ELSEVIER

FEMS Immunology and Medical Microbiology 35 (2003) 99–106

FEMS Immunology and Medical Microbiology

www.fems-microbiology.org

Staphylococcus aureus strains isolated from bovine mastitis: virulence, antibody production and protection from challenge in a mouse model

G. Leitner *, O. Krifucks, A. Glickman, A. Younis, A. Saran

National Mastitis Reference Center, Kimron Veterinary Institute, Ministry of Agriculture and Rural Development, P.O. Box 12, Bet Dagan 50250, Israel

Received 12 August 2002; received in revised form 17 November 2002; accepted 18 November 2002

First published online 23 December 2002

Abstract

Septic arthritis in mice was used as a model to evaluate the virulence of *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) isolated from cases of bovine mastitis. In addition, the model was used to evaluate the cross protection elicited by heterologous antibodies. Mice were intramuscularly inoculated with serial bacterial doses of different strains of *S. aureus* or CNS, for virulence determination; they were monitored for arthritis, gangrene or death up to 20 days. Antibody response, cross reactivity and resistance to challenge were tested by subcutaneous inoculation with a low dose of one of the *S. aureus* or CNS strains followed by challenge with two *S. aureus* strains. *S. aureus* α-hemolysin isolate was the most virulent, followed by α+β-hemolysin and β-hemolysin isolates. The least virulent isolates were the non-hemolytic *S. aureus* strains but even they were more virulent than the CNS strains tested. Antibodies against three different *S. aureus* antigens were detected by the ELISA in all mice that were inoculated with the *S. aureus* strains but not in any of those with the CNS strains. Immunoblot test against various *S. aureus* strains as antigens showed high cross-reactivity among the *S. aureus* strains but only a slight similarity, restricted to the bands above 36 kDa, with the CNS sera. Low-dose inoculation of α or α+β strains before challenge with homologous and heterologous strains protected the mice, whereas the two β strains provided only partial protection. The inoculations of non-hemolytic *S. aureus* or the CNS strains did not elicit any protection. Our findings demonstrate that pre-exposure of mice to a low dose of certain *S. aureus* strains could provide protection and that the antibodies produced could have an important protective role.

Keywords: *Staphylococcus aureus*; Mastitis; Mice model

1. Introduction

Staphylococcus aureus is a ubiquitous pathogen that causes a variety of infections in man and animals. A high degree of natural immunity to *S. aureus* has been detected in infected and non-infected human and animal hosts [1–4]. Despite this natural immunity and the existence of experimental vaccines, *S. aureus* infection continues to present a difficult problem and is the main etiological cause of mastitis in cattle, sheep and goats. The disease causes huge economic losses worldwide, estimated at $2 billion annually (or $200/cow) in the US alone [5]. Commercially available *S. aureus* vaccines against bovine mastitis have shown limited efficacy under field conditions [6–11]. In dairy cows, *S. aureus* and coagulase-negative staphylococci (CNS) cause contagious mastitis that is mainly subclinical and chronic but may appear as a per-acute event in mammary quarters. In general the former leads to the more severe damage to the mammary gland, with an enhanced inflammation response. This suggests that after *S. aureus* and/or CNS penetrate(s) into the gland, the bacterial exosecretions are potentially responsible for the severity of the disease. *S. aureus* can produce and secrete proteins that exhibit superantigen (SAg) activity; they belong to a family of pyrogenic toxins and induce massive aberrant oligoclonal activation of T lymphocytes. The various strains of *S. aureus* can produce a number of potential virulence factors such as hemolysins (α, β, γ or δ), coagulase, leukocidin, enterotoxins (A–E), toxic shock

* Corresponding author. Tel.: +972 (3) 9681637; Fax: +972 (3) 9681692.
E-mail address: gleit_vs@netvision.net.il (G. Leitner).

0928-8244/02/$22.00
doi:10.1016/S0928-8244(02)00458-3

syndrome toxin 1 (TSST-1), etc. [12,13]. The degree or severity of subclinical mastitis caused by *S. aureus*, which is partially related to these factors, can also vary among the *S. aureus* strains. In an attempt to test the role of *S. aureus* and CNS exosecretions in the bovine udder, cows were inoculated intracisternally with various strains of sterile bacterial exosecretions and were monitored for 96 h post-inoculation (PI) [14]. This study showed an association between bacterial exosecretions and the severity of the cows' response as measured by the induction of inflammation in the inoculated quarters. Mouse models, if suitable, are less costly than cows as well as being a prerequisite for experiments preceding trials in humans. Previous studies [15,16] used mouse as an animal model in order to understand which components of *S. aureus* are of important in the development and persistence of septic arthritis. Although the virulence of the bacteria in mice is not necessarily similar to that in other species, we attempted to evaluate the virulence of *S. aureus* and CNS by means of this model, which we also used in an attempt to evaluate the degree of cross protection among isolates. All strains were field isolates from bovine mastitis in Israel and at the time of isolation cows had subclinical mastitis, however, some of the cows presented acute inflammatory processes before examination that later became subclinical [17,18]. The present study addressed the questions of bacterial virulence, antibody production and protection of mice after pre-exposure to a low bacterial dose of live homologous and heterologous *S. aureus* strains.

