Journal of Dermatological Science (2006) **42**, 203—214

Journal of Dermatological SCIENCE

www.intl.elsevierhealth.com/journals/jods

REVIEW ARTICLE

Staphylococcal cutaneous infections: Invasion, evasion and aggression

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Received 13 March 2006; accepted 29 March 2006

KEYWORDS
Staphylococcus;
Toxin;
Quorum-sensing;
ET;
TSST-1;
NTED;
Virulence

Summary Staphylococcal infections cause a variety of cutaneous and systemic infections, including impetigo, furuncle, subcutaneous abscess, staphylococcal scalded skin syndrome (SSSS), toxic shock syndrome (TSS) and neonatal toxic shock syndrome-like exanthematous disease (NTED), in association with microbial virulence factors. The virulence factors produced by *Staphylococcus aureus* have a wide array of biological properties, including disruption of the epithelial barrier, inhibition of opsonization by antibody and complement, interference with neutrophil chemotaxis, cytolysis of neutrophils, and inactivation of antimicrobial peptides. Exfoliative toxins (ETs) induce the 'acantholytic' infection of *S. aureus* due to the disruption of cell-to-cell cohesion, which allows the pathogenic organisms to spread within the epithelium. Furthermore, *S. aureus* expresses exotoxins with biological properties of superantigens that induce T-cell activation with subsequent anergy and immunosuppression. Of the *S. aureus* leukotoxins, Panton-Valentine leukocidin (PVL) is involved in the development of multiple furuncles with more intense erythema, particularly in healthy young adults. TSS is an acute life-threatening illness caused by TSS toxin-1 (TSST-1) and is usually classified into two categories; menstrual TSS, originally described in association with tampon use, and nonmenstrual TSS with a variety of clinical settings. NTED is a neonatal disease induced by TSST-1 although clinical symptoms are much milder than those of TSS. In TSS and NTED, the expansion of TSST-1-reactive Vβ2-positive T cells is observed. The production of pathogenic *S. aureus* exotoxins and biofilm formation is regulated by the accessory gene regulator (agr) locus in the quorum-sensing signaling pathway. There is no doubt that targeting the quorum-sensing signaling pathway or anti-toxin therapy is a promising therapeutic approach supportive of primary antibiotic therapy.

Abbreviations: agr, accessory gene regulator; CHIP, chemotaxis inhibitory protein of staphylococci; ClfA, clumping factor A; Dsg, desmoglein; Eap, extracellular adherens protein; ET, exfoliative toxin; Map, major histocompatibilty class II analogue protein; NTED, neonatal toxic shock syndrome-like exanthematous disease; PAF-R, platelet-activating factor receptor; PVL, Panton-Valentine leukocidin; Sak, staphylokinase; SE, staphylococcal enterotoxin; SIRS, systemic inflammatory response syndrome; SSSS, staphylococcal scalded skin syndrome; TA, teichoic acid; Treg, regulatory T-cell; TSS, toxic shock syndrome; TSST-1, toxic shock syndrome toxin-1

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0923-1811/$30.00
doi:10.1016/j.jdermsci.2006.03.011

Contents

1. Introduction

Staphylococci are Gram-positive cocci colonizing the epithelial surfaces in the majority of humans. *Staphylococcus epidermidis*, a nonpathogenic member of normal cutaneous microbial flora, expresses few virulence factors under normal conditions, although the organism has the ability to survive in the host by forming biofilms on the surface of horny layers and indwelling medical devices. *Staphylococcus aureus* is usually regarded as a transient, pathogenic organism in the skin, and approximately 20% of the general population always harbors it on the nasal mucosa without any pathogenic event [1]. In certain situations, however, *S. aureus* can express a wide array of potential virulence factors, including wall teichoic acid (TA) and surface proteins that promote adherence to damaged tissue [2,3], and can diminish neutrophil functions and antibody- and cell-mediated immune responses [3,4]. Furthermore, the organism secretes exotoxins and enzymes that can cause a variety of cutaneous and systemic infections, including impetigo, furuncles, subcutaneous abscesses, staphylococcal scalded skin syndrome (SSSS), toxic shock syndrome (TSS) and neonatal toxic shock syndrome-like exanthematous disease (NTED) [5—7].

Recent studies have demonstrated that TA and surface proteins of *S. aureus* are responsible for colonization on the nasal mucosa [2,3]. In the first step of invasion, *S. aureus* must adhere to the skin or mucosal surface, and disrupt epithelial barriers comprising cell-adhesion structures such as desmosomes and adherence junctions. Many bacteria possess specific enzymes that cleave the cell—cell adhesion structures. Previous investigators have reported that the *Bacteroides fragilis* toxin is a metalloprotease that cleaves E-cadherin of adherens junctions [8], and *H. pylori* CagA protein can disrupt epithelial tight-junction scaffolding protein ZO-1, the transmembrane adhesion molecule [9] and E-cadherin/catenin-containing adherens junctions [10]. Recently, Amagai et al. have reported for the first time that staphylococcal exfoliative toxins (ETs) disrupt desmoglein 1, a desmosomal cadherin strongly expressed in the upper epidermis [11]. The resultant histologic features are exemplified in an ET-mediated acantholysis in SSSS and bullous impetigo.

Once the organisms invade human epithelium, they use a variety of strategies to allow them to survive, proliferate, spread and persist in the host. Many observations have disclosed that *S. aureus* has strategies to avoid opsonization by complement [12], neutrophil chemotaxis [13] and phagocytosis [14], and to inhibit humoral [15] and cell-mediated immune responses [16]. Even though *S. aureus* may be engulfed by neutrophils, some of the organisms are resistant to being killed by cationic, antimicrobial peptides (α-defensin) by means of neutralizing the anion charge of the bacterial cell surfaces [17].

The types of *S. aureus* exotoxins secreted, together with the toxin-induced host immune

responses, may induce characteristic clinical manifestations and influence the severity of systemic symptoms. For instance, the TSST-1 and ETs may cause TSS and SSSS in certain patients, respectively. Panton-Valentine leukocidin (PVL) has been associated with severe, deep-seated skin infections, furunculosis and necrotizing pneumonia in previously healthy children and youths [18,19]. Therefore, it is essential to investigate bacterial genes encoding these toxins, and to study the mechanism of toxin production. Recently, quorum-sensing, a signaling pathway of exotoxin production via the accessory gene regulator (agr), has been assigned a central role in the virulence of *S. aureus* infections.

