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Subcutaneous infection with *S. aureus* in mice reveals association of resistance with influx of neutrophils and Th2 response.

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Short title: Immune response to *S. aureus* infection in mice

Abbreviations: bmDC, bone marrow-derived dendritic cells; CBA, cytometric bead array; n. s., not significant; PMN, polymorphonuclear leukocytes; RPL, ribosomal protein L9; *S. aureus, Staphylococcus aureus*
ABSTRACT

*S. aureus* is the leading cause of bacterial skin infection. Once it overcomes the epithelial barrier it either remains locally controlled or spreads in the dermis causing soft tissue infection. These different courses depend not only on its virulence factors, but also on the immune response of the infected individual.

The goal of this study was to identify host factors which influence different outcomes. We therefore established comparative analysis of subcutaneous footpad infection with *S. aureus* (SH1000) in different inbred mouse strains. We found that C57BL/6 are more susceptible than BALB/c and DBA/2 mice, reflected by significantly higher footpad swelling and bacterial load, as well as increased dissemination of bacteria into inguinal lymph nodes and kidneys. This susceptibility was associated with lower influx of polymorphonuclear leukocytes, but higher secretion of CXCL-2. Remarkably, resistance correlated with *S. aureus*-specific Th2-cell response in BALB/c and DBA/2 mice, while susceptible C57BL/6 mice generated a Th1-cell response.

Since Th1-cells are able to induce release of CXCL-2, and since CXCL-2 is able to increase survival of *S. aureus* within PMN, interactions between PMN and Th1- or Th2-cells need to be considered as important mechanisms of resistance in murine soft tissue infection with *S. aureus*. 
INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is a leading cause of both hospital- and community-acquired infections worldwide causing significant morbidity and mortality (Diekema et al., 2001; Klevens et al., 2007; Lowy, 1998). The anterior nares are the principle human habitat of *S. aureus* serving as endogenous reservoir for overt clinical infections (von Eiff C. et al., 2001). The pathogen causes a broad spectrum of infections including those of skin and soft tissues ranging from superficial skin infections to serious and life-threatening deep tissue infections. The potential danger of *S. aureus* is aggravated by the increase of methicillin-resistant strains (Boucher and Corey, 2008; Kock et al., 2009).

The primary cause of skin infection is often a breach in the epidermis due to macro- or microtrauma (e.g. surgical/puncture wounds, chronic ulcers). After *S. aureus* has reached the subepidermal space it either is locally controlled or progresses within the dermis leading to soft tissue infection. These different courses depend on virulence of *S. aureus*, but also on the strength of the host’s defense. At this stage polymorphonuclear leukocytes (PMN) and macrophages have been shown to be mandatory to combat *S. aureus*-infection (Anwar et al., 2009; Gresham et al., 2000; Molne et al., 2000), because defects in their recruitment or function result in high susceptibility to soft tissue infections with *S. aureus* (Abramson et al., 1981; Liese et al., 2000; Segal et al., 2000). However, it is not known why in patients without apparent dysfunctions of PMN, *S. aureus* progresses to soft tissue infection in some, while remaining locally controlled in others.

A promising approach to reveal mechanisms of resistance is to compare immune responses between resistant and susceptible animals. Such murine models have yielded several novel insights in natural resistance in e.g. experimental leishmaniasis (Sacks and Noben-Trauth,
2002; Sacks and Sher, 2002), *Streptococcus pneumoniae*-induced pneumonia (Gingles et al., 2001) or *Pseudomonas aeruginosa*-induced keratitis (Hazlett et al., 2000).