2. Materials and methods

2.1. Mice

Female Swiss mice, 6–8 weeks of age, were maintained in the animal facility of the Kimron Veterinary Institute. Three mice were housed in each cage under standard conditions of light (12/12 h light/dark) and temperature (22°C) and were fed standard laboratory chow and water ad libitum. At the end of the experiments, mice were euthanized with CO_2

2.2. Bacterial strains

Seven strains of *S. aureus*, two strains of *Staphylococcus chromogenes* and one each of *Staphylococcus intermedius* and *Staphylococcus xylosus* were used. All strains were isolated from cases of chronic bovine mastitis [17,18]. Each strain was tested for coagulase production {(tube test) Anilab, Tal-Shachar, Israel} [19], subjected to the slide latex agglutination test (BACTI Staph, Remel, Santa Fe Drive, Lenexa, KS, USA), identified by means of the ID-32-API STAPH test (BioMerieux S.A, France), and phage typed (coagulase positive) with phages issued by the International Reference Laboratory, Colindale, UK, by the method of Blair and Williams [20] as modified by Samra et al. [21]. The strains did not produce any of the enterotoxins for which we tested (A–E) or TSST-1 as assayed by means of the *microslide-immuno-diffusion-gel* technique [22] at the Department of Food Hygiene, Ministry of Public Health, Haifa, Israel. The production of hemolysins (α or β) was confirmed by cultivating the bacteria in Columbia broth (Difco, Detroit, MI, USA) at 37°C for 10 h, centrifuging at $3000\times g$ and testing the supernatant against rabbit or sheep erythrocytes as described by Nilsson et al. [15] For challenge and for electrophoresis each bacterium was cultivated in nutrient broth (Difco, Detroit, MI, USA) at 37°C for 24 h and harvested by centrifuging at $3000\times g$ 15 min at 4°C. The pellet was resuspended in non-pyrogenic PBS (pH 7.6; 0.01 M) and washed twice, and a portion was stored at −20°C for the electrophoresis tests. For challenge, cells were resuspended to McFarland equivalence turbidity standard 4 (1.2×10^9 cells ml^{-1}) (Remel Santa Fe Dr., Lenexa, KS, USA). When a higher concentration was required ($>1.2\times\ 10^9$ cells ml^{-1}), the bacteria were repelleted and resuspended in one-quarter of the non-pyrogenic PBS. The concentration was adjusted to the appropriate level by addition of non-pyrogenic PBS, and 0.25 ml of the bacterial suspension was inoculated into the left hind leg of each mouse. The exact bacterial concentration injected to the mouse was determined by serial dilutions (×2) on blood-agar plates.

2.3. Clinical evaluation

Mice were inoculated intramuscularly (IM) with various bacteria and examined individually thereafter. The injected limb was visually inspected daily for visible erythrema gangrene. Macroscopic inspections during 20 days of observation revealed either: (0) normal appearance; (1) morbidity – erythrema gangrene; (2) mortality. Virulence was calculated as the combined percentage of mortality and morbidity among all the mice inoculated and expressed as the number of CFUs of the bacteria that resulted in death (mortality – which occurred within the first 5 days post-challenge (PC)) or erythrema gangrene (morbidity) in 50–70% of the inoculated mice.

2.4. Experimental design

Two sets of experiments were performed. The first set determined the virulence of the isolates to mice. Mice were inoculated IM: with one of the *S. aureus* strains, BS1, AU2873, AU3133, ZU452, FR2449, GL3497 or NR2602; with *S. chromogenes* (m10); with *S. intermedius* (M2). For each strain, nine mice were used receiving 5×10^6, 5×10^7 or 5×10^8 CFU. The mice were inspected for arthritis, gangrene or mortality for up to 20 days. Isolates whose virulence was not determined: mice did not show any symptoms of arthritis even with the highest dosage of

CFU and were tested again as mentioned above but the dosage of bacteria was increased to 5×10^9 CFU/mouse or decreased to 5×10^5 CFU/mouse.

The second set of experiments was conducted to study antibody responses, the cross reactivity among them, and resistance to challenges with various *S. aureus* strains. Two independent trials were conducted.

Trial 1 included five groups, each of six mice. The mice in each group were inoculated subcutaneously (SC) with 1×10^5 CFU/mouse of: one of the *S. aureus* strains, BS1, AU2837 or AU3133; *S. chromogenes* (m3); or *S. xylosus* (743)) and with the non-pyrogenic PBS as negative control. Antibody response was determined 40 days PI by ELISA.

