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DOCK8 is essential for neutrophil mediated clearance of cutaneous *S. aureus* infection

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Abstract

DOCK8 deficient patients are susceptible to skin infection with *Staphylococcus aureus* which is normally cleared by neutrophils. We examined the mechanism of this susceptibility in mice. *Dock8*$^{-/-}$ mice had delayed clearance of *S. aureus* from skin mechanically injured by tape stripping. The numbers and viability of neutrophils in infected but not in uninfected, tape stripped skin were significantly reduced in *Dock8*$^{-/-}$ mice compared to WT controls. This is despite comparable numbers of circulating neutrophils, and normal to elevated cutaneous expression of *Il17a* and IL-17A inducible neutrophil attracting chemokines *Cxcl1*, *Cxcl2* and *Cxcl3*. DOCK8 deficient neutrophils were significantly more susceptible to cell death upon *in vitro* exposure to *S. aureus* and exhibited reduced phagocytosis of *S. aureus* bioparticles but had a normal respiratory burst. Impaired neutrophil survival in infected skin and defective neutrophil phagocytosis likely underlie the susceptibility to cutaneous *S. aureus* infection in DOCK8 deficiency.

Keywords

DOCK8 deficiency; *S. aureus* ; neutrophils; phagocytosis

1. INTRODUCTION

Dedicator of cytokinesis 8 (DOCK8) is highly expressed in hematopoietic cells and forms a complex with Wiskott-Aldrich syndrome protein (WASP) to regulate WASP-dependent actin polymerization and cytoskeletal reorganization [1]. DOCK8 deficient patients suffer from severe eczema and share several characteristics with AD patients, including recurrent skin infections with bacteria, particularly *S. aureus*, and viruses, elevated serum IgE levels, eosinophilia, and a high incidence of asthma and food allergy [2, 3]. Successful hematopoietic stem cell transplantation resolves the skin lesions of DOCK8 deficient patients [4], implicating hematopoietic cells rather than stromal cells as the underlying cause of the patients' eczema and recurrent skin infections. The mechanism underlying

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the susceptibility of DOCK8 deficient patients to cutaneous *S. aureus* infection is poorly understood.

The canonical innate defense function of neutrophils is their ability to phagocytose and kill extracellular bacteria [5]. Neutropenia, as well as phagocytic and bacterial killing defects that affect neutrophils, are associated with increased susceptibility to *S. aureus* infection in humans and mice [6-8]. Knockdown of DOCK8 in neutrophils impairs their migration towards a chemotactic gradient *in vitro* [9], but neutrophils from DOCK8 deficient patients have only a mild *in vitro* migration defect [10]. The ability of DOCK8 deficient neutrophils to migrate into tissues and their capacity to eliminate bacteria *in vivo* are not known.

We have investigated the recruitment and function of DOCK8 deficient neutrophils in response to *S. aureus* skin infection. We demonstrate that DOCK8 expression is required for the survival of neutrophils in *S. aureus* infected skin and for their phagocytic function. Furthermore, we demonstrate that DOCK8 deficient neutrophils are intrinsically susceptible to *S. aureus* triggered cell death. These data illuminate the mechanisms underpinning the susceptibility to cutaneous *S. aureus* infection in DOCK8 deficiency.

2. MATERIALS AND METHODS

2.1 Mice.

DOCK8 deficient (*Dock8*$^{-/-}$) mice on C57BL/6 background were previously described [1]. Wild-type (WT) C57BL/6 mice were purchased from Charles River Laboratory. All mice were housed in a specific pathogen free environment. All experiments were performed on adult mice (age > 6 weeks) with age and sex matched controls. All procedures were performed in accordance with the Animal Care and Use Committee of the Children's Hospital Boston.

2.2 *S. aureus* preparation and quantification.

The *S. aureus* strain MNHOCH (NARSA catalog number NRS114, MLST sequence type 8, methicillin sensitive, SEB producing) was a gift from Patrick Schlievert. A single colony of *S. aureus* strain MNHOCH was cultured overnight in 5ml tryptic soy (TS) broth (Sigma-Aldrich). The overnight culture was diluted 1:50 into fresh TS broth, cultured for ~60 minutes to exponential stage and bacterial concentration was determined by measuring optical density. The bacterial pellet was resuspended at 10^8 colony forming units (CFU) per 50 μl in sterile PBS. CFUs were verified by overnight culture of inoculum on TS agar plates.

