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# Mast cells are activated by *Staphylococcus aureus in vitro* but do not influence the outcome of intraperitoneal *S. aureus* infection *in vivo*

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## Summary

*Staphylococcus aureus* is a major pathogen that can cause a broad spectrum of serious infections including skin infections, pneumonia and sepsis. Peritoneal mast cells have been implicated in the host response towards various bacterial insults and to provide mechanistic insight into the role of mast cells in intraperitoneal bacterial infection we here studied the global effects of *S. aureus* on mast cell gene expression. After co-culture of peritoneal mast cells with live *S. aureus* we found by gene array analysis that they up-regulate a number of genes. Many of these corresponded to pro-inflammatory cytokines, including interleukin-3, interleukin-13 and tumour necrosis factor-α. The cytokine induction in response to *S. aureus* was confirmed by ELISA. To study the role of peritoneal mast cells during *in vivo* infection with *S. aureus* we used newly developed Mcpt5-Cre[+] × R-DTA mice in which mast cell deficiency is independent of c-Kit. This is in contrast to previous studies in which an impact of mast cells on bacterial infection has been proposed based on the use of mice whose mast cell deficiency is a consequence of defective c-Kit signalling. *Staphylococcus aureus* was injected intraperitoneally into mast-cell-deficient Mcpt5-Cre[+] × R-DTA mice using littermate mast-cell-sufficient mice as controls. We did not observe any difference between mast-cell-deficient and control mice with regard to weight loss, bacterial clearance, inflammation or cytokine production. We conclude that, despite peritoneal mast cells being activated by *S. aureus in vitro*, they do not influence the *in vivo* manifestations of intraperitoneal *S. aureus* infection.

**Keywords:** bacterial infection; inflammation; mast cells; *Staphylococcus aureus*.

## Introduction

Mast cells are situated at the host environment interface and they are equipped with a multitude of pathogen-recognition receptors. As such they are well equipped to respond promptly to invading pathogens.[1] *Staphylococcus aureus* is generally a commensal bacteria that persistently colonizes the anterior nares of 20% of the human population.[2] Invasive *S. aureus* infection is generally a result of a break in the epithelial barriers, which allows the pathogen to invade underlying tissue.[3] After invasion of the organism, *S. aureus* can cause serious and life threatening infections including skin infections, pneumonia and sepsis.[4]

Previous studies have shown that *S. aureus* can internalize and persist within bone marrow-derived mast cells (BMMCs)[5] and that BMMCs exert anti-microbial activity against *S. aureus* by releasing extracellular traps and antimicrobial compounds.[5] Moreover, mast cells degranulate and release tumour necrosis factor-α (TNF-α) in response to *S. aureus* and it has been demonstrated that *S. aureus*-infected BMMCs have antimicrobial activity.[5] *Staphylococcus aureus* can also invade human cord blood-derived mast cells, causing TNF-α and interleukin-8 (IL-8) release.[6] However, although the above-mentioned studies have shown that mast cells respond to *S. aureus* infection, there is a lack of knowledge of the global impact of

*S. aureus* on mast cell function. Moreover, previous studies have mainly been performed using relatively immature mast cells, e.g. BMMCs, whereas the effect of *S. aureus* on terminally differentiated mast cells has been less studied. To provide a deeper understanding of how mast cells respond to infection by *S. aureus* we here chose to study the global effects of *S. aureus* on gene expression in peritoneal cell-derived mast cells (PCMCs), which is an emerging model for studies of terminally differentiated mast cells.[7] This analysis revealed extensive induction of a large number of genes in mast cells co-cultured with live *S. aureus*, many of which corresponded to cytokines such as IL-3, IL-13 and TNF-α.

Using mice that lack mast cells due to defective c-Kit signalling, e.g. Kit[W/W−v] and Kit[W−sh/W−sh] mice, mast cells have been shown to be protective against a number of different bacterial infections. In most of these studies, a role for the peritoneal mast cell population in combating bacterial infection has been suggested, in particular using the caecal ligation and puncture model.[8–15] Recently, mice in which mast cell deficiency is independent of defective c-Kit signalling have been developed.[16,17] In studies on these mice some of the findings based on using c-Kit-defective mice have been confirmed, including the role of mast cells in allergic airway hyper-responsiveness. However, there are also some conflicting data between the c-Kit-dependent and -independent mast-cell-deficient mice, e.g. with regard to the proposed role of mast cells in autoimmunity, leading to a need to also re-evaluate the role of mast cells in bacterial infections.[16,17] To provide new insight into the role of mast cells in intraperitoneal bacterial infection, we here used Mcpt5-Cre[+] × R-DTA mice in which mast cells are lacking due to mast cell-specific expression of diphtheria toxin.[18] We demonstrate that there was no difference in the course of intraperitoneal *S. aureus* [strain 8325-4 (*Φ*11)] infection in mast-cell-deficient compared with littermate mast-cell-sufficient control animals. This indicates that, despite peritoneal mast cells being activated *in vitro* by *S. aureus*, they do not influence the course of peritoneal *S. aureus* infection *in vivo*.

## Materials and methods

### Peritoneal cell-derived mast cells

The PCMCs were established according to a protocol described by Malbec *et al.*[7]

### Mice

Mast cell-deficient Mcpt5-Cre[+] × R-DTA mice were used, as described.[18] Littermate Mcpt5-Cre[−] × R-DTA mice were used as controls. All animal experiments were approved by the local ethics committee (no C118/11).