Trial 2, included 10 groups, each of eight mice. The mice in each group were inoculated SC with 1×10^5 CFU/mouse of: one of *S. aureus* strains, BS1, AU2837, AU3133, ZU452, FR2449, GL3497 or NR2602; or one of the CNSs, *S. chromogenes* (m10) or *S. intermedius* (M2) and with the non-pyrogenic PBS as negative control. Antibody response was tested twice, at 15 and 25 days PI. At 30 days PI the mice in each group were divided into two subgroups, each of which was challenged with one *S. aureus* isolate, either BS1 or NR2602. The mice were inoculated IM with 5×10^8 CFU/mouse of BS1 or with 1×10^7 CFU/mouse of NR2602, and were monitored for mortality and arthritis for up to 20 days PC.

2.5. Electrophoresis and immunoblot

One-dimension sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1-D SDS–PAGE) was performed according to Laemmli [23]. Bacterial cells were disrupted with glass beads in a homogenizer (Braun Melsungen AG, Germany) for 10–15 min. The glass beads and the remaining bacteria were removed by centrifugation ($1000\times g$, 15 min). The supernatant was filtered through 0.2-μm filters. Protein concentrations of the disrupted bacteria were determined with the Bio-Rad protein assay [24]. The samples (antigens) were adjusted to a final protein concentration of 1 mg ml^{-1} and 33 μl of each sample was mixed with 25 μl of 4× NuPAGE sample buffer, 32 μl of ultrapure water and 10 μl of reducing agent (0.5 M DTT). The sample mixtures were heated to 70°C for 10 min and loaded into the gels at 30 μl per lane. The gels were 7% [NuPAGE Tris acetate (+TA buffer)] and 10% (Bis–Tris Gel with w/MOPS) (NOVEX, San Diego, CA, USA); they were stained with coloidal blue. Molecular-mass markers for these gels were: See Blue Pre-Stained, 191–14 kDa for the 10% gel and 210–41 kDa for the 7% gel). For the immunoblot assay, a nitrocellulose membrane (0.2 μm) was blocked with 3% casein and incubated with 1:50 to 1:100 dilutions of serum from mice immunized with three different *S. aureus* strains. The blot was developed with goat anti-mouse IgG (H+L) alkaline peroxidase conjugate (1:1000), with a substrate of 3,3-diaminobenzidine tetrahydrochloride (ICN Pharmaceuticals, Inc.). Molecular-mass markers for these gels were: NOVEX Marker 12 Standard Bands (200–2.5 kDa.

2.6. Immunological assay

ELISA: 96-well immunoplates (Nunc, Kamstrup, Denmark) were coated overnight at 4°C with 200 μl/well of various strains: BS1, AU2837, AU3133, of agitation-killed *S. aureus*, suspended in carbonate-bicarbonate buffer (pH 9.6) at 5×10^8 CFU ml^{-1}. When an assay was performed, 200 μl of 3% casein were added to each well and incubated for 40 min at room temperature. The plates were washed three times for 5 min with PBS (pH 7.6) containing 0.5% Tween 20. They were then dried and 100 μl of tested serum, at 1:400 dilutions was added, and the plates were incubated for 1 h at 37°C. This procedure was carried out in duplicate. The plates were washed and affinity-purified goat anti-mice (H+L) peroxidase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) at 1:1500 dilution was added to each well and the material was incubated for 1 h at 37°C. The bound antibodies were detected by adding 100 μl of 2.2′-azino-di(3-ethyl-benzthiazolin sulfonate) peroxidase substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) after washing. The respective plates were read in a microplate autoreader (Dynatech MR5000, Guernsey, UK) at 410 nm. To each plate, positive and negative serum standards were added, prepared from hyperimmune challenged and unimmunized non-infected mice, respectively. The antibody level was (the OD of the tested serum divided by the OD of the negative serum) minus 1, i.e., (OD tested serum/OD negative serum) −1

3. Results

The virulence ratings of the isolates (Table 1) were determined by inoculation with a range of concentrations of: the *S. aureus* strains, BS1, AU2873, AU3133, ZU452, FR2449, GL3497 and NR2602; *S. chromogenes* (m10); and *S. intermedius* (M2). The most virulent isolate was the α-hemolysin, *S. aureus* NR2602 (5×10^6 CFU/mouse). Moderate virulence was shown by the one of α+β-hemolysins, isolate FR2449 (5×10^7 CFU/mouse), followed by the other α+β-hemolysin, isolate BS1 and by one of the β-hemolysin isolates, *S. aureus* AU2873 (1×10^8 CFU/mouse). The least virulent isolates were the β-hemolysin, *S. aureus* ZU452 and the non-hemolysin isolate, AU3133 ($>5\times10^9$ CFU/mouse). The non-hemolysin isolate GL3497 did not cause any mortality but the morbidity was high in all three levels of inoculation therefore, virulence was calculated as 1×10^8. Neither of the two CNSs, *S. chromogenes* (m10) or *S. intermedius* (M2), elicited any symptoms, even when the highest challenge was used.