2.3 Epicutaneous *S. aureus* application.

Mice were anesthetized, their back skin was shaved then left unmanipulated or was tape-stripped x6 with Tegaderm dressing (3M). Trans-epidermal water loss (TEWL) was measured using a TEWL probe (DermaLab) to confirm that all mice attained a similar level of skin barrier disruption post tape stripping. Using a cotton swab, 50 μl of 10^8 CFU of *S. aureus* was applied to the skin which was analyzed 48 hours later. Uninfected skin was analyzed at 6, 18, 24 and 48 hours post tape stripping.

2.4 Enumeration of *S. aureus* load *in vivo*.

To determine cutaneous *S. aureus* load *in vivo*, *S. aureus* was labeled with PSVue794 reagent kit (LI-COR), following the manufacturer's instructions. PSVue794 fluorescence was quantified using the Pearl® Trilogy Small Animal Imaging System (LI-COR). To confirm the bacterial load from skin on day 2 post infection, an 8 mm^2 skin punch biopsy was mechanically homogenized then serial dilutions were plated on CHROMagar *S. aureus* (Fisher Scientific) plates, which were cultured overnight at 37°C for enumeration of CFUs of pink *S. aureus* colonies.

2.5 Skin cell preparation and flow cytometry.

A 1 cm^2 skin section was obtained from each mouse, which was finely chopped, digested, washed and filtered as previously described [11, 12]. Skin cell suspensions were preincubated with Fcγ receptor (αCD16/αCD32) blocking Ab (clone 93) then incubated with fluorochrome-conjugated monoclonal antibodies against CD45 (104), CD3 (17A2), CD11b (M1/70), GR1 (RB6–8C5) (all BioLegend), and fixable viability dye (FVD) (Thermo Fisher). Cell counts of skin samples were determined by addition of Precision Count Beads™ (BioLegend). Cells were analyzed by flow cytometry using a BD LSRFortessa cell analyzer with FACSDiva software (BD Biosciences). Data was analyzed using FlowJo software (Tree Star Inc.).

2.6 Skin gene expression analysis.

RNA was extracted from homogenized skin tissue using the Qiagen Plus Mini RNA Extraction Kit (Qiagen). cDNA was synthesized using iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories) and analyzed by TaqMan® Universal Master Mix II and TaqMan Probes (Thermo Fisher) using a QuantStudio 5 (ThermoFisher) Real-Time PCR machine. Gene expression was normalized to *β2-microglobulin* using the $2^{-\Delta\Delta Ct}$ method. For each gene, the mean expression of the control was set to 1 for fold change calculations.

2.7 Neutrophil phagocytosis assay.

Lymphocytes were isolated from bone marrow (BM), RBCs were lysed with ACK lysis buffer (Thermo Fisher) then neutrophils were purified using the Mouse Neutrophil Isolation Kit (Miltenyi Biotec) to >95% purity, confirmed by flow cytometry. pHrodo™ Red *S. aureus* BioParticles™ Conjugate for Phagocytosis (Thermo Fisher) were opsonized for 1 hour in 10% mouse serum in sterile 1x PBS at 37°C. $1x10^5$ neutrophils were incubated with $1x10^6$ opsonized *S. aureus* BioParticles™ for 60 minutes at 37°C. Negative controls were incubated on ice for 60 minutes. Extracellular fluorescence signal was quenched with 0.05% trypan blue before analysis by flow cytometry of pHrodo™ Red uptake.

2.8 *S. aureus* triggered neutrophil cell death.

S. aureus, prepared as above, was opsonized for 1 hour in 10% mouse serum in sterile 1x PBS at 37°C. BM neutrophils, isolated as above, were cultured with opsonized bacteria at a multiplicity of infection (MOI) of 55 bacteria to 1 neutrophil, or in media alone, at 37°C. At various time points, the reaction was terminated by incubation on ice for 10 minutes,

cells were washed in 1x PBS, preincubated with Fcγ receptor (αCD16/αCD32) blocking Ab (clone 93) then stained for surface markers and FVD before analysis by flow cytometry.

2.9 Enumeration of neutrophils in circulation.

From 50 μl of blood, RBCs were lysed with ACK Lysis buffer (ThermoFisher), then samples were preincubated with Fcγ receptor (αCD16/αCD32) blocking Ab (clone 93) then stained for surface markers before analysis by flow cytometry.

2.10 Neutrophil oxidative burst.

As described previously [13], 100 μl of whole mouse blood was incubated with 2 μg/ml dihydrorhodamine 123 (DHR) (Sigma Aldrich) with or without stimulation with 0.4 μg/ml phorbol 12-myristate 13-acetate (PMA) for 30 mins at 37°C in a water bath. RBCs were lysed with ACK Lysis buffer (ThermoFisher) then cells were washed in 1x PBS before analysis by flow cytometry.

