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# Activation of mast cells in skin abscess induced by *Staphylococcus aureus* (*S. aureus*) infection in mice

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ABSTRACT

The skin abscess is a common inflammatory disease that occurs following the ubiquitous *S. aureus* infection. In our study, a skin abscess murine model was established and the dynamics of mast cells chemotaxing was evaluated. In the *S. aureus*-infected mice, severe infiltration of inflammatory cells in the dermis were observed, and mast cells were markedly accumulated in the skins. Besides, tryptase, the marker for mast cells activation, has a positive correlation with mast cell activity. The mast cells identified in the tissues were likely to be activated since they were associated with cell degranulation and the presence of tryptase. Our results suggested that mast cells and its mediator tryptase contribute to the inflammation of skin abscess induced by *S. aureus* infection.

## 1. Introduction

Skin abscesses are fairly common which usually occur in an infection both on the skin surface and within the deeper structures. An abscess may develop and enlarge, depending on whether microorganisms or leukocytes gain the upper hand in any one of a number of locations in the body (Tinelli et al., 2009). Abscess tends to get worse as time goes on, while it can spread to deeper tissues, and even enters the bloodstream to affect other organs, such as brain, liver, lungs, teeth, and tonsils(Amir et al., 2010). Skin abscess are commonly caused by infectious pathogen, such as the ubiquitous *Staphylococcus aureus* (*S. aureus*) bacteria (Chambers, 2001; Lowy, 1998). During the past 20 years, an dramatically increasing staphylococcal infections incidence and antibiotic resistance have posed severe burden to human health, in which a notable strain is called methicillin-resistant *Staphylococcus aureus* (MRSA) (Cardo et al., 2002; Grundmann et al., 2006; Lowy, 1998).

Mast cells which are main effector cells of inflammation, resident in tissues throughout the body and is abundant in the boundaries between the outside and internal world, such as the skin, mucosa of the respiratory and digestive tract (Galli et al., 2005; Metcalfe et al., 1997). Mast cells play a pivotal role in the inflammatory process. Under activation, they rapidly release its typical granules and various hormonal mediators into the interstitium, such as tryptase, histamine and 5-HT to exert multiple actions in the tissue microenvironments (Galli et al., 1999; Marshall, 2004; Wang et al., 2008). It demonstrated that mast cells can be activated after infection with RSV or cytomegalovirus in bovine and rodent models (Gibbons et al., 1990; van Schaik et al., 1999). Others have demonstrated that mast cells perform a positive role in viral invasion by dodging immunosurveillance and aggravating the inflammatory responses (King et al., 2000; Wang et al., 2008). These findings strongly suggest the importance of mast cells in the interplay between pathogens and their contribution in the induction of acute inflammation response.

Bacterial skin infections cause different outcome, such as wound infection, atomic dermatitis (AD), abscess, staphylococcal scalded skin syndrome, which is from minor annoying to deadly. However, *S. aureus* is notably and responsible for most skin inflammatory diseases, especially the increasing emergence of MRSA (Chambers, 2008; Hammond and Baden, 2008). Because of its profound impact on human and animal health, the investigations of *S. aureus*-infection are greatly appeals to biomedical community (Fournier and Philpott, 2005). However, few data were collected to delineate the kinetics of mast cells in skin abscess induced by *S. aureus* infection. In our study, we generated a skin abscess

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mouse model by administrating a clinical isolated *S. aureus strain*, and evaluated the mast cell attracting and tryptase activity in the skin inflammatory response. In addition, we quantified the dynamics of mast cell and tryptase during the MRSA infection. This study may help us to reveal the underlying mechanism in skin abscess caused by *S. aureus* infection.

## 2. Materials and methods

### 2.1. Bacterial strains

*S. aureus* ST-239, a prevalent clinical strain isolate, identified and characterized by Shanghai Huashan Hospital (Li et al., 2009), was used in this study. Firstly, *S. aureus* ST-239 was grown to mid-exponential phase in tryptone-soya broth (TSB, Oxoid Ltd., Basingtoke, Hampshire, England), and washed once with sterile phosphate buffered-saline (PBS), then resuspended in PBS at $5 \times 10^8$ CFUs/50 μl for skin abscess model.

### 2.2. Animals and skin abscess model

For the skin abscess model, thirty five-week-old (15- to 20-g), inbred, special-pathogen-free, BALB/c nude mice (SINO-British SIPPR/BK. Lab.Animal. Ltd., Shanghai, China) were used in this experiment. They were divided randomly into two groups: 10 mice as the controls, and 20 mice in the ST-239 infected group. Mice were anesthetized with isoflurane and inoculated with 50 μl PBS containing $5 \times 10^8$ live *S. aureus* or sterile PBS in the right flank by subcutaneous injection. We examined animals at 24-hour intervals for a total of 10 days with a caliper, the skin lesions value was calculated with the formula L × W (L—length, W—width). All animals were examined and weighed serially by a blinded observer. Animal infection experiments were performed at the Animal Center of Shanghai Public Health Clinical Center, and approved by the Animal Care and Use Committee of Public Health Clinical Center Affiliated to Fudan University.