In the 1-D SDS–PAGE tests, a high degree of similarity

Table 1
Virulence {number of CFUs of bacteria that induced mice death (mortality) or erythrema gangrene (morbidity) in 50% of the mice} derived from inoculation with several concentrations of various strains of *S. aureus* (BS1, AU2873, AU3133, ZU452, FR2449, GL3497 and NR2602), *S. chromogenes* (m10) or *S. intermedius* (M2)

Bacterium	CFU/mice	Mortality (%)[a]	Morbidity (%)[b]	Mortality and morbidity (%)	Virulence
BS1	5×10^8	66.7	33.3	100	1×10^8
	5×10^7	–	66.7	66.7	
	5×10^6	–	–	–	
AU2873	5×10^8	33.3	66.7	100	1×10^8
	5×10^7	–	66.7	66.7	
	5×10^6	–	–	–	
AU3133	5×10^9	–	100	100	5×10^9
	5×10^8	–	–	–	
	5×10^7	–	–	–	
ZU452	5×10^9	–	100	100	5×10^9
	5×10^8	–	66.7	66.7	
	5×10^7	–	–	–	
FR2449	5×10^8	100	–	100	5×10^7
	5×10^7	–	100	100	
	5×10^6	–	33.3	33.3	
GL3497	5×10^9	–	100	100	1×10^8
	5×10^8	–	100	100	
	5×10^7	–	66.7	66.7	
NR2602	5×10^7	100	–	100	5×10^6
	5×10^6	33.3	66.7	100	
	5×10^5	–	100	100	
S. chromogenes (m10)	5×10^9	–	–	–	$>5\times10^9$
	5×10^8	–	–	–	
	5×10^7	–	–	–	
S. intermedius (M2)	5×10^9	–	–	–	$>5\times10^9$
	5×10^8	–	–	–	
	5×10^7	–	–	–	

[a]Percentage of mice mortality during 20 days PI.
[b]Percentage of mice with arthritis and gangrene during 20 days PI.

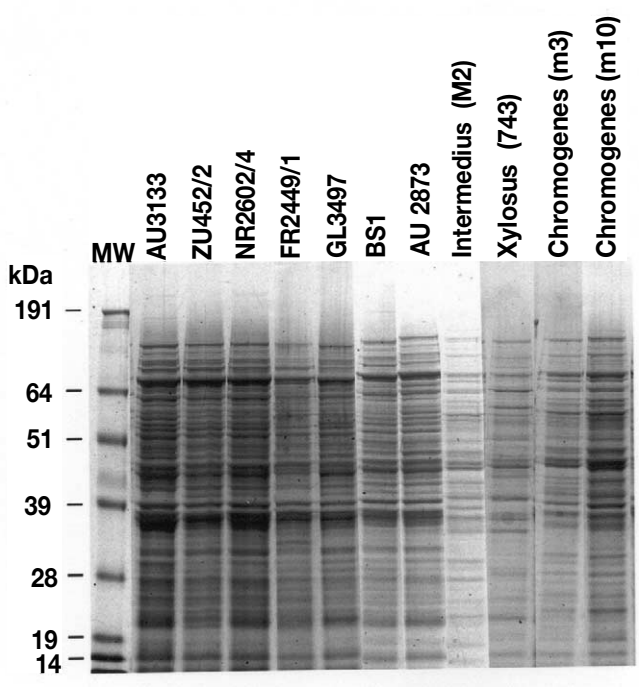

Fig. 1. 1-D SDS–gels 10% (Bis–Tris gel with w/MOPS) electrophoresis of disrupted *S. aureus* and CNS strains. Molecular-mass markers are See Blue Pre-Stained (191–14 kDa).

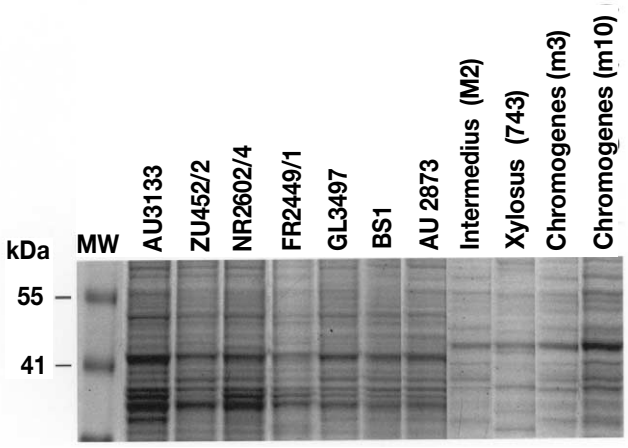

Fig. 2. 1-D SDS–gels 7% [NuPAGE Tris acetate (+TA buffer)] electrophoresis of disrupted *S. aureus* and CNS strains. Gels express the protein patterns between 55 and 36 kDa. Molecular-mass markers are See Blue Pre-Stained (210–41 kDa).

was found among the various *S. aureus* strains, in their protein bands above 42 kDa, whereas the CNS strains tested showed substantial differences in this region (Fig. 1). The 42-kDa bands identified as protein A and tested by anti-protein-A antibody (data not shown), appears only in the *S. aureus* strains. Among the *S. aureus* strains, differences were found in the range of 40–28 kDa (Fig. 2), while no differences could be observed below 28 kDa (data not shown).