So we have exploited a model of subcutaneous footpad infection with *S. aureus* for a comparative analysis of natural resistance in different inbred mouse strains and could indeed dissect novel, patterns important for immune response against *S. aureus* infection. As such we revealed that higher resistance in BALB/c and DBA/2 mice correlates with higher influx of PMN and also with a *S. aureus*-specific Th2-cell response, while susceptibility in C57BL/6 mice was associated with higher CXCL-2-expression and a Th1-cell response.
RESULTS

Host-specific factors influence the immune response to skin infection with *S. aureus*

**Footpad thickness**

To evaluate the influence of host-specific immune response to infection with *S. aureus*, we injected $2 \times 10^7$ colony forming units (CFU) of *S. aureus* strain SH1000 subcutaneously into the footpad of 3 different inbred mouse strains (BALB/c, C57BL/6, DBA/2). Swelling of footpads was monitored as a measure of severity of local infection (Figure 1a) reflecting the inflammatory response of edema and infiltrate (Sunderkotter et al., 1993; Titus et al., 1985). Within 6 days after infection, C57BL/6 mice developed significantly higher footpad thickness than BALB/c and DBA/2 mice. This significant difference continued throughout the infection as swelling reaction in BALB/c and DBA/2 mice began to decline after 6 days, while footpads reached maximum thickness from day 6 to 11 in C57BL/6 mice before decreasing after 12 days. Normal values were reached after 25 days in BALB/c and DBA/2, and after 30 days in C57BL/6 mice (data not shown). Control mice injected with PBS showed no significant swelling reaction.

**Bacterial load**

To determine whether stronger footpad swelling would correlate with higher load of *S. aureus*, we quantified bacterial loads in infected footpads. At day 7 post infection, there were significantly more bacteria in footpads of C57BL/6 mice than in those of BALB/c and DBA/2 mice (Figure 1b).

We next studied dissemination of *S. aureus* to draining lymph nodes and systemic organs. In all 3 strains, bacteria drained to popliteal lymph nodes (Figure 1c) and beyond. C57BL/6 mice continued to reveal higher bacterial loads as they contained significantly more bacteria in inguinal lymph nodes than BALB/c and DBA/2 mice 2 days after infection (Figure 1d).
Evaluating systemic dissemination, we detected low, but significantly higher bacterial loads in kidneys (Figure 1e) and liver (data not shown) of C57BL/6 than of BALB/c and DBA/2 mice 2 days after infection. Until 16 days after subcutaneous infection bacterial loads gradually decreased in systemic organs in all mouse strains, reflecting that none of the mice died of infection with \(2 \times 10^7\) CFU of *S. aureus* strain SH1000. Control mice injected with PBS did not contain *S. aureus* in different tissues (data not shown).

Thus, BALB/c and DBA/2 mice are more resistant than C57BL/6 mice because they showed faster elimination of *S. aureus* from footpads and lower bacterial loads in inguinal lymph nodes and kidneys.

**Acute inflammatory response correlates with higher influx of neutrophils, but not with higher local expression of CXCL-1 and CXCL-2**

Since presence of PMN is mandatory to combat skin infection with *S. aureus* (Anwar et al., 2009; Molne et al., 2000), we wondered if differences in resistance could be associated with differences in influx of PMN.

Utilizing activity of myeloperoxidase (MPO) in infected skin as a parameter for the number of neutrophils (Bradley et al., 1982), we found that MPO activity was significantly stronger in DBA/2 (7 and 48 hours after infection) and in BALB/c mice (48 hours after infection) than in C57BL/6 mice (Figure 2a/b), indicating a significantly higher number of PMN in footpads of DBA/2 and BALB/c mice. To confirm these findings, we performed immunohistochemical analysis. *S. aureus*-infected footpads of BALB/c and DBA/2 mice showed significantly higher absolute numbers of Ly-6G\(^+\)-cells (PMN marker) than infected footpads of C57BL/6 mice 48 hours after infection (Figure 3). In contrast, no significant differences were detected with regard to the numbers of F4/80\(^+\)-cells (monocytes/macrophages) and CD4\(^+\)- or CD8\(^+\)-T-cells (data not shown).
Thus, higher resistance in BALB/c and DBA/2 mice was associated with higher and more rapid influx of PMN in the first 48 hours of infection compared to C57BL/6 mice.