2.11 Statistical analysis.

Data were analyzed for statistical significance using GraphPad Prism 9 (GraphPad Software) using unpaired 2-tailed Student's *t* test, 1-way ANOVA or 2-way ANOVA with Sidak's multiple comparison test. The threshold for significance was $P\ value < 0.05$.

3. RESULTS AND DISCUSSION

3.1. *Dock8*$^{-/-}$ mice display delayed clearance of *S. aureus* from mechanically injured, but not intact, skin.

The skin of *Dock8*$^{-/-}$ mice and WT controls was shaved and tape stripped prior to topical application with 10^8 CFUs of *S. aureus* strain MNHOCH, labeled with the fluorescent PSVue 794 dye. The persistence of *S. aureus* bacteria on the skin was examined by *in vivo* fluorescence imaging at 0, 24 and 48 hours as well as by measuring the numbers of *S. aureus* CFUs in homogenates of whole skin biopsies. The decay in PSVue 794 fluorescence was significantly less at 24 and 48 hours post infection in *Dock8*$^{-/-}$ mice compared to WT controls (Fig. 1A), indicating impaired clearance of *S. aureus*. This was confirmed by examining the number of CFUs recovered from 1 cm^2 of skin 48 hours post infection with *S. aureus,* which were significantly higher in *Dock8*$^{-/-}$ mice compared to WT controls (Fig. 1B). These results indicate defective clearance of *S. aureus* from mechanically injured skin of *Dock8*$^{-/-}$ mice.

S. aureus releases a number of toxins that damage keratinocytes [14, 15]. One potential reason for the increased load of *S. aureus* in tape stripped skin of *Dock8*$^{-/-}$ mice is increased susceptibility of their keratinocytes to *S. aureus*. To address this question, the clearance of 10^8 CFUs of PSVue 794 labeled *S. aureus* topically applied to shaved, but not tape stripped, skin was examined in *Dock8*$^{-/-}$ mice and WT controls. As we previously reported [16], in WT mice *S. aureus* applied topically to intact skin was rapidly cleared compared to *S. aureus* applied to tape stripped skin (Fig. 1A, C). The decay in PSVue 794 fluorescence, as well as the numbers of *S. aureus* CFUs at 48 hours, were comparable in the shaved skin of *Dock8*$^{-/-}$ mice and WT controls (Fig. 1C, D). These data rule out altered susceptibility of intact skin

to *S. aureus* growth as the underlying cause for the increased susceptibility of mechanically injured skin to *S. aureus* infection in *Dock8*$^{-/-}$ mice.

3.2. Impaired accumulation and survival of neutrophils in tape stripped and *S. aureus* exposed skin of *Dock8*$^{-/-}$ mice.

Neutrophils play a critical role in clearing intradermally injected *S. aureus* [17]. There were very few neutrophils in intact skin of *Dock8*$^{-/-}$ mice and WT controls (Fig. 2A). As expected, there was robust infiltration of neutrophils in tape stripped and *S. aureus* exposed skin of WT mice (Fig. 2A). The numbers of neutrophils recovered from tape stripped and *S. aureus* exposed skin were significantly lower in *Dock8*$^{-/-}$ mice compared to WT controls (Fig. 2A). This was not due to differences in the number of circulating neutrophils as these numbers were comparable in *Dock8*$^{-/-}$ mice and WT controls both at baseline and 48 hours after application of *S. aureus* to tape stripped skin (Fig. 2B). The decrease in circulating numbers of neutrophils at 48 hours in *S. aureus* infected mice is consistent with previous observations of neutropenia following *S. aureus* infections [18].

IL-17A from TCR$\gamma\delta^+$ cells is a major driver of cutaneous accumulation of neutrophils following intradermal injection of *S. aureus,* secondary to its induction of neutrophil attracting chemokines [17, 19]. As expected, exposure of tape stripped skin to *S. aureus* caused robust local *Il17a* expression in WT mice (Fig. 2C). DOCK8 has been reported to be important for Th17 differentiation of naïve CD4$^+$ T cells [20]. *Il17a* expression in tape stripped and *S. aureus* exposed skin was comparable in *Dock8*$^{-/-}$ mice and WT controls (Fig. 2C), suggesting that DOCK8 is dispensable for *Il17a* expression by TCR$\gamma\delta^+$ cells. Furthermore, *Cxcl1* expression in tape stripped and *S. aureus* exposed skin was also comparable in the two strains, whereas expression of *Cxcl2* and *Cxcl3* were significantly higher in *Dock8*$^{-/-}$ mice compared WT controls (Fig. 2D). This is possibly due to their induction by higher levels of IL-1 and IL-6 [21], secreted by keratinocytes in *Dock8*$^{-/-}$ mice in response to their increased cutaneous load of *S. aureus*.