Table 2 presents summaries of the mortality and morbidity after inoculation, of mice pre-exposed to low concentrations of: the *S. aureus* strains, BS1, AU2873, AU3133, ZU452, FR2449, GL3497 and NR2602; *S. chromogenes* (m10); or *S. intermedius* (M2). Mice that had been pre-exposed to the α strain NR2602 or to the α+β strain BS1, were protected when inoculated with homologous and heterologous strains, while pre-exposure to the α+β strain FR2449 resulted in only a partial protection against inoculation with the NR2602 strain. No protection could be shown against the BS1 strain. Among the mice pre-exposed to the two β strains, AU2873 elicited full protection against BS1 while ZU452 failed to do so. The pre-exposure to the non-hemolytic *S. aureus* strains, AU3133 and GL3497, to the *S. chromogenes* (m10) or to *S. intermedius* (M2) did not confer any protection relative to control when the mice were inoculated with NR2602 and only poor protection when they were inoculated with BS1 strains. Moreover, the two CNS strains used: *S. chromogenes* (m10) and *S. intermedius* (M2) when challenged with NR2602, caused an increase in mortality above control (100% vs. 25% respectively). Table 3 summarizes the results of ELISA tests of antibody levels against antigens of three *S. aureus* strains: NR2602/4, AU2873 and GL3497. In both trials (1 and 2), antibodies were detected in all the mice that were inoculated with the various *S. aureus* strains while such antibodies could not be detected in any of the mice, which were inoculated with CNS. In the comparisons among the antibody levels elicited by the various antigens (cross reactivity), only strain AU3133 showed higher titer with the homologous strain than with the two heterologous strains. In all the other six *S. aureus* strains tested, antibody levels of homologous and heterologous strains showed no significant differences (Table 3).

Immunoblot tests of sera of all *S. aureus* strains, with different *S. aureus* strains used as antigens, revealed high cross-reactivity while at the same time they also showed some specific protein bands, the nature of those however are not known at this stage. Fig. 3 presents one such immunoblot with serum against strain AU2873; sera from the mice inoculated with the different CNS strains show some similarity in the immunoblot to the *S. aureus* strain, above the 36-kDa marker.

Table 2
Mortality and morbidity of mice pre-exposed to low concentrations of various strains of *S. aureus* (BS1, AU2873, AU3133, ZU452, FR2449, GL3497 and NR2602), *S. chromogenes* (m10) or *S. intermedius* (M2) and then inoculated with different concentration of the bacteria

Bacterium	Hemolysis	BS1 (5×10^8 CFU)		NR2602 (1×10^7 CFU)	
		Mortality (%)[a]	Morbidity (%)[b]	Mortality (%)	Morbidity (%)
Control[c]		25	75	25	75
BS1	α+β	0	0	0	0
AU2873	β	0	0	0	100
AU3133	–	25	50	25	50
ZU452	β	50	50	25	25
FR2449	α+β	50	50	0	25
GL3497	–	25	75	25	75
NR2602	α	0	0	0	0
S. chromogenes (m10)	–	0	100	100	–
S. intermedius (M2)	–	0	50	100	–

[a]Percentage of mice mortality during 20 days PI.
[b]Percentage of mice with arthritis and gangrene during 20 days PI.
[c]Control: mice which were not pre-exposed to low concentrations of any bacteria.

4. Discussion

The present study describes the use of a mouse model in an attempt to elucidate *S. aureus* virulence, antibody production and protection after exposure to a low bacterial dose of live homologous and heterologous *S. aureus* strains. This model may assist to find *S. aureus* strains suitable for the development of vaccines against bovine mastitis caused by *S. aureus*, as well as more information for the understanding of the mammary gland immunity complex. α-Toxin [25] or α- and γ-toxin [15] play major roles in the pathogenesis of septic arthritis. α-Toxin has been found to contribute to arthritic events by promoting adherence of neutrophils to endothelial cells [26] and macrophages, so producing proinflammatory mediators such as TNF-α. In the present study, the field strains of *S. aureus* producing the α-toxin were found to be 1.5–2 log more virulent than the α- and β-toxin and those higher than β-toxin or the non-hemolytic strains. These results support the putative role of α-toxin as the major toxin in the pathogenesis of septic arthritis, although the higher virulently of the NR2602 (α-toxins) then BS1 and FR2449 (α- and β-toxin) is not yet clear. It is possible that the level of α-toxin produced by NR2602 was higher however, the exact levels of each toxin were not titered. It has been

