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RNase 7 Protects Healthy Skin from *Staphylococcus aureus* Colonization

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TO THE EDITOR

The Gram-positive bacterium *Staphylococcus aureus* is an important pathogen that causes various skin infections (Miller and Kaplan, 2009). However, healthy skin is usually not infected by *S. aureus,* despite the high carrier rates in the normal population (Noble, 1998). This suggests that the cutaneous defense system has the capacity to effectively control the growth of *S. aureus*. There is increasing evidence that antimicrobial proteins are important effectors of the cutaneous defense system (Harder *et al.*, 2007). A recent study reported that keratinocytes contribute to cutaneous innate defense against *S. aureus* through the production of human β-defensin-3 (Kisich *et al.*, 2007). In addition to human β-defensin-3,, other antimicrobial proteins may also participate in cutaneous defense against *S. aureus*. One candidate is RNase 7, a potent antimicrobial ribonuclease that is highly expressed in healthy skin (Harder and Schröder, 2002; Köten *et al.*, 2009).

To investigate the hypothesis that RNase 7 may contribute to protect healthy skin from *S. aureus* colonization, we first incubated natural RNase 7 isolated from stratum corneum skin extracts (Harder and Schröder, 2002) with *S. aureus* (ATCC 6538). In concordance with our initial report about RNase 7 (Harder and Schröder, 2002), we verified that RNase 7 exhibited a high killing activity against *S. aureus* (lethal dose of 90% $= 3$–$6\ \mu g\ ml^{-1}$).

Recently, we reported a moderate induction of RNase 7 mRNA expression in primary keratinocytes treated with heat-killed *S. aureus* (Harder and Schröder, 2002). To assess the induction of RNase 7 by *S. aureus* in the

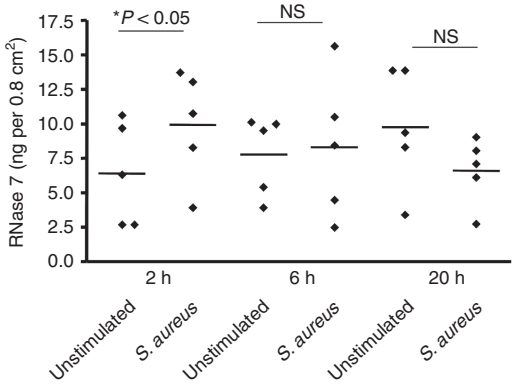

Figure 1. Induced secretion of RNase 7 on the skin surface on treatment with living *S. aureus*. Defined areas (0.8 cm^2) of skin explants derived from plastic surgery were incubated with or without approximately 1,000 colony-forming units of *S. aureus* (ATCC 6538) in 100 µl of sodium phosphate buffer. After 2, 6, and 20 hours, the concentration of secreted RNase 7 was determined by ELISA. Stimulation with *S. aureus* for 2 hours revealed a significant induction as compared with the unstimulated control after 2 hours (*$P < 0.05$, Student's *t*-test; n.s. = not significant). Data shown are means of triplicates of five skin explants derived from five donors.

context of more physiological conditions, we used human skin explants and incubated them for 2, 6, and 20 hours with living *S. aureus* (the use of skin material for this study was approved by the ethical committee of the University Kiel (A 104/06) in accordance with the Declaration of Helsinki Principles). As shown in Figure 1, only 2 hours of incubation with living *S. aureus* significantly induced the secretion of RNase 7 on the skin surface as measured by an RNase 7-specific ELISA (Köten *et al.*, 2009). This indicates a fast release of preformed material. The reduced secretion after 20 hours could be a result of cytotoxic effects from *S. aureus,* as well as of a decreased viability of skin explants. Secretion of RNase 7 after 2 hours was enhanced by increasing concentrations of *S. aureus* (Supplementary Figure S1 online).