An intrinsic defect in neutrophil migration in the skin is a potential explanation for the decreased accumulation of neutrophils in tape stripped and *S. aureus* exposed skin of *Dock8*$^{-/-}$ mice. This was assessed by examining the ability of neutrophils to accumulate in the tape stripped skin of *Dock8*$^{-/-}$ mice. As previously reported [22, 23], there was robust accumulation of neutrophils in tape stripped skin of WT mice (Fig. 2E). There was a modest but significant increase in the accumulation of neutrophils in tape stripped skin of *Dock8*$^{-/-}$ mice compared to controls (Fig. 2E), possibly because their egress from the skin may be impaired, as has been shown for DOCK8 deficient DCs [24]. Of note, the percentages of FVD$^-$ live and FVD$^+$ dead neutrophils in tape stripped skin of *Dock8*$^{-/-}$ mice were comparable to WT controls (data not shown). Altogether, the above data argue against a defect in the recruitment or migration of DOCK8 deficient neutrophils into the skin.

It has been demonstrated that DOCK8 deficient lymphocytes migrating in a 3D matrix or in virally infected skin undergo shattering cell death or cytothripsis [25]. We examined whether the survival of DOCK8 deficient neutrophils in *S. aureus* infected skin is impaired. The percentage of FVD$^-$ live neutrophils was significantly lower, whereas the percentage of FVD$^+$ dead neutrophils was significantly higher, in tape stripped and *S. aureus* exposed

skin of *Dock8*$^{-/-}$ mice compared to WT controls (Fig. 2F). These data strongly suggest that reduced neutrophil survival underlies the reduced neutrophil accumulation in *S. aureus* infected skin of *Dock8*$^{-/-}$ mice, resulting in impaired bacterial clearance and thereby increased susceptibility to cutaneous *S. aureus* infection.

S. aureus secretes factors that directly cause cell death by various mechanisms, including activation of the NLRP3 inflammasome leading to pyroptosis [26, 27]. The decreased neutrophil survival in tape stripped and *S. aureus* infected skin, but not in non-infected tape stripped skin, prompted us to examine whether DOCK8 deficient neutrophils were intrinsically more susceptible to *S. aureus* triggered cell death. Incubation with *S. aureus* triggered the death of ~20% of WT neutrophils after 1 and 2 hours of incubation (Fig. 2G). DOCK8 deficient neutrophils exhibited after 1 hour of incubation with *S. aureus* cell death that was comparable to WT neutrophils but had significantly more cell death (~60%) after 2 hours of incubation with *S. aureus* compared to WT controls (Fig. 2G), indicating that DOCK8 deficient neutrophils are intrinsically more susceptible to *S. aureus* triggered cell death. This is consistent with the previously reported role of DOCK8 in lymphocyte survival [28-30]. Susceptibility to *S. aureus* triggered cell death may contribute to the decreased accumulation and reduced viability of neutrophils in *S. aureus* infected skin of *Dock8*$^{-/-}$ mice.

3.3. DOCK8 deficient neutrophils have reduced phagocytosis of *S. aureus* bioparticles but normal respiratory burst.

Bacterial killing by neutrophils is a multi-step process starting with formation of the phagocytic cup, internalization of the phagosome, maturation of the phagolysosome and intracellular killing of the microbe, mediated by the respiratory burst with the release of reactive oxygen species [31]. The phagocytic process requires cytoskeletal reorganization which is dependent on WASP-mediated actin polymerization and actin cup formation [32, 33]. Since DOCK8 activates WASP [1], we investigated whether neutrophil phagocytic function is altered in DOCK8 deficiency. Purified neutrophils were isolated from the bone marrow of *Dock8*$^{-/-}$ mice and WT controls and cultured for 1 hour with opsonized pHrodo™ red *S. aureus* BioParticles™. These particles emit red fluorescence in the low pH environment of the phagolysosome, which can be analyzed by flow cytometry. Negative controls were incubated on ice to distinguish between particle binding and uptake. The percentage of bioparticle positive neutrophils was not significantly different between *Dock8*$^{-/-}$ mice and WT controls (Fig. 2H). However, the mean fluorescence intensity (MFI) of pHrodo™ red was significantly decreased in bioparticle positive neutrophils from *Dock8*$^{-/-}$ mice compared to WT controls (Fig. 2I), indicating that fewer particles were ingested by DOCK8 deficient neutrophils, suggesting a defect in phagocytic function. This is in contrast to the report by Mandola *et al.* [10] who observed no difference in phagocytosis by neutrophils from DOCK8 deficient patients after 10 minutes exposure to zymosan particles and analysis by microscopy. The different results are likely due to the different techniques used.