To provide a better understanding of the pathogenesis of staphylococcal cutaneous infections, this review highlights recent advances in the intriguing relationship between bacterial virulence factors and the host immune responses against them.

2. Mechanisms of *S. aureus* cell invasion

2.1. Disruption of the epithelial barrier

Almost all *S. aureus* strains have the ability to secrete a group of enzymes and exotoxins, including four hemolysins (α-, β-, δ- and γ-toxins), nucleases, proteases, lipases, hyaluronidase, and collagenase (Table 1). In addition to these microbial products, some strains produce one or more exotoxins targeting the specific molecules that constitute the cell adhesion and host immune system. In the stage of colonization, *S. aureus* adheres to epithelial cell surfaces by means of wall TA and surface proteins, and secretes α-hemolysin (α-toxin), which is capable of forming pores on the cell membrane. The *S. aureus* α-toxin may induce a wide array of cellular events in not only the infected epithelial cells but also noninfected, neighboring ones by diffusion of the toxins. The subsequent cellular events induced

Table 1 Major virulence proteins of *S. aureus* and agr regulation

Virulence factors	Human diseases	agr Regulation
Superantigens		
Enterotoxin A	Food poisoning, TSS	−
Enterotoxin B	Food poisoning, TSS	+
Enterotoxin C	Food poisoning, TSS	+
Enterotoxin D	Food poisoning, TSS	+
Enterotoxin E	Food poisoning, TSS	−
TSST-1	TSS, NTED	+
ETA*	Bullous impetigo > SSSS	+
ETB*	SSSS > bullous impetigo	+
ETD*	Deep-seated infections?	?
Cytotoxins		
α-Hemolysin	Hemolysis, necrosis	+
β-Hemolysin	Hemolysis, necrosis	+
δ-Hemolysin	?	+
γ-Hemolysin	Hemolysis, necrosis	+
PVL	Leukolysis, necrosis Deep-seated infections	+
Enzymes		
Proteases, Sak	Spread, nutrition, anti-opsonin	+
Nucleases	Spread, nutrition	−
Lipases	Spread, nutrition	+
Hyaluronidase	Spread, nutrition	−
Esterases	Inactivation of toxic fatty acids	+
Surface proteins		
Protein A	Anti-opsonin	+
Coagulase	?	+
Clumping factors	?	−
Fibronectin binding proteins	Adhesion	+

+: Synthesis changes in response to activation of agr signaling, with decreased production of surface factors and increased production of the other listed virulence factors; −: no agr effect (Ref. [42] and Table 2 in modification).

* Not yet confirmed as superantigens.

Table 2 Disruption of the epithelial cell adhesion by microbial toxins

Structures	Target molecules	Microbes	Toxins
Tight junction	Occludin	*V. cholerae*	Hemagglutinin/protease (HA/P)
	ZO-1	*H. pylori*	CagA
Adherens junction	E-cadherin	*H. pylori*	CagA
		B. fragilis	Fragilysin
		L. monocytogenes	InlA
Desmosome	Desmoglein 1	*S. aureus*	ETA, B, D

by *S. aureus* colonization include activation of phospholipase A2, phosphatidylinositol hydrolysis, production of nitric oxide, prostaglandin (PG) E2, PGI2 and thromboxane A2, activation of NF-kB, and upregulation of various inflammatory cytokines [20,21]. It has been postulated that bacterial toxins stimulates Rho family GTPases that activate the downstream signaling pathways affecting the expression of inflammatory mediators and defensins, and cytoskeleton reconstruction [22]. The activation of these signaling cascades may induce the initial step of systemic inflammatory response syndrome (SIRS) and sepsis [23].

A histologic hallmark of SSSS is acantholysis, a loss of cell-to-cell cohesion in the subcorneal layers, the features of which are identical to those of pemphigus foliaceus [5]. Amagai et al. [11] have recently demonstrated that ETs cleave human and mouse desmoglein (Dsg)-1 between the third and fourth extracellular domains after glutamic acid residue 381 as a serine protease, which results in dissociation of homophilic binding of Dsg-1 molecules, and induces acantholysis in the subcorneal layers. Therefore, the 'acantholytic' infection of *S. aureus* seems to be the result of the tissue destruction that allows the pathogenic organism to invade the epithelium (Table 2). Similarly, *H. pylori* infection of the gastric epithelium induces disruption of E-cadherin/catenin-containing adherens junctions, which is dependent on CagA/Crk signaling, a central cascade in inducing the pleiotropic cell responses to *H. pylori* infection that cause several gastric diseases, including gastric cancer [10].

2.2. 'Acantholytic' staphylococcal infections: SSSS and bullous impetigo

Generalized SSSS affects newborns (Ritter's disease), infants and children, but rarely adults. SSSS is usually diagnosed by its characteristic cutaneous manifestations, because fluid from intact bullae is generally sterile, and the infecting strain is usually recovered from distant sites such as the throat or nose. In addition to the skin lesions, the histopathologic finding of subcorneal acantholytic cleavage with minimal inflammation is diagnostic for SSSS, excluding a wide range of differential diagnoses. Generalized SSSS is thought to arise from systemic ET absorption from these sites, followed by bloodstream diffusion to the cutaneous target [5,24]. According to a recent review [25], the higher incidence of generalized SSSS in children may be due to less efficient renal clearance of the toxin and to immunological immaturity (low anti-ET antibody titers). In bullous impetigo, a localized form of SSSS, the infecting strain is generally recovered from bullae, and the ETs act at the localized areas without spreading through the bloodstream.