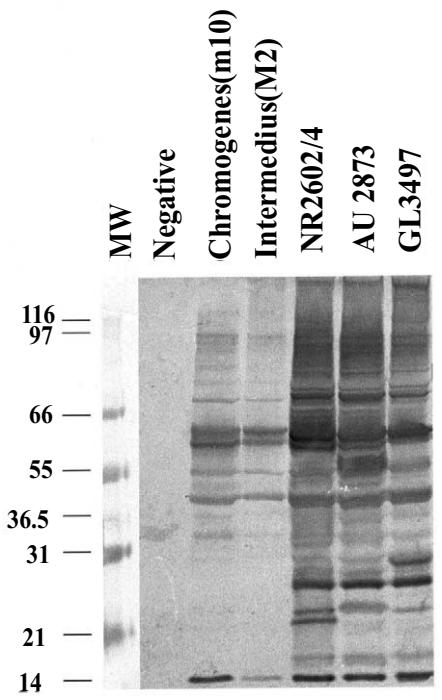

Fig. 3. 1-D SDS–gels 10% (Bis–Tris gel with w/MOPS) immunoblot assay. Electrophoresis of disrupted *S. aureus* and CNS strains. Molecular-mass markers are NOVEX Marker 12 Standard Bands (200–2.5 kDa).

Table 3
Antibody titers of mice 40 (experiment 1) and 15 and 25 (experiment 2) days after SC inoculation with a small number (5×10^5 CFU/mouse) of one of seven different strains of *S. aureus* (BS1, AU2873, AU3133, ZU452, FR2449, GL3497 and NR2602), two strains of *S. chromogenes* (m3 and m10), *S. xylosus* (743) or *S. intermedius* (M2)

Experiment	Sera	Time[a]	Antigens		
			BS1	AU2837	AU3133
1	BS1	40	0.57[b]	0.67	0.74
2		15	0.76	0.89	0.98
		25	1.30	1.10	1.88
1	AU2873	40	0.30	0.31	0.24
2		15	0.54	0.66	0.43
		25	1.12	1.52	1.37
1	AU3133	40	0.45	0.46	0.81
2		15	0.37	0.30	0.84
		25	0.80	0.92	1.66
2	ZU452	15	1.45	1.64	1.73
		25	1.98	1.88	2.46
2	FR2449	15	0.94	1.28	1.49
		25	1.12	1.79	1.45
2	GL3497	15	0.50	0.73	0.80
		25	0.54	0.81	1.50
2	NR2602	15	1.23	0.83	1.76
		25	1.56	1.62	2.30
1	*S. chromogenes* (m3)	40	0	0	0
1	*S. xylosus* (743)	40	0.06	0.09	0.08
2	*S. chromogenes* (m10)	15	0.1	0	0
		25	0.5	0.4	0.4
2	*S. intermedius*, (M2)	15	0	0	0
		25	0.2	0.5	0
1	negative	40	0	0	0
2	control	25	0	0	0

ELISA tested sera against each of the *S. aureus* strains: BS1, AU2873 and AU3133.
[a]Days post inoculation.
[b]Antibodies titer within row with underline represents homologous strain.

proposed [15] that β-toxin might have some protective properties. However, in the present study, β-toxin strains were found to be moderately virulent compared with the non-hemolytic strains of *S. aureus* and the CNS strains tested. The non-hemolytic *S. aureus* strains were more virulent than the *S. chromogenes* and *S. intermedius* strains. It seems, therefore, that although hemolysin plays a major role in the development of erythrema gangrene and mortality in mice, other, even more dominant factors may influence the pathogenesis of the disease.

Mice, which were pre-exposed to the α-toxin NR2602, or the α+β-toxin strains, BS1 and FR2449 were protected from challenge with the *S. aureus* α-toxin NR2602 strain. In contrast, pre-exposure to the β-toxin or the non-hemolytic *S. aureus* strains did not provide any protection. The β-toxin strain AU2873 did not provide any protection against challenge with the α-toxin strain NR2602 but did provide protection against the α+β BS1. These results suggest that β-toxin may have an independent pathogenesis of septic arthritis. Pre-exposure of mice to β-toxin provided a protection against β-toxin but not against the α-toxin. Interestingly, the mice pre-exposed to the two CNSs, *S. chromogenes* (m10) and *S. intermedius* (M2) exhibited a higher protection rate against the α+β BS1 strain than against the α NR2602 one. α-Toxin and probably also β-toxin play a major role in the pathogenesis of septic arthritis. However, other *S. aureus* SAg toxins such as TSST-1 also contribute to the arthritogenicity [27,28]. It has been suggested that *S. aureus* arthritis is a T-cell-dependent and SAg-mediated disease [29]. The role of B cells in this disease has been studied in B-cell-deficient mice [30] and no impact on the progress of symptoms was found. However, in the present study, our aim was to study the possibility of using a pre-exposure to low concentrations of various *S. aureus* strains, to prevent the development of symptoms of septic arthritis as a result of a subsequent higher exposure. It has been reported [31] that vaccination of mice with recombinant staphylococcal enterotoxin A provided protection. Indeed, our present results indicate that B cells were involved in the production of antibodies against the various bacteria used and that those may have had an important role in the protections process.