Respiratory burst by neutrophils is readily measured by analyzing the oxidation by hydrogen peroxide of the substrate dihydrorhodamine 123 (DHR), which yields the fluorescent

molecule rhodamine that can be detected by flow cytometry [13]. The rhodamine MFI of PMA-stimulated blood neutrophils was comparable between *Dock8*$^{-/-}$ mice and WT controls (Fig. 2J), indicating normal respiratory burst in DOCK8 deficient neutrophils. This is in agreement with Mandola *et al.* [10] who reported normal production of superoxide anions in neutrophils from DOCK8 deficient patients.

In summary, we demonstrate that in DOCK8 deficiency, injured skin is susceptible to *S. aureus* infection. The data strongly suggest that susceptibility to *S. aureus* triggered cell death and impaired phagocytic function contribute to the defective ability of neutrophils to clear *S. aureus* skin infection in DOCK8 deficiency.

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ABREVIATIONS

AD	Atopic dermatitis
CFU	Colony forming units
DOCK8	Dedicator of cytokinesis 8
EC	Epicutaneous
SEB	Staphylococcal Enterotoxin B
TEWL	Trans-epidermal water loss
WASP	Wiskott-Aldrich syndrome protein
WT	Wild type

References

[1]. Janssen E, Tohme M, Hedayat M, Leick M, Kumari S, Ramesh N, Massaad MJ, Ullas S, Azcutia V, Goodnow CC, Randall KL, Qiao Q, Wu H, Al-Herz W, Cox D, Hartwig J, Irvine DJ, Luscinskas FW, Geha RS, A DOCK8-WIP-WASp complex links T cell receptors to the actin cytoskeleton, J Clin Invest, 126 (2016) 3837–3851. [PubMed: 27599296]

[2]. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, Matthews HF, Davis J, Turner ML, Uzel G, Holland SM, Su HC, Combined Immunodeficiency Associated with DOCK8 mutations, N Engl J Med, 361 (2009) 2046–2055. [PubMed: 19776401]

[3]. Engelhardt KR, McGhee S, Winkler S, Sassi A, Woellner C, Lopez-Herrera G, Chen A, Kim HS, Lloret MG, Schulze I, Ehl S, Thiel J, Pfeifer D, Veelken H, Niehues T, Siepermann K, Weinspach S, Reisli I, Keles S, Genel F, Kutukculer N, Camcioglu Y, Somer A, Karakoc-Aydiner E, Barlan I, Gennery A, Metin A, Degerliyurt A, Pietrogrande MC, Yeganeh M, Baz Z, Al-Tamemi S, Klein C, Puck JM, Holland SM, McCabe ER, Grimbacher B, Chatila TA, Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome, J Allergy Clin Immunol, 124 (2009) 1289–1302. [PubMed: 20004785]

[4]. Cuellar-Rodriguez J, Freeman AF, Grossman J, Su H, Parta M, Murdock H, Shah N, Bollard C, Kong HH, Moutsopoulos N, Stone K, Gea-Banacloche J, Holland SM, Hickstein DD, Matched related and unrelated donor hematopoietic stem cell transplantation for DOCK8 deficiency, Biol Blood Marrow Transplant, 21 (2015) 1037–1045. [PubMed: 25636378]

[5]. Burn GL, Foti A, Marsman G, Patel DF, Zychlinsky A, The Neutrophil, Immunity, 54 (2021) 1377–1391. [PubMed: 34260886]

[6]. Dong X, Limjunyawong N, Sypek EI, Wang G, Ortines RV, Youn C, Alphonse MP, Dikeman D, Wang Y, Lay M, Kothari R, Vasavda C, Pundir P, Goff L, Miller LS, Lu W, Garza LA, Kim BS, Archer NK, Dong X, Keratinocyte-derived defensins activate neutrophil-specific receptors Mrgpra2a/b to prevent skin dysbiosis and bacterial infection, Immunity, 55 (2022) 1645–1662.e1647. [PubMed: 35882236]

[7]. Mölne L, Verdrengh M, Tarkowski A, Role of neutrophil leukocytes in cutaneous infection caused by Staphylococcus aureus, Infect Immun, 68 (2000) 6162–6167. [PubMed: 11035720]