There are three serological forms of staphylococcal ETs (ETA, ETB and ETD), all of which cleave human Dsg 1 (Table 2). Only ETA and ETB have been firmly linked to human SSSS although ETB is more frequently isolated than ETA in children with generalized SSSS [25] (Fig. 1). The *etd* gene-positive strain might be related to the development of deep-seated infections such as furuncles, cutaneous abscesses and finger pulp infection, in association with PVL production [26]. One of us (OY) has investigated a possible correlation between the clinical manifestations of SSSS and the serotype of ETs by PCR amplification of the *eta* and *etb* genes in *S. aureus* strains isolated from 103 patients with generalized SSSS and 95 patients with bullous impetigo [27]. The *eta* and *etb* genes were detected in 57 (60%) and 5 (5%) of the bullous impetigo cases, respectively. Thus, ETA is closely associated with bullous impetigo. By contrast, the *eta* and *etb* genes were detected in 31 (30%) and 20 (19%) of strains isolated from patients with generalized SSSS, respectively. In some patients with pemphigus foliaceus, the dessociation of Dsg1 may be induced by a synergistic action of anti-Dsg 1 antibodies and the colonizing ET-producing *S. aureus*, which results in the wide-spread acantholytic lesions of the erythrodermic variant [28] (Fig. 2). For better understanding of the pathogenic significance of these ETs, we should consider various interacting factors, including the protein levels of ETs in the lesions or blood, clearance of ETs, natural inhibitors against ETs and the presence of neutralizing antibodies.

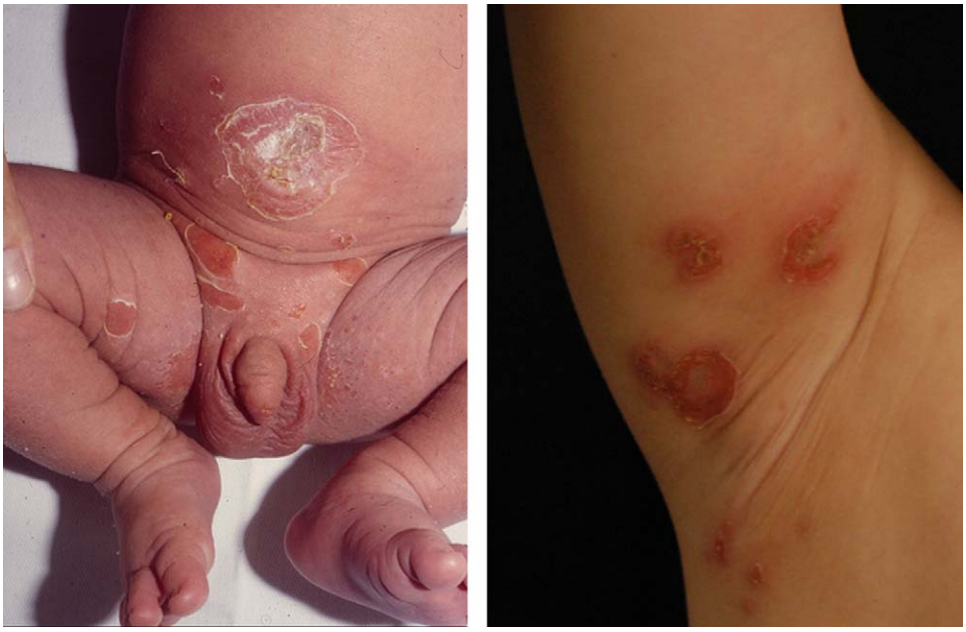

Fig. 1 Clinical manifestations of generalized SSSS (a) and bullous impetigo (b).

3. Evasion from host immunity

3.1. Interference with opsonization and chemotaxis

Complement is a family of proteins capable of releasing cytolytic peptides, recruiting effector cells against exogenous pathogens by chemotactic molecules such as C3a and C5a, and promoting phagocytosis (opsonization) by neutrophils (Fig. 3). Proliferating bacteria also produce chemoattractant molecules such as formylated peptides. *S. aureus* strains secrete the chemotaxis inhibitory protein of staphylococci (CHIP) that binds

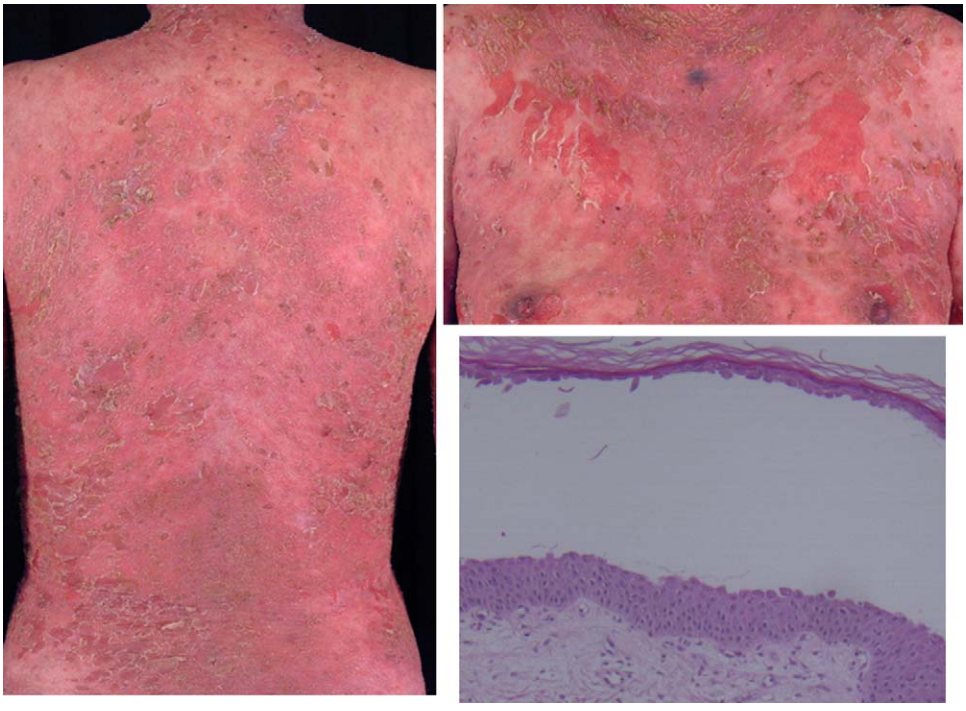

Fig. 2 Pemphigus foliaceus infected with ET-producing *S. aureus*. Erythrodermic and eroded lesions (a and b) and a histologic finding of subcorneal acantholysis (c).