### Staphylococcus aureus culture

Mice (strain C57BL/6) were infected intraperitoneally with *S. aureus* 8325-4 (*Φ*11).[19] After post mortem examination the strain was re-isolated and frozen. The *S. aureus* strain was streaked on a horse blood agar plate, incubated 37° overnight and then inoculated in 20 ml tryptone soya broth (TSB) at 37°. After overnight incubation, 200 μl was transferred to 20 ml fresh TSB and the culture was grown at 37° until the optical density at 600 nm ($OD_{600}$) reached 0·5.

### In vitro co-culture of PCMCs and S. aureus

The PCMCs were washed twice in PBS and resuspended in antibiotic-free medium at a density of $1 \times 10^6$ cells/ml and plated in 24-well tissue plates. The bacteria were washed twice in PBS and added to a final concentration of ~$2\cdot5 \times 10^7$ cells/ml; multiplicity of infection (MOI) 1 : 25. Four hours after infection, cells were collected by centrifugation. Media and cell fractions were frozen and stored at −20°.

### In vivo infection

Mice were injected intraperitoneally with 100 μl TSB medium containing ~$5 \times 10^7$ *S. aureus*. Controls were injected with 100 μl TSB medium. Body weight was monitored every day. After 4 hr, 1 day or 3 days, the mice were killed and peritoneal lavage was performed with 5-ml Tyrodes buffer. To determine the colony-forming units (CFU) in the peritoneal lavage fluid, the fluid was plated onto Baird–Parker agar medium plates (Merck, White House Station, NJ) supplemented with Egg Yolk Tellurite emulsion (Oxoid, Basingstoke, UK). The cells in the peritoneal wash were counted. Cytospins of the peritoneal cells were stained with May–Grünwald/Giemsa and differential counts were performed. The cells in the peritoneal wash were pelleted by centrifugation; the supernatant was collected and frozen at −20°.

### RNA preparation and Affymetix microarray

Total RNA from $1 \times 10^6$ cultured cells was isolated by using NucleoSpin RNA II (Macherey-Nagel, Düren, Germany). The RNA quality was evaluated with the Agilent 2100 Bioanalyzer system. The microarray analysis was performed at the Uppsala Array Platform (Uppsala, Sweden) as previously described.[20]

### Cytokine array and ELISAs

Secretion of cytokines was determined using RayBio® Mouse Cytokine Antibody Array 3 (RayBiotech, Inc., Norcross, GA) according to the manufacturer's instructions.

ELISAs for IL-3, IL-13, TNF-α (Peprotech, Rocky Hill, NJ), monocyte chemoattractant protein-1 (MCP-1) and IL-6 (eBioscience, San Diego, CA) were performed according to the manufacturer's instructions.

### Statistical analysis

Data are shown as means ± standard error of the mean. Statistical analyses were performed by using GraphPad Prism 4.0c (GraphPad Software, La Jolla, CA) and unpaired Student's *t*-test for two-tailed distributions (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

## Results

### Live *S. aureus* induces a strong pro-inflammatory response in cultured peritoneal mast cells

Most of the previous studies addressing the role of mast cells in anti-bacterial responses have mainly used relatively immature mast cells, such as BMMCs or various mast-cell-like cell lines of murine or human origin.[1,5,6] Moreover, many previous studies have investigated the effects of purified bacterial products on mast cells, rather than investigating the effects of live bacteria. Here we sought to clarify the impact of bacteria on mast cells in a physiologically more relevant setting, by examining the effect of live *S. aureus* on mature mast cells of peritoneal origin, i.e. PCMCs.

To investigate the global effect of *S. aureus* on mast cell gene expression, live *S. aureus* were co-cultured with PCMCs. After 4 hr the cells were collected, followed by RNA extraction and Affymetrix microarray analysis. As displayed in Table 1, 52 genes were significantly up-regulated with a higher than 2 log2-fold change (Table 1). Of these, several corresponded with cytokines and chemokines, indicating that *S. aureus* induces a strong pro-inflammatory response in mast cells (Table 1). The cytokine IL-3 was the gene that was induced to the highest extent of all genes, with a 5·35 log2-fold change (Fig. 1). To confirm the induction of pro-inflammatory cytokines at the protein level, ELISA was performed. As shown in Fig. 1, the expression and secretion of IL-3, IL-13 and TNF-α were confirmed by ELISA; all of these cytokines were undetectable in the medium of control cells but clearly detectable after co-culture of PCMCs with *S. aureus* (Fig. 1). Together, these findings reveal a profound induction of pro-inflammatory genes in mature mast cells exposed to live *S. aureus*.

### Mast cells do not influence the clearance of *S. aureus* in vivo

To investigate the role of mast cells in peritoneal *S. aureus* infection *in vivo*, Mcpt5-Cre⁺ × R-DTA were used, with littermate Mcpt5-Cre⁻ × R-DTA mice as mast-cell-sufficient control animals. Mast-cell-deficient and control mice were injected i.p. with *S. aureus*. To follow the course of infection, the change in body weight was monitored. As shown in Fig. 2(a), the infection with *S. aureus* caused an initial weight drop up to day 2. However, after this initial weight loss, the mice regained normal weight, suggesting recovery from infection. Notably, there was no significant difference in the weight loss or time of recovery when comparing mast-cell-sufficient and -deficient mice. To monitor the effect of mast cells on efficiency of bacterial clearance, infected mice were killed after either 4 hr, 1 day or 3 days. Clearance of *S. aureus* in the peritoneum was examined after performing peritoneal lavage, followed by plating of the fluid and quantification of CFU. This analysis showed the presence of bacteria in the peritoneum 4 hr and 1 day after infection, but there were no significant differences in the amounts of bacteria when comparing mast cell-sufficient and -deficient mice. After 3 days, bacteria were not detected in the peritoneum of either mast cell-sufficient- or deficient mice (Fig. 2b). Together, these data indicate that mast cells are dispensable for clearance of peritoneal *S. aureus* infection.