To examine, if this relevant difference in PMN influx could be explained e.g. by differential expression of chemokines we examined early local expression of PMN attracting chemokines in infected skin. A continuous increase in expression of CXCL-1-mRNA was observed in BALB/c, C57BL/6 and DBA/2 mice from 2 to 7 hours after infection. Number of RNA copies declined in all strains to base line value within 48 hours (Figure 4a). Therefore, there was no unequivocal correlation between a significant difference in higher PMN infiltration between BALB/c and C57BL/6 or DBA/2 mice and CXCL-1-expression in these mice over the first 2 days of infection. In contrast, CXCL-2-mRNA levels were significantly higher in C57BL/6 than in BALB/c and DBA/2 mice after 48 hours of infection (Figure 4b). We confirmed this result on protein level for CXCL-2 which was significantly up-regulated in C57BL/6 compared to BALB/c and DBA/2 mice (Figure 4c).

Thus, the lower influx of PMN was associated with a significantly higher expression of CXCL-2 in susceptible C57BL/6 mice compared to both resistant strains after 48 hours.

**CD4\(^+\)-T-cell response to skin infection with *S. aureus***

Since *S. aureus* infection persisted more than 2 weeks in all three mouse strains and, thus, into a time frame where adaptive immunity forms, we analyzed CD4\(^+\)-T-cell response to *S. aureus* 16 days after infection. CD4\(^+\)-T-cells from popliteal and inguinal lymph nodes of BALB/c, C57BL/6 and DBA/2 mice were isolated and co-cultured with syngeneic, antigen-presenting dendritic cells (bmDC) pulsed with heat-killed *S. aureus* or unstimulated bmDC in vitro. Subsequently, we analyzed proliferation and antigen-specific cytokine production of CD4\(^+\)-T-cells (Figure 5). While CD4\(^+\)-T-cells from all 3 strains showed an antigen-specific increase in proliferation (data not shown), they displayed differential profiles of cytokines upon re-
stimulation *in vitro*. The Th1-cytokine IFN-γ was produced in similar amounts by CD4⁺-T-cells among different mouse strains (Figure 5a). In contrary, CD4⁺-T-cells from infected BALB/c and DBA/2 mice produced high amounts of Th2-cytokines IL-4 and IL-13, while CD4⁺-T-cells from infected C57BL/6 mice released only minimal amounts (Figure 5b/c). This resulted in a IL-4/IFN-γ ratio of 12 for DBA/2 CD4⁺-T-cells and of 2 for BALB/c CD4⁺-T-cells, while C57BL/6 CD4⁺-T-cells yielded a IL-4/IFN-γ ratio of only 0.06. The latter is characteristic for Th1-cells, while the ratio of CD4⁺-T-cells from BALB/c and DBA/2 mice presents a Th2-profile (Ehrchen et al., 2008). Levels of IL-17 were similarly induced in all 3 strains (Figure 5d). Control experiments with either bmDC or CD4⁺-T-cells alone pulsed with heat-killed *S. aureus* confirmed that cytokine production was entirely due to antigen-presentation by bmDC to CD4⁺-T-cells (data not shown).

Thus, more resistant strains (BALB/c, DBA/2) displayed a Th2-cell response, while more susceptible C57BL/6 mice showed a Th1-cell response towards *S. aureus*, suggesting a beneficial role of Th2-cell response for the host in *S. aureus*-induced skin infection.
DISCUSSION

Different resistance patterns in skin infection with *S. aureus*

Utilizing a mouse model for subcutaneous soft tissue infection with *S. aureus* we revealed for the first time that different inbred mouse strains markedly differ in their susceptibility to subcutaneous *S. aureus*-infection. This phenomenon was reflected by significantly stronger footpad swelling and increased dissemination of bacteria into inguinal lymph nodes, liver and kidneys. Higher resistance in BALB/c and DBA/2 mice correlated with more rapid influx of PMN and a *S. aureus*-specific Th2-cell response compared to lesser influx of PMN, higher expression of CXCL-2 and a *S. aureus*-specific Th1-cell response in more susceptible C57BL/6 mice.

Our model of soft tissue infection with *S. aureus* demonstrates that comparison of immune responses between resistant and susceptible hosts is able to dissect mechanisms of innate and adaptive immunity which are potentially decisive on resistance.