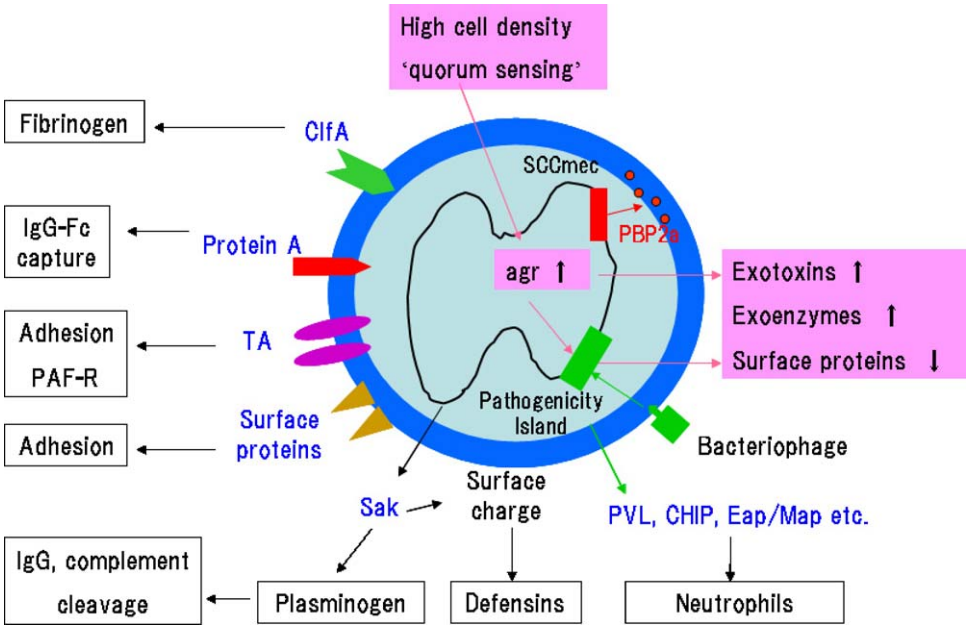

Fig. 3 Biological properties of staphylococcal surface proteins, proteases and exotoxins.

to the receptors for C5a and formyl peptides [12,29]. Extracellular adherence protein (Eap)/major histocompatibility class II analogue protein (Map) is another inhibitory molecule for neutrophil chemotaxis, blocking ICAM-1 molecules expressed by endothelial cells, thereby interfering the interaction with LFA-1 on neutrophil surfaces [13].

Although circulating antibodies to bacterial cell surface antigens including TA and peptidoglycan are present in most healthy individuals and patients with *S. aureus* infections, the existence of such anti-bacterial antibodies is insufficient for protection from infection because of a wide array of evasion mechanisms. For instance, protein A on the microbial surfaces inhibits antibody-mediated phagocytosis by blocking the Fc portion of IgG [15,30] (Fig. 3). Because protein A has the ability to bind to the V_H3 region of IgM molecules, B lymphocytes bearing IgM are led to activation-mediated apoptosis [31]. Furthermore, the extracellular staphylokinase (Sak) on the bacterial cell surface activates plasminogen, which in turn cleaves the cell-bound IgG and C3b [32,33].

3.2. Evasion from phagocytosis

S. aureus has the ability to secrete some leukotoxins that play an essential role in damaging neutrophils infiltrating at the infection sites (Table 1). There are six possible forms of γ-hemolysin/PVL, owing to the presence of three potential S and two potential F units, all of which may combine (S + F) to form a toxin molecule. Each of these subunits lacks hemolytic and leukotoxic activity when assayed alone [34]. When added as pairs to erythrocytes, however, cytolytic activity is observed to various degrees. Of these pairs of leukotoxins, PVL (LukF-PV/LukS-PV) exhibits a highly specific cytolytic activity against neutrophils, monocytes and macrophages of humans and rabbits (Fig. 4).

In the stationary phase of growth, *S. aureus* expresses abundant clumping factor A (ClfA) to produce cell-clumping and to coat the organism with fibrinogen [35], which might protect *S. aureus* from phagocytosis by biofilm formation (Table 1 and Fig. 3). Capsular polysaccharide of *S. aureus* inhibits the attachment of neutrophils to the cell

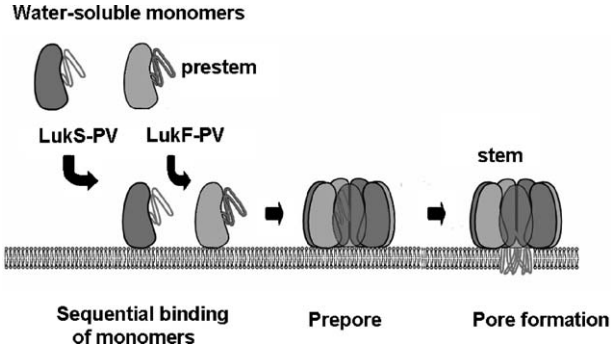

Fig. 4 Schematic model of PVL assembly for hetero-oligomeric pore formation. Water-soluble LukS-PV and LukF-PV monomers bind to putative binding sites on the membrane in this order. Sequentially, the membrane-bound monomers assemble into hetero-heptameric pre-pore. Then prestem forms beta-barrel trans membrane pore (stem) (Ref. [34]).

surface-bound complement and antibodies [36]. Neutrophils usually engulf bacterial cells and kill them by lysosomal enzymes, oxygen radicals, and cationic, antimicrobial peptide, α-defensins (human neutrophil peptide) that belong to the innate immune system. *S. aureus*, however, gains resistance to the cationic peptides by neutralizing the negative charge of the bacterial cell walls [17].

A recent mouse model of cutaneous infection by injected *S. aureus* has shown that both neutrophils and T cells with a CD4+ or CD8+ phenotype infiltrate the cutaneous lesions [37]. It has been shown that CD4 knockout mice have poor bacterial clearance compared with CD4 wild-type mice, indicating the important roles of CD4+ T cells in host defense against bacterial invasion [38]. Since many granulysin-bearing CD4+ T-cells are present in folliculitis [39], T-cell-derived antimicrobial proteins are also involved in the defense mechanism. Because *S. aureus* is able to enter and survive in phagocytic cells as well as nonphagocytic cells, including endothelial cells, epithelial cells and cultured keratinocytes, *S. aureus* surviving intracellularly may be destroyed by CD4+ T cells expressing granulysin, as observed in folliculitis [39].

