SSL7x10P the mutant of SSL7 included β1-β3 and β10-β12 of SSL10 acquired binding ability to IgG (Fig. 2G). The interaction of SSL7, SSL10 and their chimeras with human IgG was confirmed by far western blot analysis. Biotinylated hIgG bound to SSL10 and SSL7x10P immobilized on PVDF membrane but not SSL7 and SSL7x10Q (Fig. 3A). Collectively, β1-β3 and β10-β12 of SSL10 are the regions responsible for binding of SSL10 to hIgG.

DISCUSSION

In this study, we identified the two regions of SSL10 responsible for binding to hIgG by analyzing the binding ability of a series of chimera between SSL10 and its paralog SSL7 that reported to bind to IgA and C5 but not hIgG. SSL7x10, the chimera that covered C-terminal half of SSL10 and SSL10x7 that covered N-terminal half of SSL10 bound

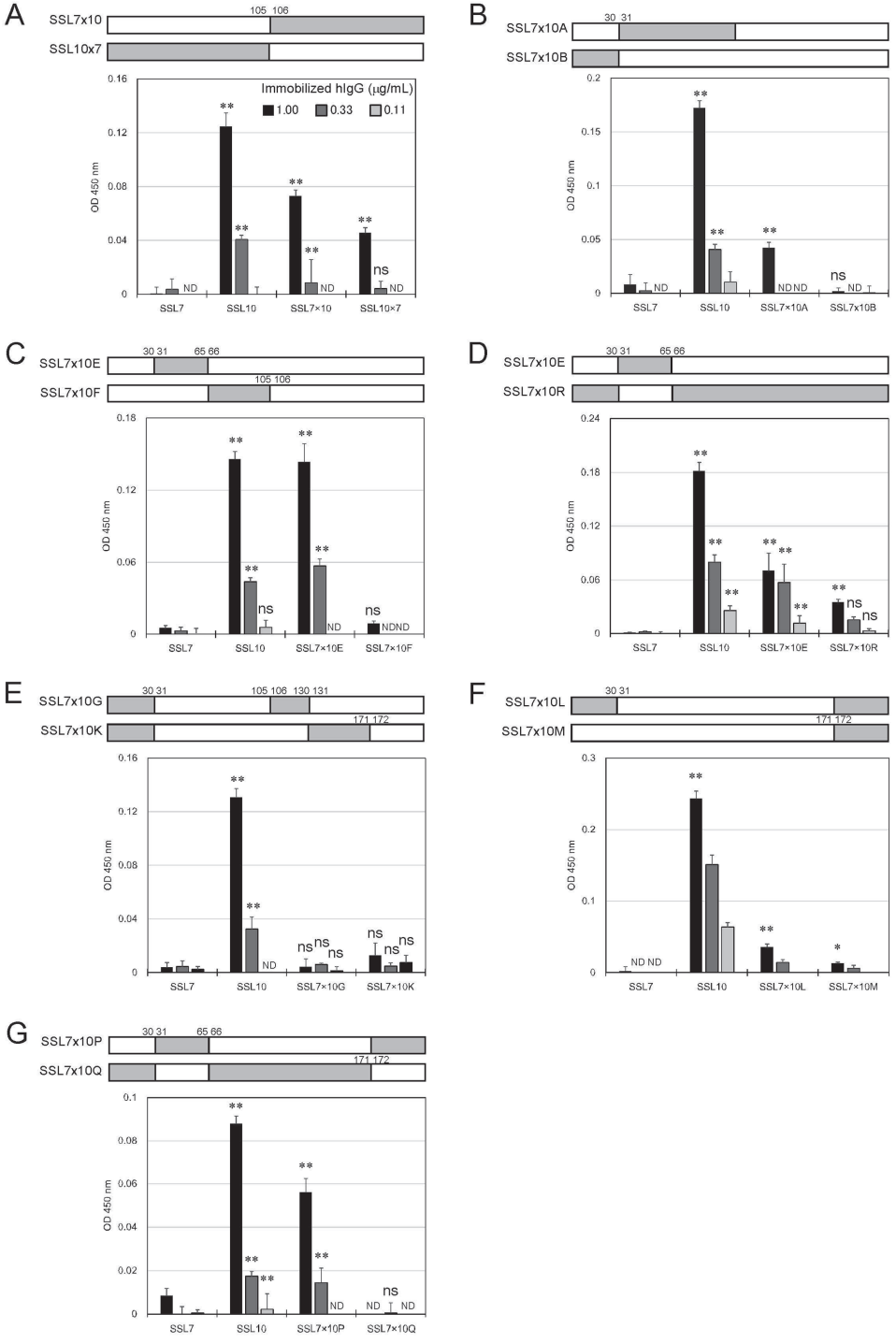

Fig. 2. Binding of SSL7, SSL10 and their chimeras to immobilized hIgG

Chimeras between SSL7 and SSL10 to immobilized hIgG was determined using solid phase binding assay as described in materials and methods. (A) The binding of SSL7x10 and SSL10x7 to immobilized hIgG, (B) SSL7x10A and SSL7x10B, (C) 7x10E and SSL7x10F, (D) 7x10E and SSL7x10R, (E) SSL7x10G and SSL7x10K, (F) SSL7x10L and SSL7x10M, and (G) SSL7x10P and SSL7x10Q. The binding of SSL10 and SSL7 to hIgG were shown as positive and negative control experiments in all experiments. Schematic representations of domain swap mutants were shown above each graph. Gray box; Region derived from SSL10. White box; Region from SSL7. The numbers indicate the amino positions of SSL10 where swapped. The date shown are representative of three independent experiments, and the values are expressed as mean ± SD of quadruplicate wells. ND=not detected. *P< 0.05, **P<0.01 and n.s.=not significant comparing with the binding of SSL7 to immobilized hIgG.