[8]. Rigby KM, DeLeo FR, Neutrophils in innate host defense against Staphylococcus aureus infections, Semin Immunopathol, 34 (2012) 237–259. [PubMed: 22080185]

[9]. Cao M, Shikama Y, Kimura H, Noji H, Ikeda K, Ono T, Ogawa K, Takeishi Y, Kimura J, Mechanisms of Impaired Neutrophil Migration by MicroRNAs in Myelodysplastic Syndromes, J Immunol, 198 (2017) 1887–1899. [PubMed: 28130497]

[10]. Mandola AB, Levy J, Nahum A, Hadad N, Levy R, Rylova A, Simon AJ, Lev A, Somech R, Broides A, Neutrophil functions in Immunodeficiency due to DOCK8 deficiency, Immunol Invest, 48 (2019) 431–439. [PubMed: 30689480]

[11]. Leyva-Castillo JM, McGurk A, Geha RS, Allergic skin inflammation and S. aureus skin colonization are mutually reinforcing, Clin Immunol, 218 (2020) 108511. [PubMed: 32569845]

[12]. Wilkie H, Janssen E, Leyva-Castillo JM, Geha RS, DOCK8 Expression in Regulatory T Cells Maintains their Stability and Limits Contact Hypersensitivity, J Invest Dermatol, 141 (2021) 1503–1511 e1503. [PubMed: 33171169]

[13]. Richardson MP, Ayliffe MJ, Helbert M, Davies EG, A simple flow cytometry assay using dihydrorhodamine for the measurement of the neutrophil respiratory burst in whole blood: comparison with the quantitative nitrobluetetrazolium test, J Immunol Methods, 219 (1998) 187–193. [PubMed: 9831400]

[14]. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, DeLeo FR, Otto M, Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA, Nat Med, 13 (2007) 1510–1514. [PubMed: 17994102]

[15]. Joubert O, Voegelin J, Guillet V, Tranier S, Werner S, Colin DA, Dalla Serra M, Keller D, Monteil H, Mourey L, Prévost G, Distinction between pore assembly by staphylococcal alpha-toxin versus leukotoxins, J Biomed Biotechnol, 2007 (2007) 25935. [PubMed: 17497023]

[16]. Leyva-Castillo JM, Das M, Kane J, Strakosha M, Singh S, Wong DSH, Horswill AR, Karasuyama H, Brombacher F, Miller LS, Geha RS, Basophil-derived IL-4 promotes cutaneous Staphylococcus aureus infection, JCI Insight, 6 (2021).

[17]. Liu H, Archer NK, Dillen CA, Wang Y, Ashbaugh AG, Ortines RV, Kao T, Lee SK, Cai SS, Miller RJ, Marchitto MC, Zhang E, Riggins DP, Plaut RD, Stibitz S, Geha RS, Miller LS, Staphylococcus aureus Epicutaneous Exposure Drives Skin Inflammation via IL-36-Mediated T Cell Responses, Cell Host Microbe, 22 (2017) 653–666 e655. [PubMed: 29120743]

[18]. Munshi HG, Montgomery RB, Severe neutropenia: a diagnostic approach, West J Med, 172 (2000) 248–252. [PubMed: 10778379]

[19]. Nakagawa S, Matsumoto M, Katayama Y, Oguma R, Wakabayashi S, Nygaard T, Saijo S, Inohara N, Otto M, Matsue H, Nunez G, Nakamura Y, Staphylococcus aureus Virulent PSMalpha Peptides Induce Keratinocyte Alarmin Release to Orchestrate IL-17-Dependent Skin Inflammation, Cell Host Microbe, 22 (2017) 667–677 e665. [PubMed: 29120744]

[20]. Keles S, Charbonnier LM, Kabaleeswaran V, Reisli I, Genel F, Gulez N, Al-Herz W, Ramesh N, Perez-Atayde A, Karaca NE, Kutukculer N, Wu H, Geha RS, Chatila TA, Dedicator of cytokinesis 8 regulates signal transducer and activator of transcription 3 activation and promotes

TH17 cell differentiation, J Allergy Clin Immunol, 138 (2016) 1384–1394 e1382. [PubMed: 27350570]

[21]. Brauweiler AM, Goleva E, Leung DYM, Staphylococcus aureus Lipoteichoic Acid Damages the Skin Barrier through an IL-1-Mediated Pathway, J Invest Dermatol, 139 (2019) 1753–1761.e1754. [PubMed: 30779913]