3.3. Biofilm formation

Bacterial glycocalyx is a polysaccharide-containing material produced by bacteria. Bacteria that adhere to implanted medical devices or damaged tissue can become the cause of persistent infections. These bacteria encase themselves in a hydrated matrix of polysaccharide and protein, forming a slimy layer known as a biofilm. Since *S. aureus* can generally produce a biofilm on damaged skin tissue, antimicrobial agents may not eradicate *S. aureus* without the help of neutrophils. The *S. aureus* glycocalyx may play a crucial role in colonization and adherence to damaged skin tissue [40].

3.4. Induction of immunomodulation

Staphylococcal TA manifests immunomodulatory effects via the platelet-activating factor receptor (PAF-R), which leads to the production of Th2 cytokine, IL-10 [41]. *S. aureus* may have different superantigen genes located on mobile genetic elements, such as pathogenicity islands, plasmids, and phages [42] [Table 1]. Superantigens mediate direct cross-linking of major histocompatibility complex class II (MHCII) molecules on antigen-presenting cells with T-cell receptors. Superantigens induce a strong proliferative response followed by clonal deletion of a substantial portion of defined Vβ T-cells [43]. Administration of superantigens to adult mice induces rapid production of interleukin-2 (IL-2) and tumor necrosis factor (TNF), and the subsequent expansion of a reactive T-cell population [44]. It has been postulated that superantigens and lipopolysaccharide (LPS) may induce severe systemic symptoms such as SIRS and TSS in a synergistic fashion, in which LPS first stimulates monocytes, after which superantigens activate a group of T-cells. After the initial phase of superantigen-induced activation in vivo, some populations of the reactive T-cells are deleted, and the remaining cells display in vitro anergy [43,45]. Both CD4+ and CD8+ T-cells exhibit a reduced capacity to proliferate in response to the superantigens after repeated stimulation [45,46].

Staphylococcus colonization is one of the aggravating factors in patients with atopic dermatitis. The Th2 inflammatory response [41] and persistent IL-18 secretion form keratinocytes [47] is induced by wall TA and protein A, respectively. Staphylococcus enterotoxins A (SEA) and B (SEB) stimulate expression of ICAM-1 and HLA-DR in normal human keratinocytes, and more than half of patients with atopic dermatitis have specific IgE antibodies to SEA and/or SEB in their serum [48]. Epicutaneous sensitization with SEB elicites a local, cutaneous inflammatory response characterized by dermal infiltration with eosinophils and mononuclear cells and an increased mRNA expression of the Th2 cytokine IL-4 but not of the Th1 cytokine IFN-γ. Epicutaneous exposure to superantigens skews the immune response towardsTh2 cells, leading to allergic skin inflammation and increased IgE synthesis that are characteristic of atopic dermatitis [49]. Patients with atopic dermatitis have significantly increased numbers of regulatory T (Treg) cells with normal immunosuppressive activity. However, after superantigen stimulation, Treg cells lose their immunosuppressive activity [50]. These data suggest a novel mechanism by which superantigens could enhance T-cell activation in patients with atopic dermatitis.

4. Mechanisms of exotoxin production and virulence

4.1. Exotoxins secreted by *S. aureus*

Although α-, β- and δ-hemolysins are important in several disease states caused by *S. aureus* infections, the pathogenic significance of such toxins in human disease has not been fully established (Table 1). Two types of bicomponent toxins generated by *S. aureus* include γ-hemolysin and PVL. Each of these toxins is made as two nonassociated secreted proteins, referred to as S and F components [34]. γ-hemolysin is made by virtually every

strain of *S. aureus*, while PVL is made by 2—3% of strains. PVL lyses neutrophils and macrophages of human and rabbit rather selectvely, whereas γ-hemolysin is able to lyse many varieties of mammalian erythrocytes as well as phagocytes.

Many genes of staphylococcal virulence factors are encoded by mobile genetic elements such as bacteriophages, plasmids and so-called "pathogenicity island" on the bacterial chromosome (Fig. 3). The pathogenicity island is believed to be formed by integration of extrachromosomal DNAs (plasmids) or by incorporation of bacteriophages carrying toxin genes by lysogenization. Incorporation of the pathogenicity island may transform a non-virulent strain into a pathogenic one. At least, three types of pathogenicity islands have been reported in staphylococcal species [51]. Many of the open reading frames encoding *S. aureus* exotoxins such as leukotoxins, enterotoxins and TSST-1 have been found in clusters on the pathogenicity islands [42,51].

4.2. Quorum-sensing signaling

Quorum-sensing is a unique regulation cascade of bacterial gene expression in response to increased cell density. When *S. aureus* cells increase in number, the cells express a signal molecule designated as 'agr' to sense the status of cell density ('quorum') [52]. The agr was first described as a pleiotropic regulator of staphylococcal exotoxins, proteases and surface proteins [53,54]. The agr system of *S. aureus* consists of 4 genes (agrA, agrC, agrD, and agrB) that are co-transcribed (RNAII), and the gene for the effector molecule of the agr system, RNAIII, which also encodes the gene for α-toxin (hld). *S. aureus* produces a series of exotoxins via agr quorum-sensing signaling, and simultaneously inhibits biofilm formation (Table 1 and Fig. 5). Thus, this pathway allows *S. aureus* cells to invade and spread from the colonization sites, where the cells are protected by biofilm, toward other tissues or blood against the host barrier and immune surveillance.

4.3. PVL-associated diseases

The PVL gene is detected in less than 5% of *S. aureus* strains of clinical origin, and is primarily associated with necrotic lesions of the skin and subcutaneous tissues such as furuncles, and also with community-acquired severe necrotizing pneumonia in previously healthy children and young adults [18,19] (Table 1 and Fig. 4). In our study [19], PVL genes were detected in 16 (40%) of the 40 *S. aureus* strains isolated from furuncles, 2 (28%) of the 7 strains isolated from carbuncles, 1 (14%) of the 7 strains isolated from abscesses, and 1 (5%) of the 20 strains isolated from folliculitis. Furuncles caused by PVL gene-positive *S. aureus* are usually multiple, and are usually associated with more intense erythema around the lesions. PVL gene-positive strains were isolated from young adults without underlying diseases, whereas PVL gene-negative strains were isolated from patients with various systemic complications, including diabetes, leukemia and autoimmune diseases. Melles et al. have reported that severe skin infections are associated with the cluster IVb strain of *S. aureus* because of lysogenization of a progenitor IVb strain with phages carrying PVL [55] (Fig. 3).