### Mast cells do not influence the inflammation in response to peritoneal *S. aureus* infection *in vivo*

Although the data above indicated that mast cells are dispensable for clearance of peritoneal *S. aureus* infection, it cannot be excluded that mast cells affect the inflammatory response at levels not necessarily manifested by differences in efficiency of bacterial clearance. To investigate the impact of mast cells on the inflammatory response, peritoneal cell populations from *S. aureus*-infected mice were recovered, followed by counting of the total number of cells, staining with May–Grünwald/Giemsa and differential counting (Fig. 3). Four hours after *S. aureus* infection, a profound increase in the number of peritoneal cells was observed compared with non-infected control animals. This was due to a significant increase in the number of neutrophils. However, there were no significant differences in the numbers of recruited neutrophils when comparing mast cell sufficient- and deficient mice. After 3 days, the number of neutrophils had declined to baseline levels and this decline was accompanied by an increase in peritoneal monocytes/macrophages. No differences in numbers of monocytes/macrophages or other peritoneal cell populations were observed at this time-point when comparing the mast-cell-sufficient and -deficient mice (Fig. 3). As expected, mast cells were readily detected in the peritoneal exudate of the Mcpt5-Cre⁻ × R-DTA mice but were undetectable in peritoneum of Mcpt5-Cre⁺ × R-DTA littermates (Fig. 3b).

**Table 1.** Genes showing significant ($P < 0.05$) up-regulation after co-culture of peritoneal cell-derived mast cells and *Staphylococcus aureus* for 4 hr