To further assess the physiological relevance of RNase 7 in cutaneous defense against *S. aureus,* we investigated whether the killing activity of skin extracts derived from stratum corneum was inhibited through blocking of the antimicrobial activity of RNase 7 by RNase 7-specific antibodies as previously described (Köten *et al.*, 2009). First, we analyzed whether RNase 7 antibodies neutralized the antibacterial activity of RNase 7 against *S. aureus*. For this purpose, we tested the activity of RNase 7 against *S. aureus* in an antibacterial microdilution assay in the presence of RNase 7 antibodies. This approach revealed that the application of RNase 7 antibodies completely blocked the *S. aureus*-killing activity of RNase 7. In contrast, the antimicrobial activity was not inhibited when equivalent concentrations of irrelevant antibodies (derived from goat preimmune serum; Köten *et al.*, 2009) were used (data not shown). Having established that RNase 7-specific antibodies neutralized the antimicrobial effect of RNase 7 against *S. aureus*, we used this approach to test the functional role of RNase 7 in cutaneous defense against *S. aureus*. To this end, we first incubated a stratum corneum skin extract with *S. aureus*. The skin extract was diluted to a ratio of 1:200 in 25 µl of sodium phosphate buffer (pH 7.4) without or with RNase 7-blocking

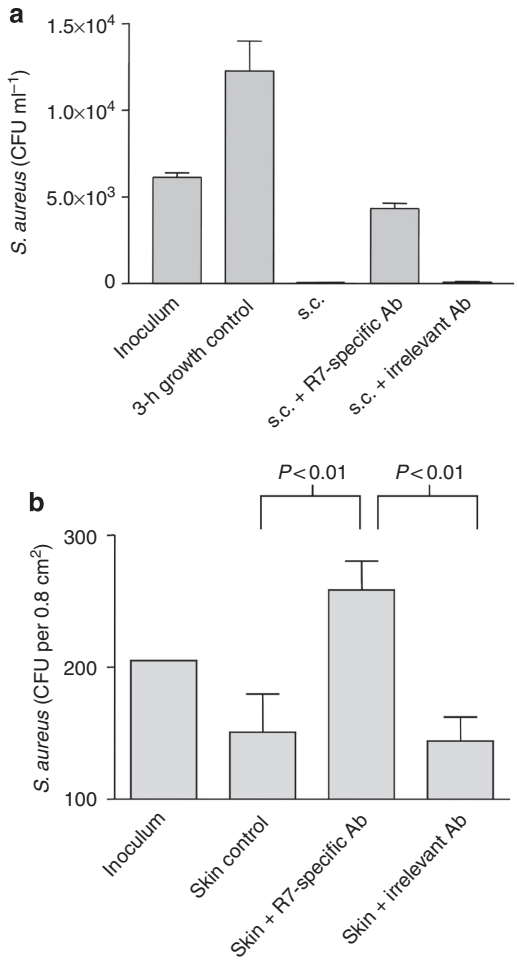

Figure 2. RNase 7 contributes to the killing activity of human skin against *S. aureus*. (**a**) Stratum corneum extracts were incubated without (subcutaneous (s.c.)) or with RNase 7-blocking antibody (s.c. + R7-specific Ab) or an irrelevant antibody (s.c. + irrelevant Ab). After 45 minutes, *S. aureus* was added and colony-forming units (CFUs) were determined after 3 hours of incubation. Data shown are means ± SD of a representative result of three independent experiments. (**b**) Defined areas (0.8 cm^2) of skin explants were incubated without (skin control) or with RNase 7 antibody (skin + R7-specific Ab) or with an equivalent concentration of an irrelevant antibody (skin + irrelevant Ab). After 30 minutes, *S. aureus* was added and CFU were determined after 2 hours of incubation. Data show means ± SD of triplicate experiments of the same donor. A representative result of three independent experiments from three donors is shown. Student's *t*-test was used for statistics.