to hIgG suggest that SSL10 possesses at least two responsible region for binding to hIgG; one of them would be in N-terminal half of OB-fold and the other would be in C-terminal half of β-grasp. The chimeras that contained either β1-β3 of SSL10 (SSL10x7, SSL7x10A, SSL7x10E and SSL7x10P) or β10-β12 of it (SSL7x10, SSL7x10R, SSL7x10L, SSL7x10M and SSL7x10P) kept binding ability to hIgG (Fig. 2 A-D, 2F and 2G). On the other hand the mutant that substituted both β1-β3 and β10-β12 of SSL10 to those of SSL7 (SSL7x10F, SSL7x10G, SSL7x10K, SSL7x10Q) lost binding ability to hIgG (Fig. 2B, 2C, 2E and 2G). Comparable results were obtained in far western blot analysis (Fig. 3A). Together with,

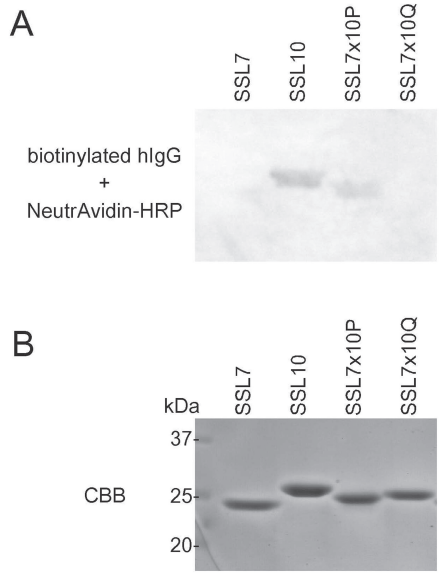

Fig. 3. Detection of the interaction between SSLs and hIgG by far western analysis

(A) SSL7, SSL10, SSL7x10P and SSL7x10Q were separated on SDS-polyacrylamide gel and transferred onto a PVDF membrane. The membrane was treated with biotinylated hIgG, then treated with NeutrAvidin-HRP followed by chemiluminescence detection. (B) The same set of samples were electrophoresed on a separate polyacrylamide gel and visualized with Coomassie Brilliant Blue.

the regions of SSL10 responsible for binding to hIgG are β1-β3 (K31-K65) and β10-β12 (G172-K205), respectively.

Interestingly the regions of SSL10 for IgG-binding revealed in this study are identical to those for prothrombin binding we identified previously.[8] It is consistent with that prothrombin bound to SSL10 with higher affinity than hIgG and the co-precipitation of IgG with SSL10-conjugate Sepharose was diminished in the presence of prothrombin.[7] Several SSLs are identified their responsible regions by resolving crystal structure and analyzing target binding ability of mutants. Excepting with the report that SSL5 interacts with prokaryotically produced MMP-9 via β1-β3 and α4-β9[9] and SSL7 recognizes C5 via β7 and loop between β6 and β7,[11] the responsible regions of SSLs have been revealed are those of β1-3 and β10-12 or adjacent regions; SSL3 binds to TLR2 via β1-β2 and β3-β4 of it;[12] SSL7 binds to IgA via β1-β2;[13] SSLs that binds to sialyllactosamine (SSL2, 3, 4, 5, 6 and 11) are recognize the oligosaccharide via β10-β11.[14,15] These regions would be an important part of SSL family for recognizing their host target molecules.

S. aureus produces the other two IgG binding proteins, SpA and Sbi. They share similar IgG binding domain,[16] whereas SSL10 shows no homology with them. SSL10 were secreted as soluble exotoxin but SpA and Sbi possess regions for associating to the surface of the cocci.[16] SSL10 specifically recognizes primate IgG but not non-primate IgG.[2,3] On the other hand SpA and Sbi bind to more broad species of IgG.[17] In other words, *S. aureus* produces three different type of IgG inhibitors those show different localizations and inhibitory mechanism.

The identification of two responsible regions of SSL10 for binding to IgG would be contribute to understanding of molecular basis of the interaction between SSL10 and IgG, and also the development of new drug and vaccines.