Murine models for *S. aureus*-induced skin infection (Miller et al., 2006; Rich and Lee, 2005), have not been exploited yet for comparative analysis of natural resistance in different inbred mouse strains such as in this study. We preferred a model of footpad infection compared to models of dorsal subcutaneous or wound infections (Chavakis et al., 2005; Goova et al., 2001; McLoughlin et al., 2006; Miller et al., 2006; Molne et al., 2000; Mori et al., 2002), because the unidirectional drainage to popliteal and inguinal lymph nodes allows better assessment of dissemination of bacteria. The SH1000 strain was chosen for this study because it has a complete virulence repertoire due to a restored *rsbU* gene in the sigB stress response pathway of *S. aureus* (Horsburgh et al., 2002) and it has been previously used in *S. aureus* infection in mouse models *in vivo* (Miller et al., 2006) and in human culture systems (Clarke et al., 2007). Yet, our model has also proven to be suitable to compare different courses of
infections in mice due to different virulence of various *S. aureus* strains (e.g. between *rsbU*<sup>+</sup>- and *rsbU*-deficient *S. aureus* strains, data not shown).

**Lower influx of PMN, but higher expression of CXCL-2 in more susceptible C57BL/6 mice**

It is generally accepted that PMN are part of the first line of defense against *S. aureus* and are critical for resolution of infection (Molne et al., 2000; Veldkamp et al., 1997). We and others have not found strain-specific differences in killing activity between PMN from C57BL/6, BALB/c and DBA/2 mice (data not shown) (von Kockritz-Blickwede et al., 2008). Thus, it is the higher influx of PMN rather than differences in killing potential which accounts for different resistance patterns. We found no correlation between the higher influx of PMN and the increased expression of CXCL-1. Thus, other not yet identified mechanisms must be involved in mediating the differential influx of PMN in our infection model. Regarding CXCL-2, it was significantly higher expressed in susceptible C57BL/6 mice. It may rather bear relevance for function than for recruitment of PMN, as it had been shown to increase intracellular survival of *S. aureus* in PMN in murine wound infections (McLoughlin et al., 2006). Correspondingly, CXCL-2 was shown to cause increased bacterial burden when applied during advanced intraperitoneal *S. aureus*-infection in mice (Gresham et al., 2000). It may thus present a susceptibility factor towards *S. aureus* in several infection models.

**Relevance of innate and adaptive immunity in skin infection with *S. aureus***

This is the first report of a *S. aureus*-specific and dichotomous CD4<sup>+</sup>-T-cell-response in subcutaneous infection, featuring a Th1-cell-response in the susceptible strain (C57BL/6), and a Th2-cell-response in resistant strains (BALB/c, DBA/2). Although C57BL/6 mice are generally more prone to develop Th1-responses, and BALB/c or DBA/2 mice to generate Th2-responses in response to microbial agents, this does not apply for all infections (e.g.
Candida albicans or Strongyloides venezuelensis). While it had been demonstrated that CD4+ T-cells were shown to exert deleterious influence on the immune response against *S. aureus* in wound infections, it was not demonstrated that this CD4+ T-cell-response was specific for *S. aureus*, let alone that it involved either Th1- or Th2-cells (McLoughlin et al., 2006; McLoughlin et al., 2008). These studies, however, already suggested an inhibitory interaction between CD4+ T-cells, PMN and *S. aureus* since they demonstrated (i) that IFN-γ released by CD4+ T-cells increases CXCL-2-production in wound tissue once the inflammatory infiltrate was established, and (ii) that this chemokine then exerts permissive effect on *S. aureus* in PMN (McLoughlin et al., 2006; McLoughlin et al., 2008). Since Th2-cell-derived IL-4 and IL-13 are known to inhibit IFN-γ-dependent processes one could speculate that Th2-cells inhibit the local, IFN-γ-dependent CXCL-2-production and its permissive effect on *S. aureus* in PMN.

Mechanisms which contribute to the Th1/Th2-cell dichotomy in subcutaneous infection of mice with *S. aureus* remain to be elucidated. Yet, the higher influx of PMN may present a factor by which the innate immune response influences adaptive immunity, because in experimental leishmaniasis an early, stronger presence of PMN is crucial for generation of the ensuing Th2-response in BALB/c mice (Tacchini-Cottier et al., 2000).