antibody (4 mg ml^{-1}) or an equivalent concentration of an irrelevant antibody. This dilution leads to a final concentration of 6 µg ml^{-1} RNase 7 in the assay system, which corresponds to the determined range of the lethal $dose_{90}$ of RNase 7 against *S. aureus* (see above). After 45 minutes, 25 µl of *S. aureus* (ATCC 6538) in sodium phosphate buffer (pH 7.4) containing 2% tryptone soya broth was added and incubated for 3 hours. Killing activity was analyzed by plating serial dilutions of the incubation mixture and determining colony-forming units the following day. The skin extract exhibited a potent *S. aureus*-killing activity, as incubation of *S. aureus* with the skin extract resulted in a complete killing of *S. aureus* (Figure 2a). The application of RNase 7-blocking antibodies to the skin extract before inoculation with *S. aureus* resulted in an outgrowth of *S. aureus* of approximately 35% as compared with the growth control (Figure 2a). In contrast, an irrelevant antibody did not inhibit the killing activity of the skin extract (Figure 2a). These data indicate an important contribution of RNase 7 to the *S. aureus*-killing activity of the skin extracts. Other skin-derived antimicrobial proteins may be responsible for the

S. aureus-killing activity, which remained after blocking the antimicrobial activity of RNase 7.

To obtain additional insight into the functional role of RNase 7 in cutaneous defense against *S. aureus,* we studied the role of RNase 7 using human skin explants. In this experimental *ex vivo* model, *S. aureus* was applied to defined areas (0.8 cm^2) of the surface of skin explants. Preincubation of skin samples with 1.5 mg ml^{-1} RNase 7-blocking antibody in 100 µl of sodium phosphate buffer (pH 7.4) led to a significant outgrowth of *S. aureus* as compared with pretreatment of skin with an equivalent concentration of irrelevant antibody (Figure 2b). These data further indicate a physiological relevance of RNase 7 for the control of *S. aureus* growth on the skin surface.

In summary, data present herein show that skin infected with living *S. aureus* responds with an increased release of RNase 7, which contributes to limit the growth of *S. aureus*. These data are in concordance with a recently published study reporting that lower RNase 7 expression in healthy skin is associated with a higher risk of *S. aureus* skin infection (Zanger *et al.*, 2009). Future studies have to evaluate whether skin infections caused by *S. aureus* may be associated with an impaired expression or function of RNase 7.

CONFLICT OF INTEREST

The authors state no conflict of interests.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Generation of Increased Numbers of HLA-DR^{high} IgG$^+$ Plasma Cells in the Peripheral Blood of Patients with Bullous Pemphigoid: NC16a-Specific Cells Belong to the Short-Lived Plasma Blast Population

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TO THE EDITOR

Bullous pemphigoid (BP) is a rare, usually severe and potentially life-threatening disease. Immunologically, this disorder is characterized by the presence of circulating IgG autoantibodies targeting distinct adhesion molecules of the dermoepidermal basement membrane zone (Hertl, 2000). Binding of autoantibodies to adhesion structures of the skin leads to a massive loss of function, resulting in blister formation. The majority of BP patients possesses high-affinity IgG autoantibodies directed against the 180 kDa BP autoantigen (BP180; Diaz *et al.*, 1990). The immunodominant site of BP180 is located within the short NC16a domain, which is recognized by more than 90% of BP patients. In the previous studies we have identified the presence of memory B cells specific for the NC16a domain, which can be induced *in vitro* to synthesize autoantibodies (Leyendeckers *et al.*, 2003). Also, it was shown that the serum level of autoantibodies to NC16a correlates with the severity of BP (Schmidt *et al*, 2000). The bulk of antibodies measurable in the serum are synthesized by terminally differentiated B lymphocytes, that is, plasma cells (PCs), mostly residing in the bone

Abbreviations: BP, bullous pemphigoid; BM, bone marrow; PB, plasma blast; PC, plasma cell