### 2.3. Measurements

Animals were weighed immediately prior to inoculation, thereafter, animals were observed at 24-hour intervals after inoculation for a total of 10 days. Lesions were measured with a caliper. Abscess size was calculated by using the formula for a spherical ellipsoid [$V = (\pi/6)$ $L.W^2$], where L is length and W is width. Areas were calculated for dermonecrosis by using the formula $A = \pi$ (L.W)/2. Lesion sizes were recorded by day of observation and graphed. For each animal, the area under the lesion size curve (lesion volume) was determined and used as a measure of disease.

### 2.4. Sample collection

On days 1, 2, 3, … 9 and 10 post-infection, one animal from each group were selected randomly and killed, and the skin tissue around the injection sites were collected. The sample of each skin tissue was fixed immediately by immersion in 2.5% (v/v) glutaraldehyde-polyoxymethylene solution and then kept at room temperature for 72 h until used for sections preparation.

### 2.5. Skin histopathology

In order to characterize the histopathology of the mice, biopsy specimens were taken after the following treatments. For histological studies, the fixed skin tissues were dehydrated by increasing concentrations of ethanol, and the tissues were embedded in paraffin. Thereafter, sections of tissue were cut at 5 μm, mounted on clean glass slides, and dried overnight at 37 °C. Sections were cleared, hydrated, and stained with hematoxylin-eosin solution for histological damage evaluation, according to the standard protocol of our lab, and the slides were coded to prevent observer bias during evaluation. All tissue sections were examined with an Motic microscope (Motic China Group Co. Ltd., Xiamen, China).

### 2.6. Distribution of mast cells by improved toluidine blue staining

Mast cells were identified by an improved toluidine blue staining method according to previous reports (Wang et al., 2008). Briefly, tissue samples were dehydrated, embedded in paraffin, deparaffinized, rehydrated, and immersed in 0.8% toluidine blue (Sigma Co.) for 15 s. Slides were next washed with distilled water for 30 s, immediately placed into 95% alcohol until the mast cells appeared deep reddish purple under the microscope, immersed for 3 min successively in 100% alcohol, alcohol-xylol (1/1, v/v), and xylol, and then mounted with neutral gums.

Quantitative analysis of mast cell density in skin samples were performed by counting the number of mast cells in 10 high-power fields (40 × magnification), and the mean was calculated. The whole section was scanned for general qualitative observations, but detailed examination focused on mast cells. Sampling of the sections was unbiased, with the samples coded and examinations performed by one investigator. Mast cell density was expressed as cells per square millimeter.

### 2.7. Measurement of mast cell-associated mediators tryptase with immunohistochemical staining

Tryptase is synthesized almost exclusively by mast cells, being released after mast cell degranulation and, because it is stored almost exclusively in mast cells, this mediator has attracted particular attention as a marker for mast cells activation in this study (Feng et al., 2007). Examinations of tryptase in tissue samples were performed by immunohistochemical analyses, based on previous report (Buffa et al., 1980), and tryptase positives in skin samples counted by Leica Qwin image soft (Leica Co. Ltd., Germany) and recorded as the percentage of tryptase positives per total.

### 2.8. Statistical analysis

Lesion volumes were calculated and data were analyzed with Microsoft Excel Statistical Software *t*-test for normally distributed data with equal variances. The results were expressed as means and standard errors. Differences were considered significant at $p < 0.05$ or $p < 0.01$.

## 3. Results

### 3.1. Clinical and gross observation of skin abscess

The skin of *S. aureus*-infected mice developed visible changes of abscess signs, such as redness and edema at the early stage of infection, and ulcer and drain at the later phase (Fig. 1A,B). Importantly, the skin abscess volume in *S. aureus*-infected mice were significantly larger than those of sterile PBS control group (Fig. 1C). Skin abscess became erythematous and fluctuant by day 3 to 4. These lesions progressed to 7 to 15 mm in diameter over 5 to 7 days, and then drained externally through the overlying epidermis (Fig. 1C).

### 3.2. Skin histopathology examination

Severe inflammatory responses were observed in the *S. aureus*-infected mice under microscope, the epidermis were thicker compared with the control mice at the early infection stage (Fig. 1G). The epidermic basal lamina were thickened and the cell components were gradually vanished (Fig. 1G,H). The dermic collagen fiber and other