**Host-specific differences in *S. aureus* infections of other murine tissues**

There are only few comparative studies on natural resistance against *S. aureus* in different mouse strains. In a keratitis model C57BL/6 were more resistant than BALB/c mice. However, identical to our model, this resistance correlated with an initially higher influx of PMN, while a specific CD4+ T-cell response was not analyzed (Hume et al., 2005). A recently described model of intravenous infection with *S. aureus* revealed higher resistance in C57BL/6 and higher susceptibility in BALB/c and DBA/2 mice (von Kockritz-Blickwede et
al., 2008). This represents a reciprocal outcome compared to our model of subcutaneous infection. Yet, during intravenous infection, resistance also depended on rapid presence of PMN in internal organs, and was independent of T-cells, which may be due to the rapid course of intravenous compared to subcutaneous infection (von Kockritz-Blickwede et al., 2008).

These results highlight the decisive importance of the immunological milieu at the site of infection. The milieu differentially influences PMN and/or CD4$^+$-T-cell functions and subsequently the course of *S. aureus* infection. The different courses of infection could be due to (i) the differing potential to generate a specific CD4$^+$-T-cell response, and/or (ii) the local inflammatory cytokine/chemokine milieu determined by resident cells at the site of infection, which influences recruitment and function of both PMN and CD4$^+$-T-cells (Metz and Maurer, 2009). Apparently, it makes a difference, whether *S. aureus* disseminates to internal organs only after it has first passed through epithelial barrier and draining lymph nodes, or after it has gained direct access to the blood, e.g. by needle or medical intervention.

In summary, differences in susceptibility and resistance to *S. aureus* in various infection models could critically depend on the route of infection and on location of interaction between PMN and CD4$^+$-T-cells. These differences also demonstrate that therapy and prophylactic measures must be tailored according to the site and route of infection.
MATERIALS AND METHODS

Bacterial strains

*S. aureus* strain SH1000 is a derivate of strain 8325-4 with restored *rsbU*-gene (Horsburgh et al., 2002). *S. aureus* SH1000 was streaked onto 5% sheep-blood-agar (BD Biosciences, Heidelberg, Germany). Colonies were grown overnight at 37°C in a shaking incubator (200 rpm) in tryptic soy broth (TSB). Mid-logarithmic-phase bacteria were obtained after 3-hour-subculture of a 1/100 dilution of overnight culture. Bacteria were pelleted, washed three times, and resuspended in phosphate buffered saline (PBS). Aliquots of *S. aureus* suspension were frozen at -80°C for further use. Number of viable bacteria (CFU) was verified by plating dilutions of the inoculum onto blood agar overnight. Heat-killed bacteria were obtained by boiling *S. aureus* suspension at 100°C for 15 minutes.

Mice

8-12-week-old BALB/c, C57BL/6, DBA/2 wild type (wt) mice were purchased from Harlan-Winkelmann (Borchen, Germany) and kept under specific-pathogen-free (SPF) conditions at the animal facility of University of Münster. All animal studies were performed with the approval of the State Review Board of Nordrhein-Westfalen (Germany).

Infection model

Mice were inoculated subcutaneously with $2 \times 10^7$ CFU of *S. aureus* into the left hind footpad. Control animals were injected with PBS. Groups of 4–5 mice were used for each experiment (n=2-3). Footpad swelling was measured daily with a micrometric caliper and specific footpad swelling was assessed by subtracting the diameter of injected (left) from non-injected (right) footpad. Mice were euthanized by CO$_2$ asphyxiation at indicated time points. Footpad
tissue, systemic organs (liver, kidney, lymph nodes) or blood were homogenized in PBS and plated after serial dilutions on blood agar to determine bacterial loads. Colonies were counted after incubation overnight at 37°C.

