present study blocked C-cascade events normally resulting in cell lysis is quite unclear. Several mechanisms should be considered. Perhaps the carbohydrates bind to complement components themselves, rendering them unable to bind cell membrane receptors. Related to this suggestion would be the possibility that carbohydrate-binding to certain complement molecules may inhibit enzymatic events needed to complete the sequences of the cascade necessary for cell lysis. However, it seems equally reasonable that the carbohydrates may bind to cell membrane receptors for complement in a fashion that either blocked complement attachment or impeded further activation events at the cell surface. This latter mechanism suggests that components of the C-cascade and carbohydrates may compete for common binding sites at the cell membrane, an attractive possibility in light of other studies which demonstrated that C-fragments bind to conglutinin by carbohydrate moieties found on the complement molecule, and the direct lytic action of melittin, a hydrophobic peptide thought to disrupt membranes in an analogous manner to the C-cascade, was inhibited in a competitive fashion by galactosamine and glucosamine[17]. No matter what mechanism, the interference was specific with regard to both the availability of a reactive C-2 group on the sugars, and the observations that neither isomers, such as mannose, nor related molecules, such as N-acetylated carbohydrates blocked C-mediated lysis.

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Human specific immunoglobulin protects against infection with common *Staphylococcus* in mice

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Summary. Mice infected with non-capsulated *Staphylococcus aureus* strains highly resistant to methicillin survived after the administration of specific immunoglobulin extracted from pooled human sera by using homologous capsular type strains, but no protective effect was shown with a conventional immunoglobulin preparation and methicillin, even with high doses.
Key words. Immunoglobulin; *Staphylococcus aureus;* methicillin.

Despite the fact that therapeutic effects of conventional immunoglobulin preparations vary considerably, they have been widely applied for infectious diseases[1]. Recently, we extracted specific immunoglobulin from pooled human sera against several species of capsulated bacterial strains including *Staphylococcus aureus,* and significant prophylactic effects of the preparation were observed in mice[2]. For the practical use of immunoglobulin preparations against *S. aureus,* however, the preparation would also be required to overcome antibiotic-resistant non-capsulated strains, since the majority of causative organisms are of this type. The experiments were designed to observe whether the specific immunoglobulin preparations were effective against infection with common *S. aureus* strains.

Four *S. aureus* strains were used for challenge infection in mice. The minimal inhibitory concentration of methicillin for these strains was more than 100 μg per ml, tested by the method described in the Manual of Clinical Microbiology[3]. Although they were non-capsulated as determined by the criteria proposed by Yoshida and Minegishi[4], the strains MRSA-198 and MRSA-580 both had a capsular type antigen A and B, while both strains MRSA-121 and MRSA-125 were of the bivalent capsular type, A plus B, tested by the method of Yoshida et al.[5]. For the extraction of the specific immunoglobulin, eluate containing specific immunoglobulins was obtained from an antigen-antibody complex using propionic acid in the presence of 5% sucrose by a method noted elsewhere[2]. Antigen used for elution was Smith surface antigen[6] (SSA), the protection-inducing antigen of the Smith strain, capsular type A, and whole cells of the ATCC-21734 strain, capsular type B, both of which strains were capsulated and methicillin-sensitive. The capsular types were determined by the method of Yoshida[7]. Protein and immunoglobulin levels in the eluate were measured by the method of Lowry et al.[8] and by using immunoplates (Hyland).

Mice infected with MRSA-198 strain (capsular type A) were completely protected from lethal effects by treatment with an amount of eluate from SSA containing 0.06 mg protein. The amount was the same doses as that for the Smith strain in mice. With challenge by the MRSA-580 strain (capsular type B) plus treatment with an eluate obtained using the ATCC-21734 strain, containing 0.12 mg protein, all animals survived. Eluate containing double the amount of protein content was required for complete protection against ATCC-21734 strain. For the challenge infection with strains MRSA-121 or MRSA-125 (bivalent capsular type) the animals were primarily treated with eluates obtained using the SSA or ATCC-21734 strain; however, the animals succumbed even when given high doses of those eluates. Minimum protein amounts of the eluates capable of protecting against challenge with the single capsular type of the capsulated strains A and B were combined and administered. Animals survived otherwise lethal infections with strains MRSA-121 or MRSA-125 with these doses (table 1). In these experiments, no effect was shown even with 2.36 mg of Venoglobulin I (Midori-Juji Pharmaceutical Co. Ltd., Tokyo), a preparation that includes biological properties of IgG and does not contain isolated