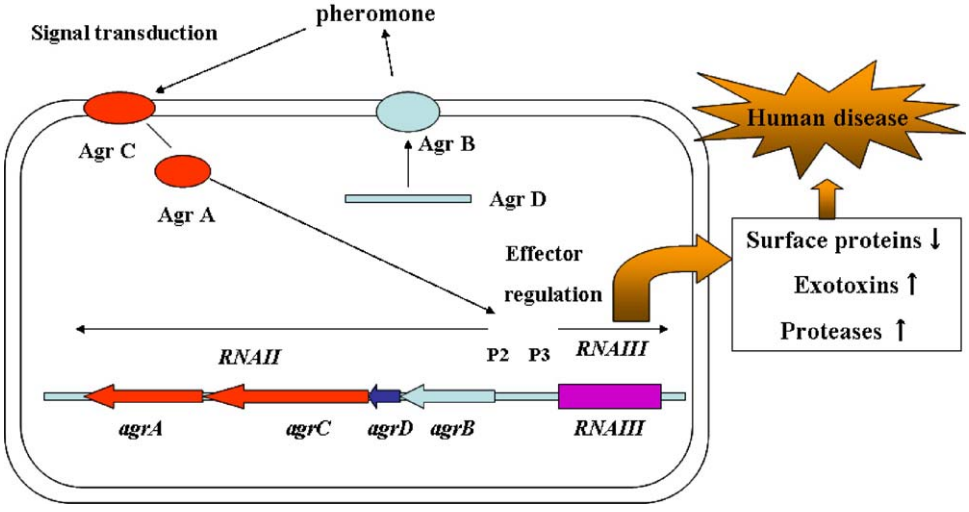

Fig. 5 A schematic model of the agr quorum-sensing system. The agr up-regulates the expression of exotoxins, and down-regulates the expression of surface proteins.

4.4. TSS

TSS is an acute life-threatening illness caused by TSST-1 produced by *S. aureus*. TSS is defined as an acute and potentially fatal illness characterized by high fever, a diffuse erythematous rash, desquamation of the skin 1—2 weeks after onset, hypotension and involvement of three or more organ systems. A massive expansion in the number of TSST-1-reactive Vβ2-positive T cells is observed in patients with TSS. Cytokines produced by T cells activated by TSST-1 have been implicated in the pathogenesis of this illness [10,41—43]. Exposure to TSST-1-producing *S. aureus* is commonplace in the general population, but TSS rarely occurs because anti-TSST-1 antibodies play a protective role against the development of TSS in adults [56].

TSS is usually classified into two categories: menstrual TSS, originally described in association with tampon use, and nonmenstrual TSS, which occurs in a variety of clinical settings and has a tendency to recur. Kain et al. [57] have observed that patients with nonmenstrual TSS may have higher frequencies of previous antibiotic treatment and hospital exposure than patients with menstrual TSS, indicating the pathogenic significance of a predisposition to colonization with TSST-1-producing *S. aureus*. In both conditions, colonization or infection with TSST-1-producing *S. aureus* induces a systemic inflammatory response if a sufficient amount of toxin-neutralizing antibody is lacking. In a previous study, more than 90% of patients with menstrual TSS had low or negative titers of TSST-1-specific antibodies in acute-phase serum samples [58]. TSS-like systemic symptoms indistinguishable from typical TSS might be caused by toxins other than TSST-1, especially SEB [56].

It has been reported that *S. aureus* requires certain conditions for the production of TSST-1, including animal protein, a low level of glucose, a temperature of 37—40 °C, a pH of 6.5—8, and oxygen. All of these requirements except oxygen are present in the human vagina during menstruation in the absence of tampons [59]. Therefore, the usage of tampons may introduce oxygen into the normally anaerobic vagina. Kass et al. [60] have proposed that some tampons bind sufficient magnesium, which alters the intravaginal growth kinetics of TSST-1-producing *S. aureus*. It has been postulated that proteolytic cleavage of menstrual blood may release sufficient oxygen to produce TSST-1 in patients with menstrual TSS without tampon use.

Recurrent menstrual TSS may occur, probably due to the persistent colonization of TSST-1-producing *S. aureus*, the continuation of tampon use, and, most importantly, the persistent immunologic susceptibility to TSST-1. It has been reported that less than half of patients with menstruation TSS develop seropositivity to TSST-1 within 2 months of their illness [58], and some individuals have been found to have a continuing predisposition, even after repeated episodes of TSS.

4.5. Neonatal toxic shock syndrome-like exanthematous disease (NTED)

NTED is a disease of neonates characterized by an exanthematous eruption caused by TSST-1 [60]. Although neonates exhibit a marked polyclonal expansion of Vβ2-positive T-cells in the acute phase of this illness, this neonatal disease does not match the clinical criteria for TSS because NTED regresses spontaneously without anti-staphylococcal treatment, and the prognosis is good. The disease, therefore, is designated NTED [61]. Similar to the development of TSS, the occurrence of NTED also depends on anti-TSST-1 IgG antibody titers derived from maternal antibodies [62].

5. Perspectives

5.1. Inhibition of colonization

Inhibition of bacterial adherence and colonization is the first line of prevention of bacterial infections. Previous studies have indicated that staphylococcal TA and surface proteins are responsible for the establishment of colonization on nasal mucosa. *S. aureus* colonizes persistently in approximately 20% of the population and transiently in 60%, and never colonizes in the remaining 20% [3]. It is obvious that the differences in the individual defense mechanisms on the nasal mucosa are a key to preventing colonization in the host.

Therapeutic procedures to selectively eradicate pathogenic *S. aureus*, with simultaneous protection of *S. epidermidis*, an example of normal flora on body surfaces, have been used clinically.

These procedures include the application of a low-pH cream and a gluco-oligosaccharide that inhibits the attachment of *S. aureus* cells on the epithelial surfaces [63].

5.2. Vaccination

A number of investigations are focusing on active or passive immunization against *S. aureus* infections, using a capsular polysaccharide vaccine [64] and humanized monoclonal antibodies [65]. The use of toxoid vaccines may overcome the mechanism of resistance in patients with recurrent TSS who fail to develop immunity to TSST-1.