**Myeloperoxidase Assay**

Myeloperoxidase (MPO) activity from skin lesion was obtained from tissue homogenates of *S. aureus* or PBS-injected footpads. Tissue was pulverized in liquid nitrogen and then homogenized in 1 ml homogenization-buffer (0.5% hexadecyl-trimethyl-ammonium-bromide, 5 mM EDTA in modified PBS [w/o Ca$^{2+}$, Mg$^{2+}$, pH=6.0]. Homogenates were centrifuged at 12,000 x g for 20 minutes at 4°C. Supernatants were analyzed in doublets using 10 µl mixed with 100 µl 3,3',5,5'-tetramethylbenzidine (TMB) in a 96-well-plate (Nunc, Langenselbold, Germany). Reaction was stopped with 100 µl 0.2 N H$_2$SO$_4$ after 5 minutes. Absorbance was measured at 450 nm in a microplate reader (Dynatech Laboratories, Denkendorf, Germany). Values were normalized to tissue weight.

**Generation of DC and isolation of CD4$^+$-T-cells**

Dendritic cells (bmDC) were generated from bone-marrow cells as reported previously (Krummen et al., 2006; Varga et al., 2007; Varga et al., 2010). Purity was tested by CD11c-staining (anti-CD11c, BD Biosciences, Heidelberg, Germany) and assessed by FACS. After 5 days part of bmDC was incubated with heat-killed bacteria (bacteria : DC = 5:1) for 24 hours. Before co-culture experiments bmDC were harvested and washed twice in PBS.

For isolation of CD4$^+$-T-cells popliteal and inguinal lymph nodes of mice were taken 16 days after infection. CD4$^+$-T-cells were separated by MACS technology (magnetic cell sorting, Miltenyi Biotech, Bergisch-Galdbach, Germany) using CD4$^+$-T-cell Isolation Kit (Miltenyi Biotech, Bergisch-Galdbach, Germany) according to the manufacturer’s instructions.
Resulting CD4\(^+\)-T-cells were > 95% pure according to FACS analysis of CD4-staining (anti-CD4 [L3T4], BD Pharmingen, Heidelberg, Germany). For co-culture experiments CD4\(^+\)-T-cells were used with bmDCs at a ratio of 40:1. After 7 days supernatants were taken for analysis.

For FACS analysis cells were stained with 1 µg of indicated antibodies (30 min, 4°C), and analyzed using FACSCalibur equipped with CellQuestPro software (BD Biosciences, Heidelberg, Germany).

**Cytokine and Chemokine Determination**

Production of IFN-\(\gamma\), IL-13, IL-4, IL-17 was analyzed from supernatants of co-cultures using CBA FlexSet technology (BD Biosciences, Heidelberg, Germany) according to the manufacturer’s instructions and measured by FACS.

CXCL-2 (MIP-2) production was determined from lavage of feet. Dissected feet were flushed with PBS. CXCL-2 (MIP-2) was determined using ELISA (R&D, Wiesbaden-Nordenstadt, Germany) according to the manufacturer’s protocol.

**RNA Isolation, Reverse Transcription, Quantitative real-time PCR**

RNA isolation was done as described (Ehrchen et al., 2010), quality and quantity was determined using UV/vis-spectral photometer NanoDrop ND-1000 (Peqlab, Erlangen, Germany) and the microfluidics system (Agilent 2100 Bioanalyzer, Agilent Technologies).

Gene-expression was confirmed by quantitative real-time PCR (RT-PCR) as described before (Ehrchen et al., 2007). Following primers were used: RPL: 5’-TGG TCC CTG CTC TCA AG-3’, 5’-GGC CTT TTC CTT CCG TTT CTC-3’; CXCL-1: 5’-GCG AAA AGA AGT GCA GAG AGA TAG AG-3’, 5’-CGT GCG TGT TGA CCA TAC A AT ATG-3’; CXCL-2: 5’-GTC CCT CAA CGG AAG AAC CAA-3’, 5’-ACT CTC AGA CAG CGA GGC ACA T-
3’. Relative expression was normalized to housekeeping gene ribosomal protein L9 (RPL) as endogenous control.

**Histology**

Immunohistochemical staining was performed as described previously (Sunderkötter et al., 1993). Briefly, footpads were fixed with formalin for staining with antibodies to Ly-6G and F4/80 (BD Pharmingen, Heidelberg, Germany), or frozen for staining for CD4 and CD8 (Serotec, Düsseldorf, Germany). In four random fields of four sections per group total numbers of cells (data not shown) and absolute numbers of positively stained cells were determined in blinded fashion using light microscope with ocular grid (Zeiss Axioskop, Göttingen, Germany). Percentages of positive stained cells were calculated compared to total numbers of cells.