| Probe set ID | Gene title | Gene symbol | log2-fold change | *P*-value |
|---|---|---|---|---|
| 10385918 | Interleukin 3 | Il3 | 5·35 | 9·78E–06 |
| 10427035 | Nuclear receptor subfamily 4, group A, member 1 | Nr4a1 | 5·28 | 3·56E–07 |
| 10560481 | FBJ osteosarcoma oncogene B | Fosb | 4·93 | 1·26E–05 |
| 10450369 | Heat-shock protein 1A | Hspa1a | 4·62 | 1·01E–05 |
| 10397346 | FBJ osteosarcoma oncogene | Fos | 4·27 | 3·34E–06 |
| 10504838 | Nuclear receptor subfamily 4, group A, member 3 | Nr4a3 | 4·15 | 3·80E–06 |
| 10450367 | Heat-shock protein 1B/heat-shock protein 1A | Hspa1b/1a | 3·99 | 1·02E–05 |
| 10385837 | Interleukin 13 | Il13 | 3·78 | 4·30E–05 |
| 10449284 | Dual specificity phosphatase 1 | Dusp1 | 3·57 | 6·49E–05 |
| 10565819 | Solute carrier organic anion transporter family, member 2b1 | Slco2b1 | 3·54 | 1·28E–05 |
| 10482772 | Nuclear receptor subfamily 4, group A, member 2 | Nr4a2 | 3·48 | 2·17E–06 |
| 10545588 | Hexokinase 2 | Hk2 | 3·34 | 0·00059 |
| 10545130 | Growth arrest and DNA-damage-inducible 45α | Gadd45a | 3·33 | 6·13E–05 |
| 10584580 | Small nucleolar RNA, C/D box 14E | Snord14e | 3·28 | 2·75E–05 |
| 10536794 | RIKEN cDNA 2310016C08 gene | 2310016C08Rik | 3·23 | 0·00032 |
| 10520862 | Fos-like antigen 2 | Fosl2 | 3·21 | 2·01E–05 |
| 10451198 | Vascular endothelial growth factor A | Vegfa | 3·17 | 0·00026 |
| 10584576 | Heat-shock protein 8/small nucleolar RNA, C/D box 14D/small nucleolar RNA, C/D box 14C | Hspa8/Snord14d/14c | 3·14 | 1·55E–05 |
| 10352448 | Dual specificity phosphatase 10 | Dusp10 | 3·08 | 0·00021 |
| 10580282 | Jun-B oncogene | Junb | 3·08 | 0·00062 |
| 10449741 | Salt-inducible kinase 1 | Sik1 | 2·98 | 0·00015 |
| 10551891 | Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, delta | Nfkbid | 2·91 | 0·00051 |
| 10373912 | Oncostatin M | Osm | 2·85 | 0·00031 |
| 10531073 | UDP glucuronosyltransferase 2 family, polypeptide B38 | Ugt2b38 | 2·78 | 0·04599 |
| 10515399 | Polo-like kinase 3 (*Drosophila*) | Plk3 | 2·76 | 2·43E–05 |
| 10350516 | Prostaglandin-endoperoxide synthase 2 | Ptgs2 | 2·71 | 2·41E–05 |
| 10550906 | Plasminogen activator, urokinase receptor | Plaur | 2·62 | 9·61E–05 |
| 10508723 | Small nucleolar RNA, H/ACA box 61 | Snora61 | 2·60 | 0·00229 |
| 10489204 | Transglutaminase 2, C polypeptide | Tgm2 | 2·56 | 0·00024 |
| 10377439 | Period homologue 1 (*Drosophila*) | Per1 | 2·55 | 0·00012 |
| 10545200 | Similar to Igk-C protein | LOC100046894 | 2·55 | 0·02605 |
| 10597758 | Cysteine-serine-rich nuclear protein 1 | Csrnp1 | 2·53 | 2·82E–05 |
| 10546450 | A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 9 | Adamts9 | 2·53 | 0·00519 |
| 10361091 | Activating transcription factor 3 | Atf3 | 2·52 | 0·00011 |
| 10389231 | Chemokine (C-C motif) ligand 3 | Ccl3 | 2·49 | 0·00015 |
| 10450501 | Tumour necrosis factor | Tnf | 2·48 | 0·00044 |
| 10472923 | Adenylate kinase 3-like 1 | Ak3 l1 | 2·48 | 6·94E–05 |
| 10358408 | Regulator of G-protein signalling 1 | Rgs1 | 2·38 | 1·37E–05 |
| 10374197 | Receptor (calcitonin) activity modifying protein 3 | Ramp3 | 2·35 | 0·00067 |
| 10531057 | UDP glucuronosyltransferase 2 family, polypeptide B5 | Ugt2b5 | 2·31 | 0·04142 |
| 10379518 | Chemokine (C-C motif) ligand 7 | Ccl7 | 2·30 | 1·29E–05 |
| 10364030 | Adenosine A2a receptor | Adora2a | 2·30 | 1·66E–05 |
| 10429926 | Diacylglycerol O-acyltransferase 1 | Dgat1 | 2·25 | 0·02014 |
| 10520452 | Interleukin 6 | Il6 | 2·21 | 0·00863 |
| 10523156 | Chemokine (C-X-C motif) ligand 2 | Cxcl2 | 2·20 | 0·00217 |
| 10373918 | Leukaemia inhibitory factor | Lif | 2·19 | 0·00071 |
| 10563659 | SPT2, Suppressor of Ty, domain containing 1 (*Saccharomyces cerevisiae*) | Spty2d1 | 2·13 | 9·43E–05 |
| 10368277 | Ribosomal protein S12 | Rps12 | 2·07 | 0·00381 |
| 10503334 | GTP-binding protein (gene over-expressed in skeletal muscle) | Gem | 2·06 | 0·00154 |
| 10523547 | 1-acylglycerol-3-phosphate O-acyltransferase 9 | Agpat9 | 2·05 | 0·04722 |
| 10478890 | CCAAT/enhancer binding protein (C/EBP), β | Cebpb | 2·05 | 0·00246 |
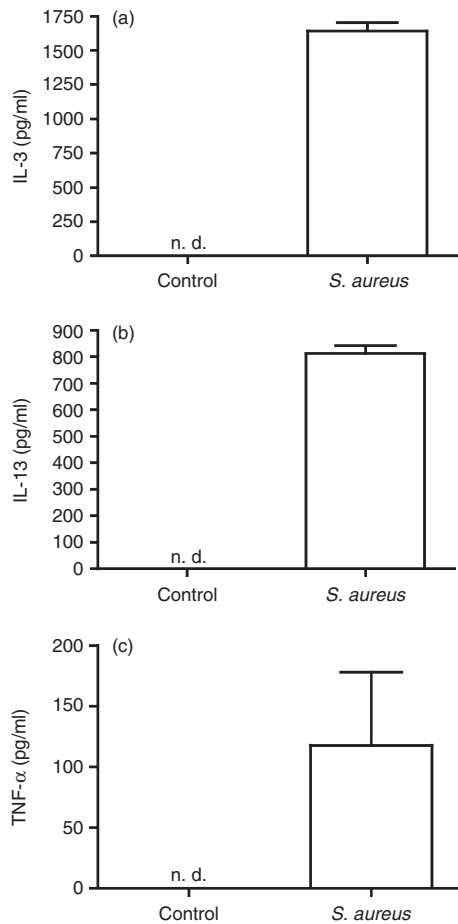
**Figure 1.** Co-culture of peritoneal cell-derived mast cells (PCMCs) and *Staphylococcus aureus* induces release of cytokines. PCMCs ($1 \times 10^6$ cells/ml) were cultured either alone (control) or together with *S. aureus* (MOI = 25) At 4 hr, medium samples were collected and analysed for content of interleukin-3 (IL-3) (a), IL-13 (b) or tumour necrosis factor-$\alpha$ (TNF-$\alpha$) (c) by ELISA. Mean $\pm$ SEM ($n = 3$).