In the mammary gland, *S. aureus* and CNS are the predominant pathogenic bacteria that cause chronic infection, and the disease is generally more severe when caused by *S. aureus* than when caused by CNS strains. Other studies [32,33] have demonstrated the role of SAgs as one of the virulence factors that modulate the immune response and promote survival of the bacterium. Therefore, the mechanisms by which SAgs cause septic arthritis in mice and the severity of mastitis in cows appear to be the same. If so, protecting mice from septic arthritis and elucidating the mechanism involved may contribute to the development of anti-*S. aureus* immunization in cows. One may question the relevance of the mouse model to the problem of bovine mastitis caused by *S. aureus* strains. Therefore, a composition comprising three strains of *S. aureus* derived from cows affected with mastitis was evaluated by means of this model. The said composition was found to be highly efficacious in protecting mice against both homologous and heterologous strains. This composition, with an adjuvant was subsequently used as a vaccine, administered to cows, which were then challenged with a virulent strain of *S. aureus*, and it was found to confer significant immunity on the cows [34]. It seems, therefore, that this mouse model, in spite the differences between the clinical symptoms in mice and cows, can serve as a useful tool for the evaluation of the virulence of *S. aureus* isolates derived from wounds or mastitis cases. Moreover, it can be used as a tool for evaluating the degree of immunity conferred by potential vaccines designed against *S. aureus* strains.

References

[1] Adlab, C. and Easman, C.S.F. (1983) Immunity and hypersensitivity to staphylococcal infection. In: Staphylococci and Staphylococcal Infections (Easman, C.S.F. and Adlam, C., Eds.). p.275–323. Academic Press, Inc., New York.

[2] Bayer, A.S., Tillman, B.D., Concepcion, N. and Guze, L.B. (1980) Clinical value of teichoic acid antibody titers in the diagnosis and management of staphylococcemias. West. J. Med. 132, 294–300.

[3] Granstrom, M., Julander, I. and Mobly, R. (1983) Serological diagnosis of deep *Staphylococcus aureus* infections by enzyme-linked immunosorbent assay (ELISA) for staphylococcal hemolysins and teichoic acid. Scand. J. Infect. Dis. 41 (Suppl.), 132–139.

[4] Henru, A., Verbrugh, A.H., Peters, R., Rozenberg-Aszka, M., Peterson, P.K. and Verkoef, J. (1981) Antibodies to cell wall peptidoglican of *Staphylococcus aureus* in patients with serious staphylococcal infections. J. Infect. Dis. 144, 1–9.

[5] Pankey, J.W. (1990) In: Proceedings of the 29th Annual Meeting of the National Mastitis Council. National Mastitis Council. Washington, DC, 167.

[6] Nordhaug, M.L., Nesse, L.L., Norcross, N.L. and Gudding, R. (1994) A field trial with an experimental vaccine against *Staphylococcus aureus* mastitis in cattle. I. Clinical parameters. Dairy Sci. 77, 1267–1275.

[7] Pankey, J., Boddie, N.T., Watts, J.I. and Nickerson, S.C. (1985) Evaluation of protein A and a commercial bacterin as vaccines against *Staphylococcus aureus* mastitis by experimental challenge. J. Dairy Sci. 68, 726–731.

[8] Watson, D.L. (1984) Evaluations of attenuated, live staphylococcal mastitis vaccine in lactating heifers. J. Dairy Sci. 67, 2608–2613.

[9] Watson, D.L. (1992) Vaccination against experimental staphylococcal mastitis in dairy heifers. Res. Vet. Sci. 53, 346–353.

[10] Watson, D.L., McColl, M.L. and Davies, H.I. (1996) Field trial of a staphylococcal mastitis vaccine in dairy heifers: clinical, subclinical and microbiological assessments. Aust. Vet. J. 74, 447–450.

[11] Yoshida, K., Ichiman, Y., Narikawa, S. and Evans, G.B. (1984) Staphylococcal capsular vaccine for preventing mastitis in two herds in Georgia. J. Dairy Sci. 67, 620–627.

[12] Iandolo, J.J. (1990) The genetics of staphylococcal toxins and virulence factors. Bacteria 11, 399–426.

[13] Smeltzer, M.S., Hart, E.M. and Iandolo, J.J. (1993) Phenotypic characteristization of xpr, a global regulator of extracellular virulence factors in *Staphylococcus aureus*. Infect. Immun. 61, 919–925.

[14] Younis, A., Krifucks, O., Heller, D.E., Samra, Z., Glickman, A.,

Saran, A. Leitner, G. (2002) *Staphylococcus aureus* exosecretions and bovine mastitis. J. Vet. Med. B. (accepted).

