HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2012 November 01.

Published in final edited form as:

J Invest Dermatol. 2012 May ; 132(5): 1513–1516. doi:10.1038/jid.2011.462.

Genetic Requirement for ADAM10 in Severe *Staphylococcus aureus* Skin Infection

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TO THE EDITOR

Staphylococcus aureus is a leading cause of human skin infections, contributing to disease in both healthy and immunocompromised individuals, also complicating burn and surgical wound sites and lesions of atopic dermatitis (Lowy, 1998; Ong and Leung, 2010). Host defense against staphylococcal skin infection is multifaceted, relying on local immunologic control through T_H17 and IL-1β–driven recruitment of neutrophils in addition to the protective actions of antimicrobial peptides and physical properties of the cutaneous barrier (Miller and Cho, 2011). Pathogen virulence in staphylococcal skin infection is likewise multifactorial (Weidenmaier *et al.*, 2010), relying in part on the action of α-hemolysin, a pore-forming cytotoxin secreted by almost all strains of *S. aureus*. Hla is required for dermonecrotic changes during infection, also contributing to abscess size (Kennedy *et al.*, 2010). Immunization strategies targeting Hla protect against dermonecrosis (Kennedy *et al.*, 2010), however the molecular mechanism by which the toxin causes pathologic disturbance of the epithelial barrier is ill-understood.

We recently identified the zinc-dependent metalloprotease ADAM10 as the cellular receptor for Hla (Wilke and Bubeck Wardenburg, 2010). ADAM10 regulates epithelial function through its ability to cleave E-cadherin, severing the protein-based adherens junction tether between adjacent cells (Maretzky *et al.*, 2005). ADAM10 knockout mice exhibit embryonic lethality (Hartmann *et al.*, 2002), while conditional skin knockout of ADAM10 during gestation or in the postnatal epidermis results in marked dysregulation of epithelial differentiation and barrier function (Weber *et al.*, 2011). To examine the contribution of ADAM10 to skin infection, we generated conditional knockout mice in which exon 3 of ADAM10 is flanked by *loxP* sites (Tian *et al.*, 2008), excised in the presence of a Cre recombinase expressed under control of the keratin 14 promoter (Vasioukhin *et al.*, 1999). Topical application of tamoxifen (TAM) to a $1cm^2$ skin area for 5 days leads to localized ADAM10 genomic excision (Fig. S1a and b), abrogating epidermal ADAM10 expression

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CONFLICT OF INTEREST

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(Fig. 1a, ADAM10$^{-/-}$). In contrast to littermate controls, ADAM10$^{-/-}$ mice did not develop dermonecrotic lesions following subcutaneous infection with 3×10^7 colony forming units of the epidemic *S. aureus* USA300/LAC (Figs. 1b and c). Abscess size was reduced in ADAM10$^{-/-}$ mice (Fig. 1d), however lesional bacterial recovery was unaltered (Fig. S1c).

The ability of ADAM10 to cleave E-cadherin suggested that Hla may utilize its receptor to cause epithelial barrier injury, not merely to facilitate binding. Toxin treatment of A431 keratinocytes led to upregulation of cell-associated metalloprotease activity measured in a fluorogenic substrate assay; the non-pore-forming Hla_{H35L} mutant did not elicit this response (Fig. 1e). Enhanced metalloprotease activity correlated with E-cadherin cleavage, detectable by immunoblot analysis of E-cadherin precipitates from A431 lysates as the toxin-induced loss of full-length (Fl) protein and C-terminal fragment (Ctf) accumulation produced by ADAM10-dependent cleavage (Fig. 1f). Ctf accumulation was also observed in cultured primary keratinocytes upon toxin treatment (Fig. S1d). These molecular events occurred at subcytolytic concentrations of Hla (10 μg per ml, data not shown), wherein a disturbance of cell-cell interactions in an A431 monolayer was manifest as a loss of resistance to the passage of an electrical current using electrical cell-substrate impedance sensing (ECIS, Fig. 1g).

These findings suggest that the principal role of the toxin-receptor complex may be to disrupt the epithelial barrier. Within 24 hours following *S. aureus* infection, loss of E-cadherin surface expression in epidermal tissue overlying the infection was evident in control mice as compared to ADAM10$^{-/-}$ mice (Fig. 1h). Inflammatory cell infiltration at the infection site was similar in both control and ADAM10$^{-/-}$ mice (Fig. 1i, upper, arrows), however epidermal loss was apparent overlying the abscess site only in control mice (Fig. 1i, lower). Together with CFU analysis, these results indicate that the Hla-ADAM10 complex does not markedly alter the host response to infection or bacterial accumulation. Epithelial injury induced by the toxin-receptor complex likely contributes to tissue edema and increased lesional size observed in control mice (Fig. 1d).

