Pathogenicity of *Staphylococcus aureus* phage type 3A/3C/55/71 and *Staphylococcus sciuri* in germfree euthymic and athymic mice after intravenous infection

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The clinical importance of *Staphylococcus aureus* to humans and animals is well documented. On the other hand, it is not long ago that the coagulase-negative staphylococci (CNS) were regarded as apathogenic but there is now no doubt that they play an important role as pathogens in immunocompromised patients and those with implanted prosthetic devices (Fleer & Verhoff, 1984). An increasing number of publications deal with this problem which is for example reviewed by Pulverer (1985) and Pulverer *et al.* (1987).

Except for *S. epidermidis* (Cohn, 1962; De Navasquez, 1950; Smith & Dubois, 1956; Yoshida *et al.*, 1976, Baddour *et al.*, 1987; Costerton *et al.*, 1987), little is known about the pathogenicity of CNS for laboratory animals. It is becoming increasingly evident that there is a need to evaluate the health risk of CNS for SPF animals.

S. sciuri formerly *S. sciuri* sub. *sciuri* (Schleifer *et al.* 1983) is considered to be a rodent specific *Staphylococcus* due to its wide distribution in this family (Kloos, 1980; Wullenweber-Schmidt *et al.* 1987) but its pathogenic potential, particularly for immunoincompetent rodent strains, has not yet been determined.

This organism could be of special interest since it might, as a component of rodent commensal flora analogous to the human *S. epidermidis,* act as antagonist to *S. aureus. S. aureus* (phage type 3A/3C/55/71) causes losses due to pyogenic infections in our NMRI and NMRI-nu colonies, and we therefore established a separate SPF colony of the nude mice also colonised with *S. sciuri* (Wullenweber-Schmidt et al., 1988) to test this possibility under field conditions.

The purpose of this study was to compare the pathogenicity of *S. sciuri* with *S. aureus* (phage type 3A/3C/55/71) in germfree NMRI euthymic and athymic mice. Both staphylococcal strains are widely spread in our rodent colonies.

MATERIAL AND METHODS

Staphylococcal strains

The *Staphylococcus aureus* strain originated from a liver abscess of a Han:NMRI mouse. Phage typing which was kindly done by Dr. W. Lenz, University of Bonn, revealed the pattern 3A/3C/55/71 (100 × RTD).

The *Staphylococcus sciuri* strain 908/1 was isolated from the upper respiratory tract of a healthy Han:NMRI mouse.

Cultivation of bacterial strains and preparation of inoculum

Freeze-stored (-70°C) cell preparations were cultivated overnight at 37°C on Columbia agar (Oxoid, 4230 Wesel, F.R.G.) containing 5% (v/v) sheep blood, and subcultures were prepared the following day. After overnight incubation, cells were harvested by rinsing the agar with sterile saline. Thereafter, bacterial titers were adjusted by turbidity measurement (OD at 546 nm) and viable counts were done on Columbia agar from appropriate dilutions in saline.

Animals and experimental design

Three to four months old, germfree, female, euthymic Han:NMRI and athymic Han:NMRI-nu/nu were used to compare the pathogenicity of *S. aureus* and *S. sciuri.* Each group contained 5 equally distributed litter mates.

Appropriate bacterial suspensions of 0.1 ml were administered i.v. (tail vein), control animals got 0.1 ml sterile saline only (Tables 1 and 2).

Table 1. Comparative pathogenicity of *S. aureus* (3A/3C/55/71) and *S. sciuri* after i.v.application into germfree, female Han:NMRI mice.

Species	Inf.dose(cfu)	Dead mice/total	Death (dpi)	purul.Infl./total
S. aureus	8×10^7	5/5	2-4	5/5
	4×10^7	4/5	5-6	5/5
	1×10^7	2/5	3	4/5
	4×10^4	1/5	7	1/5
	1×10^4	0/5		0/5
S. sciuri	4×10^7	0/5		0/5
	2×10^7	0/5		0/5
	0.5×10^7	0/5		0/5
	4×10^4	0/5		0/5
	0.5×10^4	0/5		0/5

Inf. dose = infection dose; cfu = colony forming unit;
dpi = day post infection; purul. Infl. = purulent inflammation.

During the 8 day experimental period, the mice were kept in sterile cages with filterhoods in Trexler isolators at room temperature. Sterile diet (type Han MR 5, Altromin, 4937 Lage, F.R.G.) and water were fed ad libitum. Moribund animals were sacrificed during the experiment, surviving mice at the end of the assay.
According to our guidelines (Anonymus, 1986) material from the following body sites was examined bacteriologically: conjunctiva, nares, trachea, lung, liver, kidney, spleen, heart, vagina, blood, urine and caecal contents as well as from pyogenic lesions. Bacterial concentrations were evaluated semi-quantitatively.
Sections of lung, liver, heart, kidney and spleen, stained with haematoxylin and eosin, were examined following routine histological preparation.