### Mast cells do not influence the cytokine induction in response to peritoneal *S. aureus* infection *in vivo*

Since the co-culture of peritoneal mast cells with *S. aureus* was shown to induce a robust cytokine response (see Fig. 1 and Table 1), it was also of interest to investigate whether the peritoneal mast cell population contributes to the total cytokine output in response to infection of mice with *S. aureus*. For this purpose we used cytokine/chemokine membrane arrays to obtain an unbiased view of the cytokine profile induced by the bacterial infection. As seen in Fig. 4, *S. aureus* infection caused elevated levels of various cytokines in the peritoneum, including IL-6, macrophage inflammatory protein-1$\gamma$, granulocyte colony-stimulating factor, MCP-1 and sTNF RI but no difference was seen between the mast-cell-sufficient and -deficient mice. To verify these results, ELISA for IL-6 and MCP-1 was performed. This analysis demonstrated a significant up-regulation of these cytokines at 4 hr after infection and
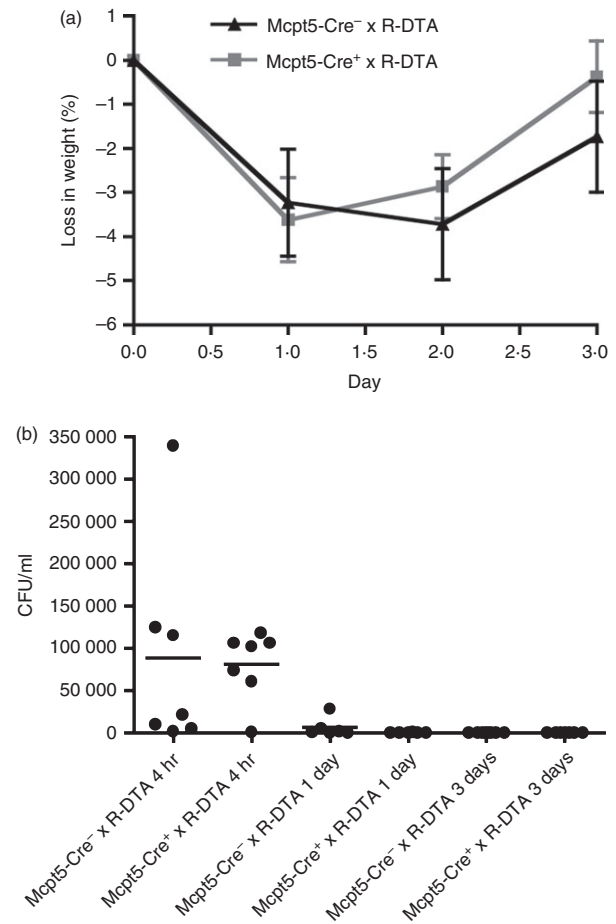


**Figure 2.** Mast cells do not influence the weight loss of *Staphylococcus aureus*-infected mice or the clearance of *S. aureus*. (a) Mcpt5-Cre$^+$ $\times$ R-DTA and Mcpt5-Cre$^-$ $\times$ R-DTA were injected intraperitoneally with ~5 $\times$ 10$^7$ CFU of *S. aureus* and monitored for change in weight. As a control, TSB medium only (bacterial growth medium) was injected. (b) After 4 hr, 1 day or 3 days, the mice were killed and peritoneal lavage was performed with subsequent determination of *S. aureus* counts (CFU) in the lavage fluid. Mean $\pm$ SEM ($n = 7$ to $n = 11$). Results shown are a representative of four independent experiments.

confirmed that there were no differences in the levels of IL-6 or MCP-1 as a consequence on mast cell presence or absence (Fig. 5a,b). In addition, we analysed for possible effects of mast cells on the levels of IL-3 and IL-13, i.e. two of the cytokines that were profoundly induced upon co-culture of mast cells with *S. aureus in vitro* (see Table 1, Fig. 1). However, as displayed in Fig. 5(c,d), no significant differences in the levels of these cytokines were seen due to the absence or presence of mast cells.

### Discussion

It has been shown that mast cells produce certain cytokines, e.g. TNF-$\alpha$ and IL-6, in response to *S. aureus*.[5,6,21] Here we extend these findings by unbiased technology
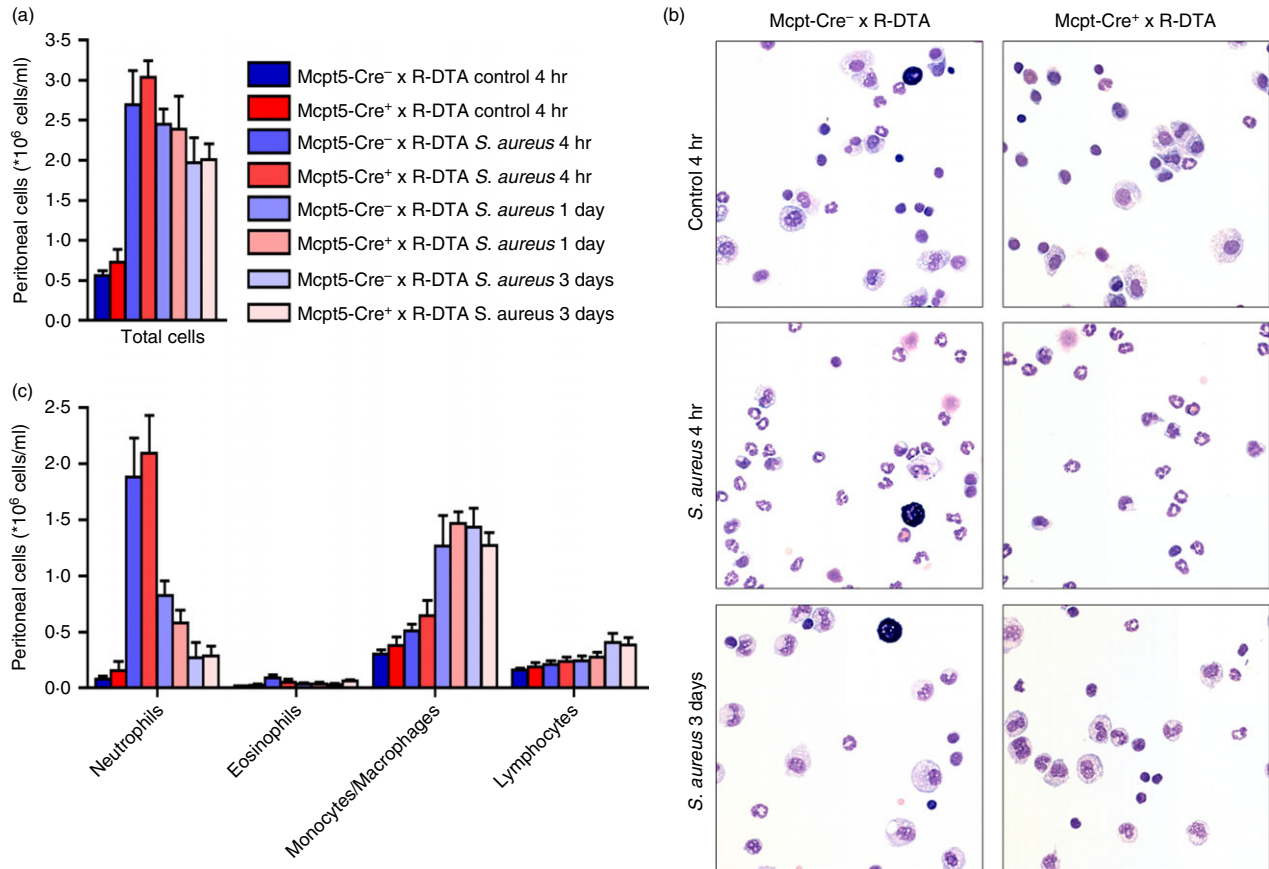
**Figure 3.** Mast cells do not influence the inflammation after *Staphylococcus aureus* infection. Mcpt5-Cre[+] × R-DTA and Mcpt5-Cre[−] × R-DTA were infected intraperitoneally with *S. aureus*. As a control, TSB medium only (bacterial growth medium) was injected. After either 4 hr, 1 day or 3 days, peritoneal lavage was performed. The cells in the peritoneal lavage were counted (a), stained with May–Grünwald/Giemsa (b) and differential counts were performed (c). Mean ± SEM ($n = 5$ to $n = 11$). Results shown are a representative of four independent experiments.