[15] Nilsson, I.M., Hazttord, O., Foster, T. and Tarkowski, A. (1999) Alpha-toxin and gamma-toxin jointly promote *Staphylococcus aureus* virulence in murine septic arthritis. Infect. Immun. 67, 1045–1049.

[16] Tao, M., Yamashita, H., Watanabe, K. and Nagatake, T. (1999) Passible virulence factors of *Staphylococcus aureus* in a mouse septic model. FEMS Immunol. Med. Microbol. 23, 135–146.

[17] Chaffer, M., Leitner, G., Winkler, M., Glikman, A., Krifuks, O., Ezra, E. and Saran, A. (1999) Coagulase-negative staphylococci and mammary gland infections in cows. J. Vet. Med. B. 46, 707–712.

[18] Yonis, A., Leitner, G., Heller, D.E., Samra, Z., Gadba, R., Lubashevsky, E., Chaffer, M., Yadlin, N., Winkler, M. and Saran, A. (2000) Phenotypic characteristics of *Staphylococcus aureus* isolated from bovine mastitis in Israeli dairy herds. J. Vet. Med. B. 47, 591–597.

[19] Koneman, E.W., Allen, D.S., Bowell Jr., R.V., Janda, M.W., Sommers, M.H. and Winn Jr., C.W. (1988) Color and Textbook of Diagnostic Microbiology, 3rd edn. J.B. Lippincott Company, Philadelphia, PA, 3, 311–364.

[20] Blair, J.E. and Williams, O.E.R. (1961) Phage type of staphylococci. Bulletin World Health Organization 24, 771–784.

[21] Samra, Z. and Gadba, R. (1993) Antibiotic susceptibility and phage typing of Methicillin resistant *Staphylococcus aureus* clinical isolation from blood cultures of 962 patients in 15 Israeli hospitals. Eur. J. Epidemiol. 9, 559–562.

[22] Su, Y.C. and Wong, C.L.A. (1997) Current perspectives on detection of Staphylococcal enterotoxins. J. Food Prot. 60, 195–202.

[23] Laemli, U.K. (1970) Nature 227, 680–685.

[24] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

[25] Gemmel, C.G., Goutcher, C., Reid, R. and Sturrock, D. (1997) Role of certain virulence factors in a murine model of *Staphylococcus aureus* arthritis. J. Med. Microbiol. 46, 208–213.

[26] Krull, M., Dold, C., Hippenstiel, S., Rosseau, S., Lohmeyer, J. and Suttorp, N. (1996) *Escherichia coli* hemolysin and *Staphylococcus aureus* α-toxin potently induce neutrophil adhesion to cultured human endothelial cells. J. Immunol. 157, 4133–4140.

[27] Abdelnour, A.T., Bremell, T., Holmdahl, R. and Tarkowski, A. (1994) Clonal expansion of T lymphocytes causes arthritis and mortality in mice infected with toxic shock syndrome toxin-1-producing staphylococci. Eur. J. Immunol. 24, 1161–1166.

[28] Abdelnour, A., Bremell, T. and Tarkowski, A. (1994) Toxic shock syndrome toxin 1 contributes to the arthrritogenicity of *Staphylococcus aureus*. J. Infect. Dis. 170, 94–99.

[29] Abdelnour, A., Bremell, T., Holmdahl, R. and Tarkowski, A. (1994) Role of T lymphocytes in experimental *Staphylococcus aureus* arthritis. Scand. J. Immunol. 39, 403–408.

[30] Gjertsson, I., Hultgren, H.O., Stenson, M., Holmdahl, R. and Tarkowski, A. (2000) Are B lymphocytes of importance in severe *Staphylococcus aureus* infection. Infect. Immun. 68, 2431–2434.

[31] Nilsson, I.M., Verdrengh, M., Uirich, G.R., Bavari, S. and Tarkowski, A. (1999) Protection against *Staphylococcus aureus* sepsis by vaccination with recombinant staphylococcal enterotoxin A devoid of superantigenicity. J. Infect. Dis. 180, 1370–1373.

[32] Ferens, W.A., Davis, W.C., Hamilton, M.J., Park, Y.H., Deobold, C.F., Fox, L. and Bohach, G. (1998) Activation of bovine lymphocyte subpopulations by staphylococcal enterotoxin C. Infect. Immun. 66, 573–589.

[33] Park, Y.H., Fox, L.K., Hamilton, M.J. and Davis, W.C. (1993) Suppression of proliferative response of BoCD4$^+$ T lymphocytes by activated BoCD8$^+$ T lymphocytes in the mammary gland of cows with *Staphylococcus aureus* mastitis. Vet. Immunol. Immunopathol. 36, 137–151.

[34] Leitner, G., Lubashevsky, E., Nachmeas, E., Glickman, A., Winkler, M., Saran, A. and Trainin, Z. (2000) Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows: Animal model, field trials and therapeutic effect. In: Proceedings of Symposium on immunology of the ruminant mammary gland (Zecconi, A., Ed.), pp. 418–425. Stresa, Italy.