Table 1. Effect of eluates containing specific immunoglobulins in mice against challenge with strains MRSA-198, MRSA-580, MRSA-121 and MRSA-125 of *Staphylococcus aureus*

Treated with eluate (protein mg) extracted from capsular type strain		Challenged with strain (capsular type) A		B		A/B	
		MRSA 198	Smith	MRSA 580	ATCC-21734	MRSA 121	MRSA 125
Smith (A)	0.12	0/5*	0/5	5/5	5/5	5/5	5/5
	0.06	0/5	0/5	5/5	5/5	5/5	5/5
	0.03	2/5	3/5	5/5	5/5	5/5	5/5
	0.01	5/5	5/5	5/5	5/5	5/5	5/5
ATCC-21734 (B)	0.12	5/5	5/5	0/5	0/5	5/5	5/5
	0.06	5/5	5/5	0/5	2/5	5/5	5/5
	0.03	5/5	5/5	5/5	5/5	5/5	5/5
	0.01	5/5	5/5	5/5	5/5	5/5	5/5
Smith ATCC-21734	0.06 + 0.12					0/5	0/5
	0.03 + 0.06	ND	ND	ND	ND	3/5	2/5
	0.01 + 0.03					5/5	5/5
Untreated		5/5	5/5	5/5	5/5	5/5	5/5

* No. of deaths/No. of experimental animals. ND, not done.

Table 2. Protein and immunoglobulin amounts (mg) in 1.0-ml eluates obtained by using Smith surface antigen, extracted from strain Smith, and whole cells of strain ATCC-21734 of *Staphylococcus aureus*

Substance	Eluate obtained by using Smith surface antigen	ATCC-21734
Protein	2.24	5.38
IgG	1.64	2.57
IgA	0.24	0.26
IgM	0.28	0.63
Immunoglobulin content (%)	96.43	64.31

Fc fragment, as well as 250 mg per kg of methicillin. Serological properties of the eluates obtained from the SSA and ATCC-21734 strain showed that 96.4 and 64.3% of the protein in them consisted of three major immunoglobulins (table 2).

Owing to the appearance of multi-antibiotic-resistant bacterial strains, immunoglobulin therapy has been applied for infection with those strains, despite the variable effects actually obtained with conventional immunoglobulins. Therefore the development of specific immunoglobulin preparations is desirable for practical clinical use. In these experiments, human immunoglobulins specific for capsulated *S. aureus* strains protected mice against infection with non-capsulated strains which produced homologous capsular type antigen, at a rate almost similar to that in capsulated strains. In general, the population of capsulated strains of *S. aureus* has been assumed to be extremely low. However, non-capsulated strains were capable of converting to capsulated organisms in vivo[9], and a large amount of the non-capsulated Smith strain either actively or passively induced resistance in mice[10] against homologous infection. In organisms defined according to our criteria[4] as non-capsulated strains of *S. aureus*, partial or small capsules were electron-microscopically demonstrated[4], and 99.9% of fresh isolates of non-capsulated strains were typable for capsular type[11]. In previous papers by the present authors, polyvalent capsular vaccine protected against infection with *S. aureus* in bovine mastitis in field experiments[12] and protective activity against capsulated *S. aureus* in normal human sera was shown to be 64%[14]. Further, induction of resistance of the strains was capsular type-specific[13]. These findings indicate that capsulation is a common phenomenon in *S. aureus* although the capsular size and amount of capsule varies depending upon the strain, and that induction of resistance by immunoglobulins specific for homologous capsular strains against infection with non-capsulated strains is a reasonable concept. With the method of isolation of specific immunoglobulins applied in these experiments, a preparation containing IgM extracted from 100 ml human sera was capable of protecting 4.4 kg mouse weight against infection with otherwise lethal doses of capsulated Smith strains[2]. These preparations show the possibility of therapeutic effects in human staphylococcal infection.

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