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Author Contributions Author contributions: S. I. conceived and supervised the study, S. I., S. H., T. T. and K. O. designed experiments, T. N. and S. I. performed experiments, S. I. analyzed data and wrote the manuscript, and S. H., T. T. and K. O. made manuscript revisions.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Atkins KL, Burman JD, Chamberlain ES, Cooper JE, Poutrel B, Bagby S, Jenkins AT, Feil EJ, van den Elsen JM. S. aureus IgG-binding proteins SpA and Sbi: host specificity and mechanisms of immune complex formation. *Mol. Immunol.*, **45**, 1600–1611 (2008).
2) Patel D, Wines BD, Langley RJ, Fraser JD. Specificity of staphylococcal superantigen-like protein 10 toward the human IgG1 Fc domain. *J. Immunol.*, **184**, 6283–6292 (2010).
3) Itoh S, Hamada E, Kamoshida G, Yokoyama R, Takii T, Onozaki K, Tsuji T. Staphylococcal superantigen-like protein 10 (SSL10) binds to human immunoglobulin G (IgG) and inhibits complement activation via the classical pathway. *Mol. Immunol.*, **47**, 932–938 (2010).
4) Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. *Nat. Rev. Microbiol.*, **13**, 529–543 (2015).
5) Walenkamp AM, Boer IG, Bestebroer J, Rozeveld D, Timmer-Bosscha H, Hemrika W, van Strijp JA, de Haas CJ. Staphylococcal superantigen-like 10 inhibits CXCL12-induced human tumor cell migration. *Neoplasia*, **11**, 333–344 (2009).
6) Itoh S, Yokoyama R, Murase C, Takii T, Tsuji T, Onozaki K. Staphylococcal superantigen-like protein 10 (SSL10) binds to phosphatidylserine and apoptotic cells. *Microbiol. Immunol.,* **56**, 363–371. (2012).
7) Itoh S, Yokoyama R, Kamoshida G, Fujiwara T, Okada H, Takii T, Tsuji T, Fujii S, Hashizume H, Onozaki K. Staphylococcal superantigen-like protein 10 (SSL10) inhibits blood coagulation by binding to prothrombin and factor Xa via their gamma-carboxyglutamic acid (Gla) domain. *J. Biol. Chem.*, **288**, 21569–21580 (2013).
8) Itoh S, Takii T, Onozaki K, Tsuji T, Hida S. Identification of the blood coagulation factor interacting sequences in staphylococcal superantigen-like protein 10. *Biochem. Biophys. Res. Commun.*, **485**, 201–208 (2017).
9) Kohno K, Itoh S, Hanai A, Takii T, Fujiwara T, Onozaki K, Tsuji T, Hida S. Identification of matrix metalloproteinase 9-interacting sequences in staphylococcal superantigen-like protein 5. *Biochem. Biophys. Res. Commun.*, **497**, 713–718 (2018).
10) Arcus VL, Langley R, Proft T, Fraser JD, Baker EN. The Three-dimensional structure of a superantigen-like protein, SET3, from a pathogenicity island of the Staphylococcus aureus genome. *J. Biol. Chem.*, **277**, 32274–32281 (2002).
11) Laursen NS, Gordon N, Hermans S, Lorenz N, Jackson N, Wines B, Spillner E, Christensen JB, Jensen M, Fredslund F, Bjerre M, Sottrup-Jensen L, Fraser JD, Andersen GR. Structural basis for inhibition of complement C5 by the SSL7 protein from Staphylococcus aureus. *Proc. Natl. Acad. Sci. USA*, **107**, 3681–3686 (2011).
12) Koymans KJ, Feitsma LJ, Brondijk TH, Aerts PC, Lukkien E, Lossl P, van Kessel KP, de Haas CJ, van Strijp JA, Huizinga EG. Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3). *Proc. Natl. Acad. Sci. USA*, **112**, 11018–11023 (2015).

13) Ramsland PA, Willoughby N, Trist HM, Farrugia W, Hogarth PM, Fraser JD, Wines BD. Structural basis for evasion of IgA immunity by Staphylococcus aureus revealed in the complex of SSL7 with Fc of human IgA1. *Proc. Natl. Acad. Sci. USA*, **104**, 15051–15056 (2007).
14) Baker HM, Basu I, Chung MC, Caradoc-Davies T, Fraser JD, Baker EN. Crystal structures of the staphylococcal toxin SSL5 in complex with sialyl Lewis X reveal a conserved binding site that shares common features with viral and bacterial sialic acid binding proteins. *J. Mol. Biol.*, **374**, 1298–1308 (2007).
15) Chung MC, Wines BD, Baker H, Langley RJ, Baker EN, Fraser JD. The crystal structure of staphylococcal superantigen-like protein 11 in complex with sialyl Lewis X reveals the mechanism for cell binding and immune inhibition. *Mol. Microbiol.*, **66**, 1342–1355 (2007).
16) Smith EJ, Visai L, Kerrigan SW, Speziale P, Foster TJ. The Sbi protein is a multifunctional immune evasion factor of Staphylococcus aureus. *Infect. Immun.*, **79**, 3801–3809 (2011).
17) Zhang L, Jacobsson K, Vasi J, Lindberg M, Frykberg L. A second IgG-binding protein in Staphylococcus aureus. *Microbiology*, **144**, 985–991 (1998).