[22]. Oyoshi MK, He R, Li Y, Mondal S, Yoon J, Afshar R, Chen M, Lee DM, Luo HR, Luster AD, Cho JS, Miller LS, Larson A, Murphy GF, Geha RS, Leukotriene B4-driven neutrophil recruitment to the skin is essential for allergic skin inflammation, Immunity, 37 (2012) 747–758. [PubMed: 23063331]

[23]. Gregorio J, Meller S, Conrad C, Di Nardo A, Homey B, Lauerma A, Arai N, Gallo RL, Digiovanni J, Gilliet M, Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons, J Exp Med, 207 (2010) 2921–2930. [PubMed: 21115688]

[24]. Harada Y, Tanaka Y, Terasawa M, Pieczyk M, Habiro K, Katakai T, Hanawa-Suetsugu K, Kukimoto-Niino M, Nishizaki T, Shirouzu M, Duan X, Uruno T, Nishikimi A, Sanematsu F, Yokoyama S, Stein JV, Kinashi T, Fukui Y, DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses, Blood, 119 (2012) 4451–4461. [PubMed: 22461490]

[25]. Zhang Q, Dove CG, Hor JL, Murdock HM, Strauss-Albee DM, Garcia JA, Mandl JN, Grodick RA, Jing H, Chandler-Brown DB, Lenardo TE, Crawford G, Matthews HF, Freeman AF, Cornall RJ, Germain RN, Mueller SN, Su HC, DOCK8 regulates lymphocyte shape integrity for skin antiviral immunity, J Exp Med, 211 (2014) 2549–2566. [PubMed: 25422492]

[26]. Paget C, Doz-Deblauwe E, Winter N, Briard B, Specific NLRP3 Inflammasome Assembling and Regulation in Neutrophils: Relevance in Inflammatory and Infectious Diseases, Cells, 11 (2022).

[27]. Muñoz-Planillo R, Franchi L, Miller LS, Núñez G, A critical role for hemolysins and bacterial lipoproteins in Staphylococcus aureus-induced activation of the Nlrp3 inflammasome, J Immunol, 183 (2009) 3942–3948. [PubMed: 19717510]

[28]. Lambe T, Crawford G, Johnson AL, Crockford TL, Bouriez-Jones T, Smyth AM, Pham TH, Zhang Q, Freeman AF, Cyster JG, Su HC, Cornall RJ, DOCK8 is essential for T-cell survival and the maintenance of CD8+ T-cell memory, Eur J Immunol, 41 (2011) 3423–3435. [PubMed: 21969276]

[29]. Randall KL, Chan SS, Ma CS, Fung I, Mei Y, Yabas M, Tan A, Arkwright PD, Al Suwairi W, Lugo Reyes SO, Yamazaki-Nakashimada MA, Garcia-Cruz Mde L, Smart JM, Picard C, Okada S, Jouanguy E, Casanova JL, Lambe T, Cornall RJ, Russell S, Oliaro J, Tangye SG, Bertram EM, Goodnow CC, DOCK8 deficiency impairs CD8 T cell survival and function in humans and mice, J Exp Med, 208 (2011) 2305–2320. [PubMed: 22006977]

[30]. Kearney CJ, Randall KL, Oliaro J, DOCK8 regulates signal transduction events to control immunity, Cell Mol Immunol, 14 (2017) 406–411. [PubMed: 28366940]

[31]. Underhill DM, Goodridge HS, Information processing during phagocytosis, Nat Rev Immunol, 12 (2012) 492–502. [PubMed: 22699831]

[32]. Lorenzi R, Brickell PM, Katz DR, Kinnon C, Thrasher AJ, Wiskott-Aldrich syndrome protein is necessary for efficient IgG-mediated phagocytosis, Blood, 95 (2000) 2943–2946. [PubMed: 10779443]

[33]. Park H, Cox D, Cdc42 regulates Fc gamma receptor-mediated phagocytosis through the activation and phosphorylation of Wiskott-Aldrich syndrome protein (WASP) and neural-WASP, Mol Biol Cell, 20 (2009) 4500–4508. [PubMed: 19741094]

1. *Dock8*$^{-/-}$ mice have reduced clearance of *S. aureus* applied to mechanically injured skin
2. Neutrophils numbers are decreased in *S. aureus* infected skin of *Dock8*$^{-/-}$ mice
3. DOCK8 protects neutrophils from *S. aureus* triggered cell death
4. DOCK8 is important for the phagocytic function of neutrophils