5.3. Anti-exotoxin and biofilm therapy

Although antibiotic therapy is the first line treatment for *S. aureus* infections, there has been little consideration of anti-toxin therapy. Recent advances in understanding the molecular mechanisms underlying toxin-induced tissue injury and host immune reactions have enabled us to develop a new approach to anti-toxin treatments: direct neutralization of exotoxins and inhibition of the agr quorum-sensing pathway to produce exotoxins [52]. The disclosure of the agr-induced inhibition of biofilm formation (Fig. 5) might provide us a novel strategy against biofilm-associated infections.

Acknowledgement

We would like to thank Emeritus Professor, Jiro Arata (Okayama University, Okayama) and Dr. Hisanori Akiyama (Okayama City Hospital, Okayama) for their critical reading of our manuscript and suggestions.

References

[1] Peacock SJ, de Silva L, Lowy FD. What determines nasal carriage of *Staphylococcus aureus*? Trends Microbiol 2001; 9:605—10.
[2] Weidenmaier C, Kokai-Kun JF, Kristian SA, et al. Role of teichoic acid in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med 2004; 10:243—5.
[3] Foster TJ. Immune evasion by staphylococci. Nat Rev Microbiol 2005;3:943—58.
[4] Fedtke I, Gotz F, Peschel A. Bacterial evasion of innate host defenses: the *Staphylococcus aureus* lesson. Int J Med Microbiol 2004;294:189—94.
[5] Ladhani S, Joannou CL, Lochrie DP, Evans RW, Poston SM. Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev 1999;12:224—42.
[6] Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. Clin Microniol Rev 2000;13:16—34.
[7] Takahashi N. Neonatal toxic shock syndrome-like exanthematous disease (NTED). Pediatr Int 2003;45:233—7.
[8] Wu S, Lim KC, Huang J, Saidai RF, Sears CL. Bacteroides fragilis enterotoxin cleaves the zonula adherens protein E-cadherin. Proc Natl Acad Sci USA 1998;95:14979—84.
[9] Amieva MR, Vogelmann R, Covacci A, et al. Disruption of the epithelial apical—junctional complex by Helicobacter pylori CagA. Science 2003;300:1430—4.
[10] Suguki M, Mimuro H, Suzuki T, et al. Interaction of CagA with Crk plays an important role in Hlicobacter pylori-induced loss of gastric epithelial cell adhesion. J Exp Med 2005;202:1235—47.
[11] Amagai M, Matsuyoshi N, Wang ZH, Andl C, Stanley JR. Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. Nat Med 2000;6: 1275—7.
[12] de Haas CJ, Veldkamp KE, Peschel A, et al. Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial anti-inflammatory agent. J Exp Med 2004;199:687—95.
[13] Chavakis T, Hussain M, Kanse SM, et al. *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. Nat Med 2002;8:687—93.
[14] Gresham HD, Lowrance JH, Caver TE, et al. Survival of *Staphylococcus aureus* inside neutrophils contributes to infection. J Immunol 2000;164:3713—22.
[15] Foster TJ, McDevitt D. Surface-associated proteins of *Staphylococcus aureus*: their possible rules in virulence. FEMS Microbiol Lett 1994;118:199—205.
[16] Lee LY, Miyamaot YJ, McIntyre BW, et al. The *Staphylococcus aureus* Map protein is an immunomodulator that interferes with T cell-mediated responses. J Clin Invest 2002;110: 1461—71.
[17] Collins LV, Kristian SA, Weidenmaier C, et al. *Staphylococcus aureus* strains lacking D-alanine modifications of teichoic acid are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J Infect Dis 2002;186: 214—9.
[18] Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999;29:1128—32.
[19] Yamasaki O, Kaneko J, Morizane S, et al. The association of Staphylococcus aureus strains carrying Panton-Valentine leukocidin genes with the development of deep-seated follicular infections. Clin Infect Dis 2005;40:381—5.
[20] Dragneva Y, Anuradha CD, Valeva A, et al. Subcytocidal attack by staphylococcal alpha-toxin activates NF-kappaB and induces interleukin-8 production. Infect Immun 2001; 69:2630—5.
[21] Rose F, Dahlem G, Guthmann B, et al. Mediator generation and signaling events in alveolar epithelial cells attacked by *S. aureus* alpha-toxin. Am J Physiol Lung Cell Mol Physiol 2002;282:L207—14.
[22] Boquet P, Lemichez E. Bacterial virulence factors targeting Rho GTPases: parasitism or symbiosis? Trends Cell Biol 2003; 13:238—46.
[23] Backert S, Konig W. Interplay of bacterial toxins with host defence: molecular mechanisms of imunomoculatory signaling. Int Med Microbiol 2005;295:519—30.
[24] Lina G, Gillet Y, Vandenesch F, Jones M, Floret D, Etienne J. Toxin involvement in staphylococcal scalded skin syndrome. Clin Infect Dis 1997;25:1369—73.
[25] Ladhani S. Understanding the mechanism of action of the exfoliative toxins of *Staphylococcal aureus*. FEMS Immunol Med Microbiol 2003;39:181—9.
[26] Yamasaki O, Tristan A, Yamaguchi T, et al. Distribution of the exfoliative toxin D gene in clinical *Staphylococcus aureus* isolates in France. Clin Microbiol Infect, in press.
[27] Yamasaki O, Yamaguchi T, Sugai M, et al. Clinical manifestations of staphylococcal scalded skin syndrome depends on the serotypes of exfoliative toxins. J Clin Microbiol 2005;43: 1890—3.
[28] Akiyama H, Hamada T, Huh W-K, et al. Confocal laser scanning microscopic observation of glycocalyx production by *Staphylococcus aureus* in skin lesions of bullous impetigo, atopic dermatitis and pemphigus foliaceus. Br J Dermatol 2003;148:526—32.
[29] Haas PJ, de Haas CJ, Kleibeuker W, et al. N-terminal residues of the chemotaxis inhibitory protein of *Staphylococcus aureus* are essential for blockin formylated peptide receptor but not C5a receptor. J Immunol 2004;173: 5704—11.