These data raised the possibility that an enzymatic inhibitor of ADAM10 may protect the epithelium. Mice receiving systemic treatment with the ADAM10 inhibitor GI254023X (Ludwig *et al.*, 2005) (200 mg per kg per day, five days) did not demonstrate dermonecrotic lesions following infection compared to mice treated with the DMSO vehicle (Fig. 2a). Topical application of GI254023X also prevented skin breakdown (Fig. 2b, 100 mg per kg per day, five days). Both routes of treatment led to a reduction in abscess size (data not shown). In vitro, GI254023X treatment preserved E-cadherin expression and epithelial barrier function in toxin-treated A431 cells (Figs. 2c and d).

These studies provide insight into pathogenesis of staphylococcal skin infection demonstrating a genetic requirement for ADAM10 expression to mediate epithelial barrier injury, which to our knowledge is previously unreported. The Hla-ADAM10 complex thereby disables the most innate host defense of the skin. These findings extend recent observations that demonstrate a role for ADAM10 in Hla-mediated injury to the lung epithelium and vascular endothelium (Inoshima *et al.*, 2011; Powers *et al.*, 2011), defining a generalized principle of toxin action in which Hla utilizes its cellular receptor as a means to

bind host cells and cause barrier injury. As ADAM10 cleaves muItiple cellular substrates, it is of considerable interest to now understand the potential role of these proteins in staphylococcal disease pathogenesis.

These observations have important implications for *S. aureus* skin disease. First, Hla may facilitate an initial breach of intact skin to permit pathogen invasion. Second, ADAM10 polymorphisms that alter toxin binding or enzymatic activity may exist, impacting susceptibility to infection. Finally, in disease states characterized by a compromise of epithelial barrier function such as atopic dermatitis, *S. aureus* may potentiate or incite barrier disruption, contributing to pathology and facilitating niche establishment. Indeed, *S. aureus* is the most common bacterial pathogen complicating atopic dermatitis lesions (Ong and Leung, 2010). Studies implicating ADAM10 in atopic dermatitis pathogenesis (Maretzky *et al.*, 2008), together with our observations, suggest that the Hla-ADAM10 interaction may contribute to epidermal pathology over a continuum of staphylococcal skin disease ranging from acute infection to chronic barrier insults. Therapies impacting ADAM10 activity may therefore demonstrate broad clinical utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank C. Labno for microscopy support, T. Li for immunohistochemistry support, and the Integrated Microscopy and Immunohistochemistry Facilities at the University of Chicago. This work was supported by the Depts. of Pediatrics and Microbiology at the Univ. of Chicago and the University of Chicago Institute for Translational Medicine (NIH award UL1RR024999 from the National Center for Research Resources). The authors acknowledge membership in and support from the Region V 'Great Lakes' RCE (NIH award 2-U54-AI-057153).