and show that peritoneal mast cells also up-regulate a number of additional pro-inflammatory compounds when encountering *S. aureus*, including IL-3, IL-13, Oncostatin M, Lif, and chemokines Ccl3, Ccl7 and Cxcl2. Among other genes, we note a profound up-regulation of all members of the Nr4a family of nuclear receptors, i.e. Nr4a1, -2 and -3 in response to live *S. aureus*. We previously showed that all of these genes were strongly up-regulated in more immature mast cells, i.e. BMMCs, which were exposed to either group C streptococci or to IgE receptor cross-linking.[22] Hence, the findings presented here indicate that a profound up-regulation of the Nr4a family members is a general consequence of mast cell activation in response to a broad range of activating stimuli, and in mast cells of different states of maturity. We also note, similarly to our previous findings on BMMCs activated by either IgE receptor cross-linking or by group C streptococci,[23] that PCMCs exposed to *S. aureus* display a strong up-regulation of the gene for ADAMTS9, a metalloprotease implicated in extracellular matrix remodelling.

Based on the strong induction of numerous cytokines, e.g. IL-3 and IL-13, in PCMCs co-cultured with *S. aureus*

*in vitro*, we anticipated that the absence of mast cells may cause a reduction in the levels of these cytokines following intraperitoneal infection of mice with *S. aureus*. In particular, because the mast cells used for the *in vitro* experiments were mature and of peritoneal origin, i.e. having a phenotype closely resembling that of the mast cells of the peritoneal cavity *in vivo*,[7] we anticipated that the i.p. infection with *S. aureus* would affect the peritoneal mast cells in a fashion similar to that observed in the *in vitro* co-culture setting. Intriguingly though, we did not see any effects of mast cell deficiency on the levels of these cytokines. Furthermore, the absence of mast cells did not affect the levels of a range of additional cytokines, including IL-6 and MCP-1. Hence, mast cells do not contribute significantly to the total pool of these pro-inflammatory cytokines during intraperitoneal *S. aureus* infection *in vivo*. One potential explanation for these findings may be that although peritoneal mast cells may express these cytokines in response to *S. aureus in vivo*, their total output is negligible in comparison with contributions from other cells, e.g. monocytes/macrophages. In this context it is important to point out that mast cells represent a relatively minor population of
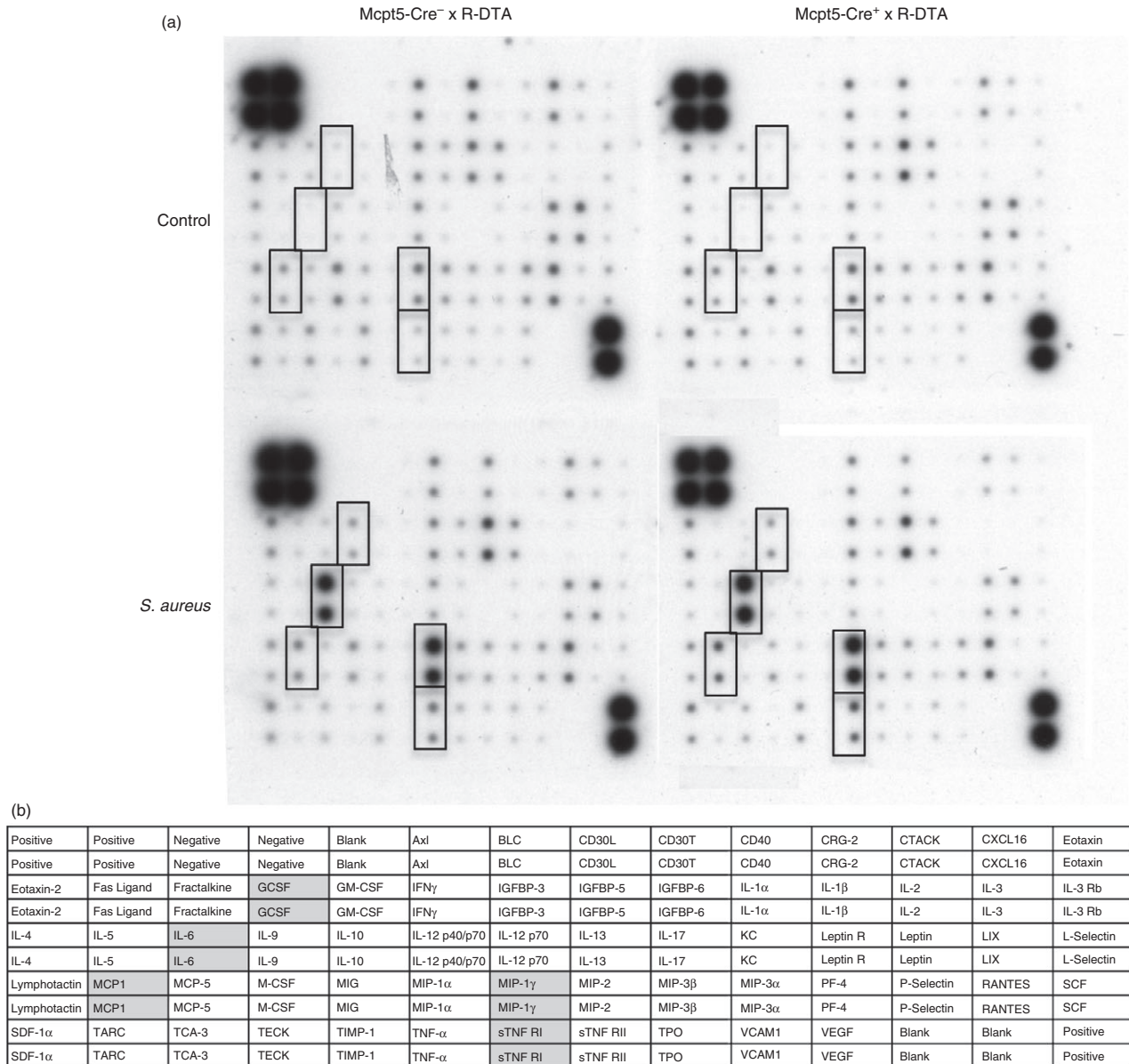
(a)