**Statistical analysis**

All data are expressed as mean ± SEM (standard error of mean). Each experiment was performed independently at least three times. Data were compared with Student’s t-test. Values of *p < 0.05, **p < 0.001 were considered statistically significant.
CONFLICT OF INTEREST

The authors state no conflict of interest.

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FIGURE LEGEND

Figure 1. Susceptibility to *S. aureus*-induced skin infection in inbred mouse strains.

Different inbred mouse strains were inoculated s. c. with 2x10⁷ CFU of *S. aureus* strain SH1000 or PBS. 

a) Time course of footpad swelling of *S. aureus*-infected BALB/c (open squares), C57BL/6 (black squares) and DBA/2 (grey squares) mice is shown. 

b) - d) Bacterial loads in systemic organs of *S. aureus*-infected BALB/c (open bars), C57BL/6 (black bars) and DBA/2 (grey bars) mice at different time points after infection. 

b) Bacterial loads of *S. aureus*-infected footpads, c) draining popliteal lymph nodes d) and inguinal lymph nodes of *S. aureus*-infected mice. 

e) Bacterial loads in kidneys of *S. aureus*-infected mice. Bars represent the mean +/- SEM of ten mice per group of three independent experiments. *p<0.05; **p<0.001.

Figure 2. Acute inflammatory response to skin infection with *S. aureus*.

MPO activity was determined as a parameter for the amount of neutrophils in footpad tissue homogenates a) 7 hours and b) 48 hours after injection of 2x10⁷ CFU of *S. aureus* strain SH1000 or PBS into the footpad of BALB/c (open bars), C57BL/6 (black bars) and DBA/2 (grey bars) mice. Data represent the mean +/-SEM of 12 mice per group from three independent experiments. *p<0.05.

Figure 3. Influx of PMN in *S. aureus*-infected skin.

a) Representative sections of staining for Ly-6G of footpads 48 hours after infection with 2x10⁷ CFU of *S. aureus* strain SH1000 or PBS. 

b) Numbers of infiltrated Ly-6G⁺-cells per field. 

c) Percentage of Ly-6G⁺-cells compared to total numbers of cells. BALB/c (open squares), C57BL/6 (black squares) and DBA/2 (grey squares) mice. n = 4/group, *p<0.05. Scale bar = 0.2 mm.
Figure 4. **Expression of chemokines in *S. aureus*-infected tissue.**

Time course of a) CXCL-1- and b) CXCL-2-mRNA-expression in footpad tissue of BALB/c (open squares), C57BL/6 (black squares) and DBA/2 (grey squares) mice s.c. injected with $2 \times 10^7$ CFU of *S. aureus* strain SH1000. PCR data were normalized to RPL expression and relative copies and mean +/-SEM were calculated of nine mice per group of three independent experiments. c) Secreted CXCL-2 concentration in lavage of footpad tissues of BALB/c (open bars), C57BL/6 (black bars) and DBA/2 (grey bars) mice 48 hours after infection with $2 \times 10^7$ CFU of *S. aureus* strain SH1000 or PBS. Results represent mean +/-SEM of nine mice per group of three independent experiments. * p<0.05.

Figure 5. **Cytokine production by CD4$^+$-T-cells isolated from *S. aureus*-infected mice.**

CD4$^+$-T-cells were isolated from draining lymph nodes of *S. aureus*-infected BALB/c (open bars), C57BL/6 (black bars) and DBA/2 (grey bars) mice 16 days after infection and co-cultured with syngeneic bmDC stimulated with heat-killed *S. aureus* (SH1000 AG) (ratio of 40:1 CD4$^+$-T-cells : bmDCs) or unstimulated syngeneic bmDC (w/o AG). After 7 days the cytokine production was assessed from supernatants of the co-cultures. a) IFN-γ, b) IL-4 and c) IL-13 d) IL-17. Results represent the mean +/-SEM of ten mice per group of three independent experiments. * p<0.05.