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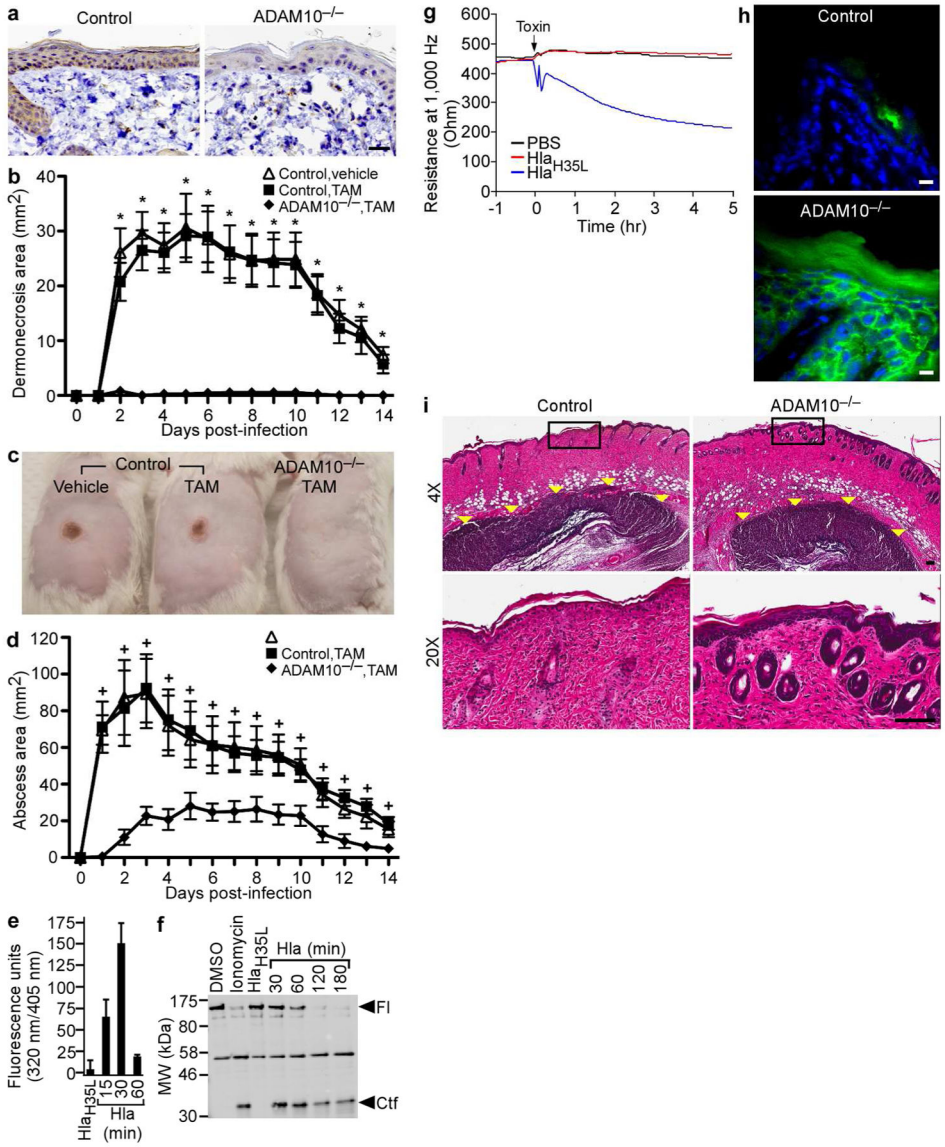

Fig. 1.

ADAM10 mediates α-hemolysin dependent epithelial injury. **(a)** Epidermal expression of ADAM10 visualized by anti-mouse ADAM10 immunohistochemical staining of tissues derived from ADAM10$^{-/-}$ mice in which topical treatment with tamoxifen (TAM, dissolved in ethanol, 1 mg per mouse per day for 5 days, applied to a 1 cm^2 area) induces loss of ADAM10 expression through Cre recombinase-mediated excision of *ADAM10loxP* alleles or control mice treated with ethanol alone. **(b)** Dermonecrosis area recorded from control mice that received topical treatment with vehicle alone (n = 13) or TAM (n = 8), or ADAM10$^{-/-}$ mice treated with TAM (n = 13) followed by subcutaneous infection at the site of vehicle or TAM application with 3 × 10^7 *S. aureus* USA300/LAC, where * denotes P < 0.001. **(c)** Image of mice treated as described in **(b)** and **(d). (d)** Abscess area recorded from mice detailed in **(b)** where + denotes P < 0.05. Area in **(b)** and **(d)** was calculated based on the formula A = [π/2] × length × width where error bars represent SEM. All animal studies

were reviewed, approved, and supervised by the Institutional Animal Care and Use Committee at the University of Chicago. **(e)** Cell-associated metalloprotease activity measured in A431 keratinocytes following treatment with 10 Rg ml^{-1} (300 nM) active Hla or the non-toxigenic mutant Hla_{H35L} at the time points indicated. Activity was quantified by detection of the product derived from cleavage of the fluorogenic peptide substrate Mca-PLAQAV-Dpa-RSSSR-NH2 (10 RM, R&D Systems, Minnesota) diluted in 25 mM Tris, pH 8.0. **(f)** Immunoblot analysis of full-length (Fl) E-cadherin and accumulation of the C-terminal cleavage fragment (Ctf) following treatment of A431 cells with controls DMSO and ionomycin compared to 10 Rg ml^{-1} Hla_{H35L} or Hla over the time course indicated. **(g)** Electrical cell substrate impedance sensing (ECIS, Applied Biophysics, New York) recordings of A431 monolayers treated with PBS (black), the Hla_{H35L} mutant (10 Rg ml^{-1}, red), or active Hla (10 Rg ml^{-1}, blue). **(h)** E-cadherin (green) immunofluorescence microscopy analysis of tissue from control or $ADAM10^{-/-}$ mice 24 hours following infection with 3×10^7 *S. aureus* delivered by subcutaneous route. Nuclei (blue) are stained with the fluorescent DNA stain DAPI. **(i)** Hematoxylin and eosin staining of tissues from mice treated as described in **(h)**, shown at 4X (upper) with a 20X image of the highlighted area (lower). The site of infection and inflammatory cell recruitment is marked by yellow arrows. Scale bars in **(a)** = 50 μm, **(h)** = 10 μm, **(i)** = 100 μm.