Results

The results of the infection experiment with *S. aureus* phage type 3A/3C/55/71 and *S. sciuri* in germfree, female Han:NMRI mice are shown in Table 1. *S. aureus*-infected mice exhibited a dose-dependent morbidity and mortality. Deaths occurred between day 2 and 7 post infection (dpi) and were associated with massive abscess formation in the kidneys and occasionally in the myocardium, lungs and the peritoneum. Moreover, *S. aureus* could be recovered in high numbers from all internal organs and body fluids tested. Surviving animals injected with 4 resp. 1×10^4 cfu were usually free from *S. aureus* with regard to internal organs, blood and urine, but exhibited high bacterial concentrations in the caecum which was the only intestinal segment examined bacteriologically. The mucosa of the upper respiratory tract and vagina were moderately colonized.
In comparison, *S. sciuri*-injection (Table 1) neither caused disease nor death. At the end of the experiment (dpi 8), *S. sciuri* was not isolated from internal organs but could be detected

Table 2. Comparative pathogenicity of *S. aureus* (3A/3C/55/71) and *S. sciuri* after i.v.application into germfree, female Han:NMRI-nu/nu mice.

Species	Inf.dose(cfu)	Dead mice/total	Death (dpi)	purul.Infl./total
S aureus	1×10^7	5/5	3-6	5/5
	1×10^6	4/5	5-6	5/5
	4×10^4	0/5	7	3/5
S sciuri	1×10^7	0/5		2/5
	1×10^6	0/5		0/5
	4×10^4	0/5		1/5

Abbreviations see Table 1.

in the caecum. Vaginal and respiratory mucosa were uniformly and densely colonized.

Similar results were obtained with athymic mice (Han:NMRI-nu/nu) as shown in Table 2. *S. aureus*-infected animals again showed a dose-dependent morbidity and mortality. As in the euthymic mice, all diseased animals had abundant abscess formation in the kidneys and 7 out of 10 mice of the 10^7 and 10^6-group had abscesses of the myocardium. Furthermore, most of the mice derived from these two groups displayed haemorrhagic alterations of the gut which were not observed in the euthymic mice. None of the *S. sciuri*-infected Han:NMRI-nu/nu died during the experiment but, in three animals, pathological processes could be detected by histological examination. Two mice from the highest concentration group had a nephritis and a pyelitis respectively. In the latter case, *S. sciuri* could be isolated in high numbers from the kidneys. In the lowest concentration group, one animal showed a mild bronchitis but no bacteria were isolated.

In general, the recovery of staphylococci from different body sites was the same as in the experiment with the euthymic mice.

A statistical evaluation of comparable bacterial concentrations (1×10^7, 4×10^4) by means of the χ^2-test could only confirm low significant differences in the mortality between *S. aureus*-infected Han:NMRI and Han:NMRI-nu/nu mice at an infective dose of 1×10^7 cfu. Differences concerning the morbidity are not significant.

Control mice of both strains which received sterile saline only did not show clinical signs of disease. The bacteriological examination at the end of the experiment could confirm their germfree status.

Discussion

The main objective of this study was to get an indication of the pathogenic potential of *S. sciuri* in two distinct types of outbred mice in order to see whether a strain of this staphylococcal species might be included in our association flora to overcome health problems due to *S. aureus* phage type 3A/3C/55/71 (lysogroup II). This strain causes health problems in our NMRI and NMRI-nu/nu mice, particularly abscesses and mastitis but also sporadically spontaneous cases of purulent arthritis (paper in preparation) could be diagnosed.

Bacteriological monitoring of our rodent colonies which include healthy and diseased animals leads to the conclusion that *S. sciuri* is apathogenic but so far we know the pathogenicity of this species hat not yet been investigated in the mouse or rat until this study was conducted.

To our knowledge, the only experimental study of the pathogenicity of *S. sciuri* was done by Miedzobrodzki *et al.* (1985) who investigated the virulence of 34 strains of coagulase-negative staphylococci (CNS) after inoculation into the allantoic cavity in 8-day-old chick embryos. The LD_{50} (as $\log_{10}$ number of bacteria/ml) at 48 hours for two different *S. sciuri* strains was 5.18 resp. 6.18. In comparison, *S. aureus* WOOD 46 had an LD_{50} of 2.30 and *S. aureus* strain 316 one of 6.90, representing the two extremes of the scale. Moreover, the heterogenous reaction patterns in this model indicate that there is no direct relation between the taxonomic position of the CNS and their virulence. Obviously, strain specificities have to be taken into account.

With regard to other CNS, Yoshida *et al.* (1976) could demonstrate strain differences in the pathogenicity of *S. epidermidis* in female DD-mice after i.p. infection. Cohn (1962) found out that NCS-mice infected i.p. with high doses of a strain (»Greaves«) of *S. epidermidis* (»*S. albus*«) survived the infection.