Mcpt5-Cre⁻ x R-DTA                    Mcpt5-Cre⁺ x R-DTA

Control

*S. aureus*



(b)

| Positive | Positive | Negative | Negative | Blank | Axl | BLC | CD30L | CD30T | CD40 | CRG-2 | CTACK | CXCL16 | Eotaxin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Positive | Positive | Negative | Negative | Blank | Axl | BLC | CD30L | CD30T | CD40 | CRG-2 | CTACK | CXCL16 | Eotaxin |
| Eotaxin-2 | Fas Ligand | Fractalkine | GCSF | GM-CSF | IFNγ | IGFBP-3 | IGFBP-5 | IGFBP-6 | IL-1α | IL-1β | IL-2 | IL-3 | IL-3 Rb |
| Eotaxin-2 | Fas Ligand | Fractalkine | GCSF | GM-CSF | IFNγ | IGFBP-3 | IGFBP-5 | IGFBP-6 | IL-1α | IL-1β | IL-2 | IL-3 | IL-3 Rb |
| IL-4 | IL-5 | IL-6 | IL-9 | IL-10 | IL-12 p40/p70 | IL-12 p70 | IL-13 | IL-17 | KC | Leptin R | Leptin | LIX | L-Selectin |
| IL-4 | IL-5 | IL-6 | IL-9 | IL-10 | IL-12 p40/p70 | IL-12 p70 | IL-13 | IL-17 | KC | Leptin R | Leptin | LIX | L-Selectin |
| Lymphotactin | MCP1 | MCP-5 | M-CSF | MIG | MIP-1α | MIP-1γ | MIP-2 | MIP-3β | MIP-3α | PF-4 | P-Selectin | RANTES | SCF |
| Lymphotactin | MCP1 | MCP-5 | M-CSF | MIG | MIP-1α | MIP-1γ | MIP-2 | MIP-3β | MIP-3α | PF-4 | P-Selectin | RANTES | SCF |
| SDF-1α | TARC | TCA-3 | TECK | TIMP-1 | TNF-α | sTNF RI | sTNF RII | TPO | VCAM1 | VEGF | Blank | Blank | Positive |
| SDF-1α | TARC | TCA-3 | TECK | TIMP-1 | TNF-α | sTNF RI | sTNF RII | TPO | VCAM1 | VEGF | Blank | Blank | Positive |

**Figure 4.** Mast cells do not influence the production of cytokines in the peritoneum after *Staphylococcus aureus* intraperitoneal infection. Mcpt5-Cre⁺ × R-DTA and Mcpt5-Cre⁻ × R-DTA were infected intraperiteonally with *S. aureus*. As a control, TSB medium only (bacterial growth medium) was injected. After either 4 hr, peritoneal lavage was performed. Peritoneal lavage fluid from three different animals in each group was pooled and analysed using cytokine membranes. Cytokines that were up-regulated in the infected mice are marked with rectangles (a). A scheme of the cytokine membrane; cytokines that were up-regulated in the infected mice are marked with grey (b).

the peritoneum, resident mast cells accounting for approximately 2% of the total peritoneal cells. Hence, even though mast cells may in fact express significant amounts of the respective cytokines, their relative contribution is too low to be detectable by the methods used here. Another potential explanation for the apparent discrepancy between the cytokine responses seen *in vivo* versus *in vitro* is that the mechanism of mast cell activation may differ between the *in vitro* situation and *in vivo*, such that genes that are up-regulated after contact with *S. aureus in vitro* may not necessarily be induced during the *in vivo* conditions.