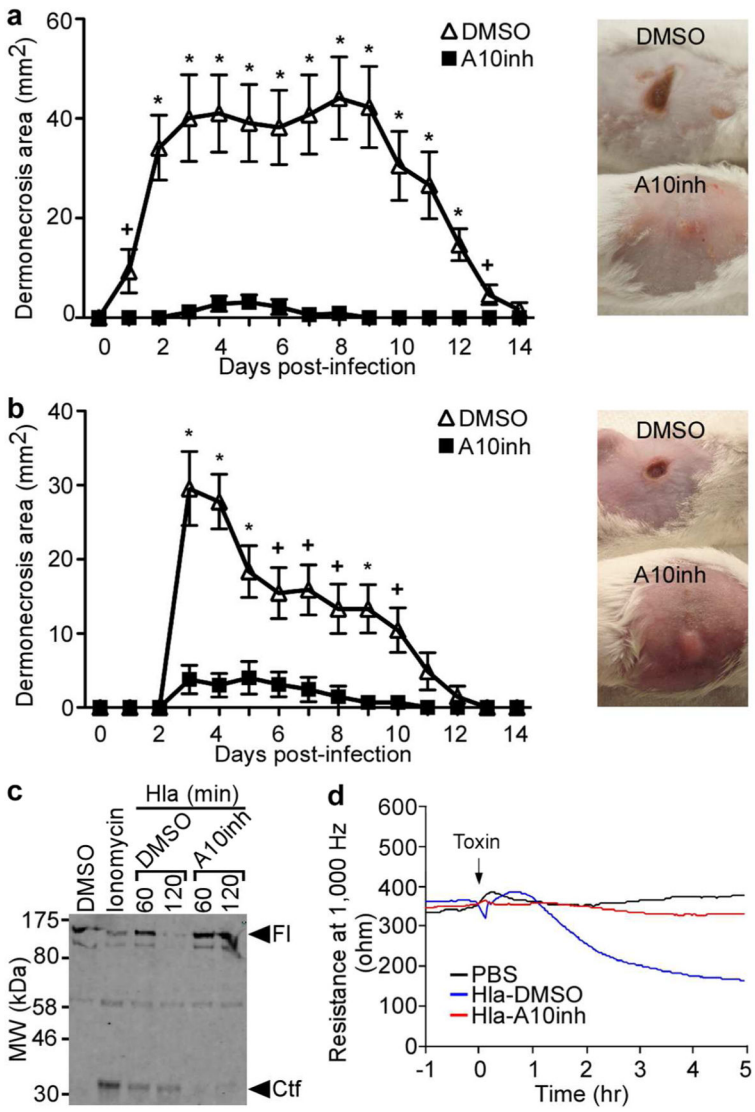

Fig. 2.

An ADAM10 inhibitor protects against Hla-induced injury. **(a)** Dermonecrosis area recorded from wild-type mice that received a five-day course of once-daily intraperitoneal injection with vehicle alone (DMSO) or the ADAM10 inhibitor GI254023X (200 mg per kg per day, Okeanos, China), followed by subcutaneous infection with 3×10^7 *S. aureus* USA300/LAC (n = 10 mice per group). **(b)** Dermonecrosis area recorded from wild-type mice that received a five-day course of once-daily topical application with vehicle alone (DMSO) or the ADAM10 inhibitor GI254023X (100 mg per kg per day), followed by subcutaneous infection with 3×10^7 *S. aureus* USA300/LAC (n = 10 mice per group), where + denotes $P < 0.05$ and * denotes $P < 0.001$ in **(a)** and **(b). (c)** Immunoblot analysis of full-length E-cadherin (Fl) and accumulation of the C-terminal cleavage fragment (Ctf) following pre-treatment of A431 cells with DMSO or GI254023X, then exposed to 10 Rg ml^{-1} Hla for the time periods indicated. **(d)** Electrical cell substrate impedance sensing (ECIS) recordings of

A431 monolayers treated with PBS (black), or Hla (10 Rg ml^{-1}) following pre-treatment with control DMSO (blue) or GI254023X (20 Rg ml^{-1}, red).