[30] Uhlen M, Guss B, Nilsson B, et al. Complete sequence of the staphylococcal gene encoding protein A. A gene evolved through multiple duplications. J Biol Chem 1984;1695—702.
[31] Goodyear CS, Silverman GJ. Staphylococcal toxin induced preferential and prolonged in vivo deletion of innate-like B lymphocytes. Proc Natl Acad Sci USA 2004;101:11392—7.
[32] Rooijakkers SH, van Wamel WJ, Ruyken M, et al. Anti-opsonic properties of staphylokinase. Microbes Infect 2005;7: 476—84.
[33] Harpel P, Sullivan R, Chang TS. Binding and activation of plasminogen on immobilized immunoglobulin G: identification of the plasmin-derived Fab as the plasminogen-binding fragment. J Biol Chem 1989;264:616—24.
[34] Kaneko J, Kamio Y. Bacterial two-component and heterohepatamic pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. Biosci Biotechnol Biochem 2004;68:981—1003.
[35] Loughman A, Fitzgerald JR, Brennan MP, et al. Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. Mol Microbiol 2005;57:804—18.
[36] O'Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol Rev 2004;17:218—34.
[37] Molne L, Tarkowski A. An experimental model of cutaneous infection induced by superantigen-producing *Staphylococcus aureus*. J Invest Dermatol 2000;114:1120—5.
[38] Mohammed KA, Nasreen N, Ward MJ, et al. Induction of acute pleural inflammation by *Staphylococcus aureus*. I. CD4+ T cells play a critical role in experimental empyema. J Infect Dis 2000;181:1693—9.
[39] Oono T, Morizane S, Yamasaki O, et al. Involvement of granulysin-producing T cells in the development of superficial microbial folliculitis. Br J Dermatol 2004;150:904—9.
[40] Akiyama H, Huh W-K, Yamasaki O, Oono T, Iwatsuki K. Confocal laser scanning microscopic observation of glycocalyx production by *Staphylococcus aureus* in mouse skin: does *S. aureus* generally produce a biofilm on damaged skin? Br J Dermatol 2002;147:879—85.
[41] Zhang Q, Mousdicas N, Yi Q, et al. Staphylococcal lipoteichopic acid inhibits delayed-type hypersensitivity reaction via the platelet-activating factor receptor. J Clin Invest 2005;115:2855—61.
[42] Lyon GJ, Novick RP. Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. Peptide 2004; 25:1389—403.
[43] Wang ZQ, Orlikowsky T, Dudhane A, et al. *Staphylococcal* enterotoxin B-induced T-cell anergy is mediated by regulatory T cells. Immunology 1998;94:331—9.
[44] Dohlsten M, Bjorklund M, Sundstedt A, et al. Immunopharmacology of superantigen staphylococcal enterotoxin A in T-cell receptor V beta 3 transgenic mice. Immunology 1993; 79:520—7.
[45] Sundstedt A, Grundstrom S, Dohlsten M. T cell- and perforin-dependent depletion of B cells in vivo by staphylococcal enterotoxin A. Immunology 1998;95:76—82.
[46] Grundstrom S, Dohlsten M, Sundstedt A. IL-2 unresponsiveness in anergic CD4+ T cells is due to defective signaling through the common gamma-chain of the IL-2 receptor. J Immunol 2000;164:1175—84.
[47] Nakano H, Tsutsui H, Terada M, et al. Persistent secretion of IL-18 in the skin contributes to IgE response in mice. Int Immunol 2003;15:611—21.
[48] Morishita Y, Tada J, Sato A, et al. Possible influences of *Staphylococcus aureus* on stopic dermatitis: the colonization features and the effects of staphylococcal enterotoxins. Clin Exp Allergy 1999;29:1110—7.
[49] Laouini D, Kawamoto S, Yalcindag A, et al. Epicutaneous sensitization with superantigen induces allergic skin inflammation. J Allergy Clin Immunol 2003;112:981—7.
[50] Ou LS, Goleva E, Hall C, Leung DY. T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. J Allergy Clin Immunol 2004;113:756—63.
[51] Kuroda M, Ohta T, Uchiyama I, et al. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet 2001;357:1225—40.
[52] Otto M. Quorum-sensning control in *Staphylococci*: a target for antimicrobial drug therapy? FEMS Microbiol Lett 2004;241:135—41.
[53] Lindsay JA, Foster SJ. Interactive regulatory pathways control; virulence determinant production and stability to environmental conditions in *Staphylococcus aureus*. Mol Gen Genet 1999;262:323—31.
[54] Vuong C, Saenz HL, Gotz F, Otto M. Impact of the agr quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis 2000;182:1688—93.
[55] Melles DC, Gorkink RF, Boelens HA, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. J Clin Invest 2004;114:1732—40.
[56] Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev 2000;13:16—34.
[57] Kain KC, Schulzer M, Chow AW. Clinical spectrum of nonmenstrual toxic shock syndrome (TSS): comparison with menstrual TSS by multivariate discriminant analyses. Clin Infect Dis 1993;17:292—3.
[58] Stolz SJ, Davis JP, Vergeront JM, et al. Development of serum antibody to toxic shock syndrome toxin among individuals with toxic shock syndrome in Wisconsin. J Infect Dis 1985; 151:883—9.
[59] Schlievert PM, Blomster DA. Production of staphylococcal pyogenic exotoxin type C: influence of physical and chemical factors. J Infect Dis 1983;147:236—42.
[60] Kass EH, Schlievert PM, Parsonnet J, Mills JT. Effect of magnesium on production of toxic-shock syndrome toxin-1: a collaborative study. J Infect Dis 1988;158:44—51.
[61] Takahashi N, Nishida H, Ino M, et al. A new exanthmatous disease in newborn infants. Acta Neonat Jpn 1995;31: 371—7.
[62] Takahashi N, Kato H, Imanishi K, et al. Immunopathophysiological aspects of an emerging neonatal infectious disease induced by a bacterial superantigen. J Clin Invest 2000; 106:1409—15.
[63] Akiyama H, Oono T, Huh W-K, et al. Actions of gluco-oligosaccharide against *Staphylococcus aureus*. J Dermatol 2002; 29:580—6.
[64] Fattom A, Fuller S, Propst M, et al. Safety and immunogenicity of a booster dose of *Staphylococcus aureus* type 5 and 8 capsular polysaccharide conjugate vaccine (Staph VAX) in hemodialysis patients. Vaccine 2004;23:656—63.
[65] Domanski PJ, Patel PR, Bayer AS, et al. Characterization of a humanized monoclonal antibody recognizing clumping factor A expressed by *Staphyococcus aureus*. Infect Immun 2005;73:5229—32.

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