Mast cells have been shown to be protective to a number of different bacterial infections, but we failed to see any significant contribution of mast cells in the course of peritoneal *S. aureus* infection. One potential explanation for this seeming discrepancy could be related to the fact that previous studies have been performed on Kit^{W/W−v} and Kit^{W−sh/W−sh} mice, in which the mast cell absence is caused by defective signalling through c-Kit. These mice have, in addition to being mast cell-deficient, a number of different abnormalities including complex alterations of many haematopoietic compartments. To prove that
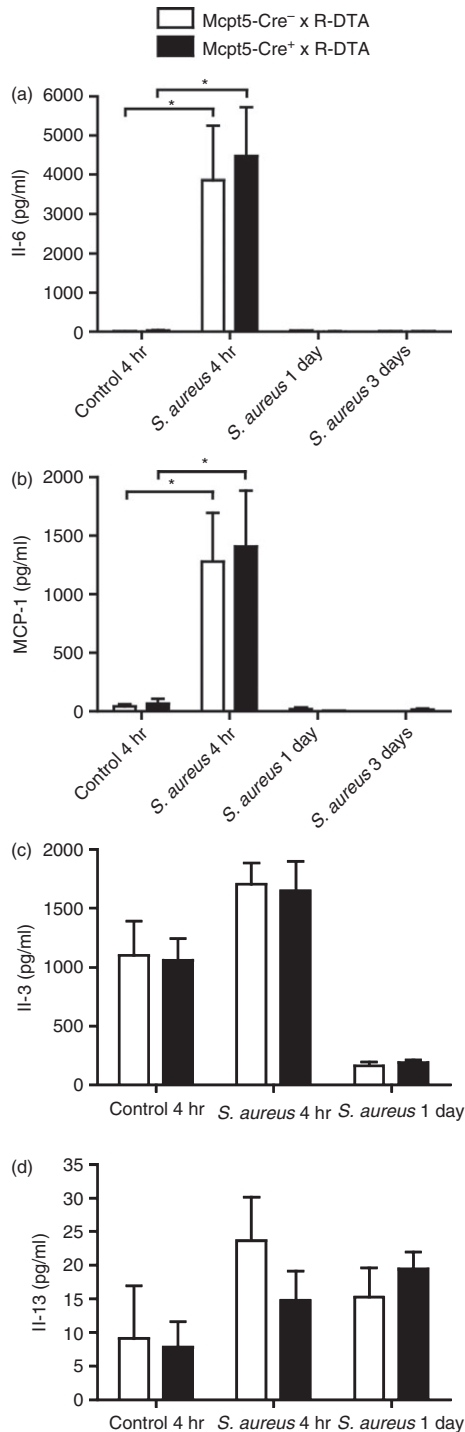
**Figure 5.** Mast cells do not influence the production of interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP-1), IL-3 and IL-13 in the peritoneum after *Staphylococcus aureus* infection. Mcpt5-Cre$^+$ × R-DTA and Mcpt5-Cre$^-$ × R-DTA were infected intraperitoneally with *S. aureus*. As a control, TSB medium only (bacterial growth medium) was injected. After 4 hr or 1 day, peritoneal lavage was performed. Peritoneal lavage fluids were analysed for levels of IL-6 (a), MCP-1 (b), IL-3 (c) and IL-13 (d) by ELISA. Mean ± SEM ($n = 5$ to $n = 11$). *$P < 0.05$.

any effects seen in Kit$^{W/W-v}$ and Kit$^{W-sh/W-sh}$ mice are in fact the result of their lack of mast cells rather than other effects of defective c-Kit, it has therefore been essential to reconstitute the mast cell niche of these mice and to show that this reverses the phenotype to that of mast-cell-sufficient mice. However, it is not certain that the distribution and function of the reconstituted mast cells will be a reflection of mast cells in wild-type mice.[17,24] Moreover, the recent development of c-Kit-independent mast-cell-deficient mice has provided some conflicting data in comparison with those generated based on Kit$^{W/W-v}$ and Kit$^{W-sh/W-sh}$ mice. For example, the role of mast-cell-derived IL-10 in contact hypersensitivity[18,25] and the role of mast cells in autoimmunity[26–28] has been questioned (reviewed in Rodewald *et al.*[17] and Reber *et al.*[16]). This has led to a need to re-evaluate a range of proposed mast cell functions derived from the c-Kit-defective mice, such as their protective role in bacterial infections. The data reported here may therefore question the importance of mast cells in the host defence towards bacterial infections. On the other hand, even though the present data did not reveal any significant role of mast cells in intraperitoneal *S. aureus* infection, we cannot exclude that mast cells can influence the host defence towards other pathogenic bacteria, or to bacterial infection occurring through routes of administration other than the peritoneum. Neither can we exclude that mast cells may have an impact on the host defence towards other strains of *S. aureus* than the one used here, for example strains of higher virulence. It is also important to emphasize that much of the previous work on this topic has been based on the caecal ligation and puncture model, a model of severe sepsis. In contrast, the model used in this study represents a milder course of disease, so we cannot exclude that a protective role of mast cells is more evident in severe than in mild infections.

In conclusion, we have shown that peritoneal mast cells are activated by *S. aureus in vitro* whereas they do not play a role in intraperitoneal *S. aureus* infection *in vivo*. However, to make more general conclusions about the bona fide role of mast cells in bacterial infection, it will be imperative to perform more extensive studies on the novel c-Kit-independent mast-cell-deficient mice, using different bacterial strains, different administration routes for bacteria, and different experimental setups.

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## Disclosures

The authors declare that they have no conflict of interests.

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