The pathogenicity of *S. aureus* after natural and experimental infection is already well documented, but remarkable strain differences exist. Karczewska *et al.* (1985) found out that 90% of mice died within 3 days post i.p. infection with S. *aureus* strain »Smith diffuse« whereas the »Smith compact« strain was harmless. Easmon & Glynn (1976) showed the importance of the capsule and alpha-hemolysin as pathogenicity factors in the mouse model.

In 1963, Gorrill and McNeil produced similar results to ours, using *S. aureus* NCTC 8354. They demonstrated that about 90% of i.v. in-

fected »albino mice« died within 24 h when challenged with 2×10^8 cfu, and 60% died within 14 days post infection with 1×10^7 cfu. In agreement with our results, they also showed that peak organ counts of *S. aureus* were obtained from the kidneys, most probably due to a multiplication in this organ.

Trends in differences of susceptibility to *S. aureus* infection between NMRI and NMRI-nu/nu can be shown in our experiments, although the use of statistical methods is limited, since the groups were relatively small. Whereas 5 out of 5 nude mice died after exposure to *S. aureus* with 1×10^7 cfu, only 2 out of 5 NMRI mice died. On the other hand, none of the NMRI-nu/nu died after infection with 4×10^4 cfu but one of the NMRI did. The situation is reversed with regard to the development of pygenic processes. Also, the haemorraghic alterations of the intestine after *S. aureus* infection could only be seen in the nude mice.

In conclusion, it seems that the immundeficiency of the nude mouse strain is responsible for its greater susceptibility to an infection with *S. aureus*. In contrast, the *S. sciuri* strain 908/1 can be regarded as largely apathogenic after i.v. infection, and this is supported by our observations derived from the routine monitoring of our rodent colonies. *S. sciuri* has therefore been incorporated into our association flora for SPF mice.

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Summary

To evaluate the possibility of protecting our colonies of small laboratory animals against *Staphylococcus aureus* infections by preassociation with the rodent specific *Staphylococcus sciuri,* it was first of all necessary to determine the pathogenicity of the *S. sciuri* strain under consideration. Germfree euthymic Han:NMRI and athymic (T-cell deficient) Han:NMRI-nu/nu mice were injected intravenously with increasing doses of *S. sciuri* strain 908/1. *S. aureus* (phage type 3A/3C/55/71) which is known to cause health problems in SPF colonies of the above mentioned strains of mice was used for comparison. *S. aureus* infections exhibited a dose-dependent morbidity and mortality in both strains of mice. In contrast, *S. sciuri* did not cause any lethal infection but produced sporadically mild purulent processes in the euthymic mice. Due to the use of relatively small groups a statistical evaluation can only show tendencies.

Sammendrag

For at undersøge muligheden for at beskytte vore kolonier af små forsøgsdyr mod *Staphylococcus aureus* infektioner ved præassociering med den gnaverspecifikke *Staphylococcus sciuri* var det først og fremmest nødvendigt at bestemme patogeniciteten af den valgte *S. sciuri* stamme. Kimfri euthymiske Han:NMRI og athymiske Han:NMRI-nu/nu mus blev injiceret i.v. med stigende doser af *S. sciuri* stamme 908/1. *S. aureus* (phag type 3A/3C/55/71), som vides at forårsage sundhedsproblemer i SPF-kolonier i de ovennævnte musestammer, anvendtes til sammenligning.

S. aureus infektion udviste en dosisafhængig morbiditet og mortalitet i begge musestammer. I modsætning hertil forårsagede *S. sciuri* ikke letale infektioner, men kun sporadiske milde purulente processer i de euthymiske mus. På grund af lille gruppestørrelse kan den statistiske evaluering kun vise tendenser.

Yhteenveto / K. Pelkonen

Tässä työssä tutkittiin mahdollisuutta pienjyrsijäkoloniat Staphylococcus aureus-infektioilta assosioimalla ne ensin jyrssijäspesifisellä Staphylococcus sciwrilla. Ensin piti kwitenkin määrittää käytetyn S. sciurikannan patogeenisyys. Mikrobivapaisiin eutyymisiin Han:NMRI-hiiriin ja atyymisiin (T-solut puuttuvat) Han:NMRI-nu/nu-hiriin injisoitiin i. v. nousevia annoksia S. Sciuri kantaa 908/1. S. aureus (faagityyppi 3A/3C/55/71) kantaa, jonka tiedetiin aiheuttavan terveysongelmia edellämainittujen kantojen SPF-kolonioissa, käytettiin vertailukantana.

S. aureus infektiot aiheuttivat annosriippuvaisen sairastuvuuden ja kuolevuuden kummassakin hiirikannassa. Sitävastoin S. sciuri ei aiheuttanut tappavia infektioita, mutta satunaisesti lieviä märkäisiä paiseita eutyymisissä hiirissä. Pienestä eläinmäärästä johtuen tilastollinen analyysi antoi vain viitteitä merkitsevistä eroista.

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