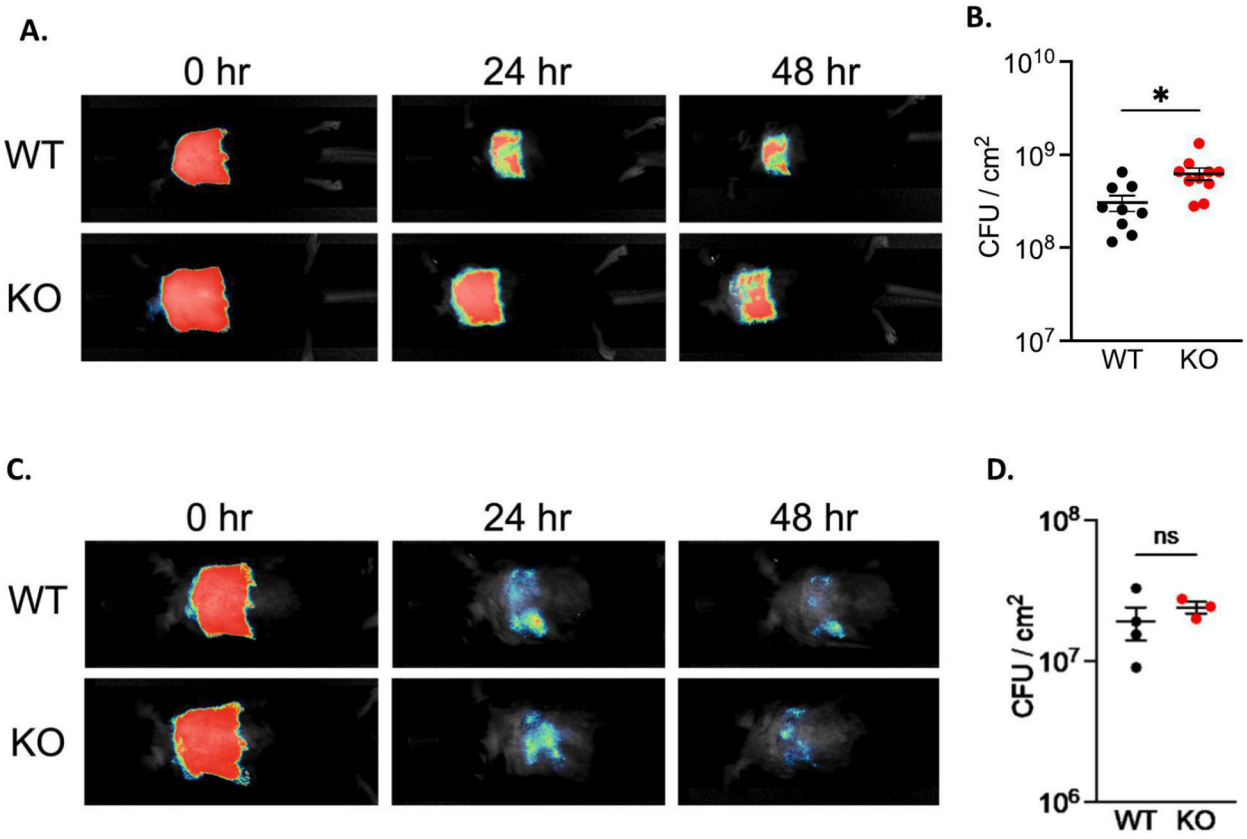

Figure 1. *Dock8*$^{-/-}$ mice have delayed clearance of *S. aureus* from mechanically injured skin

A. Representative *in vivo* fluorescence imaging of PSVue 794 fluorescence at 0 hours, 24 hours and 48 hours after application of 10^8 CFUs of PSVue 794-labeled *S. aureus* to 1 cm^2 of shaved and tape stripped skin of *Dock8*$^{-/-}$ (KO) mice and WT controls. **B.** Numbers of *S. aureus* CFUs in skin homogenates 48 hours after application of 10^8 CFUs of *S. aureus* to shaved and tape stripped skin of KO and WT mice. **C.** Representative *in vivo* fluorescence imaging of PSVue 794 fluorescence at 0 hours, 24 hours and 48 hours after topical application of 10^8 CFUs of PSVue 794-labeled *S. aureus* to 1 cm^2 of shaved skin of KO and WT mice. **D.** Numbers of *S. aureus* CFUs in skin homogenates 48 hours after topical application of 10^8 CFUs of *S. aureus* to shaved skin of KO and WT mice. Results display mean ± SEM and are representative of one experiment repeated at least twice. ns, not significant, * $p < 0.05$, by Student's T-test (B, D).

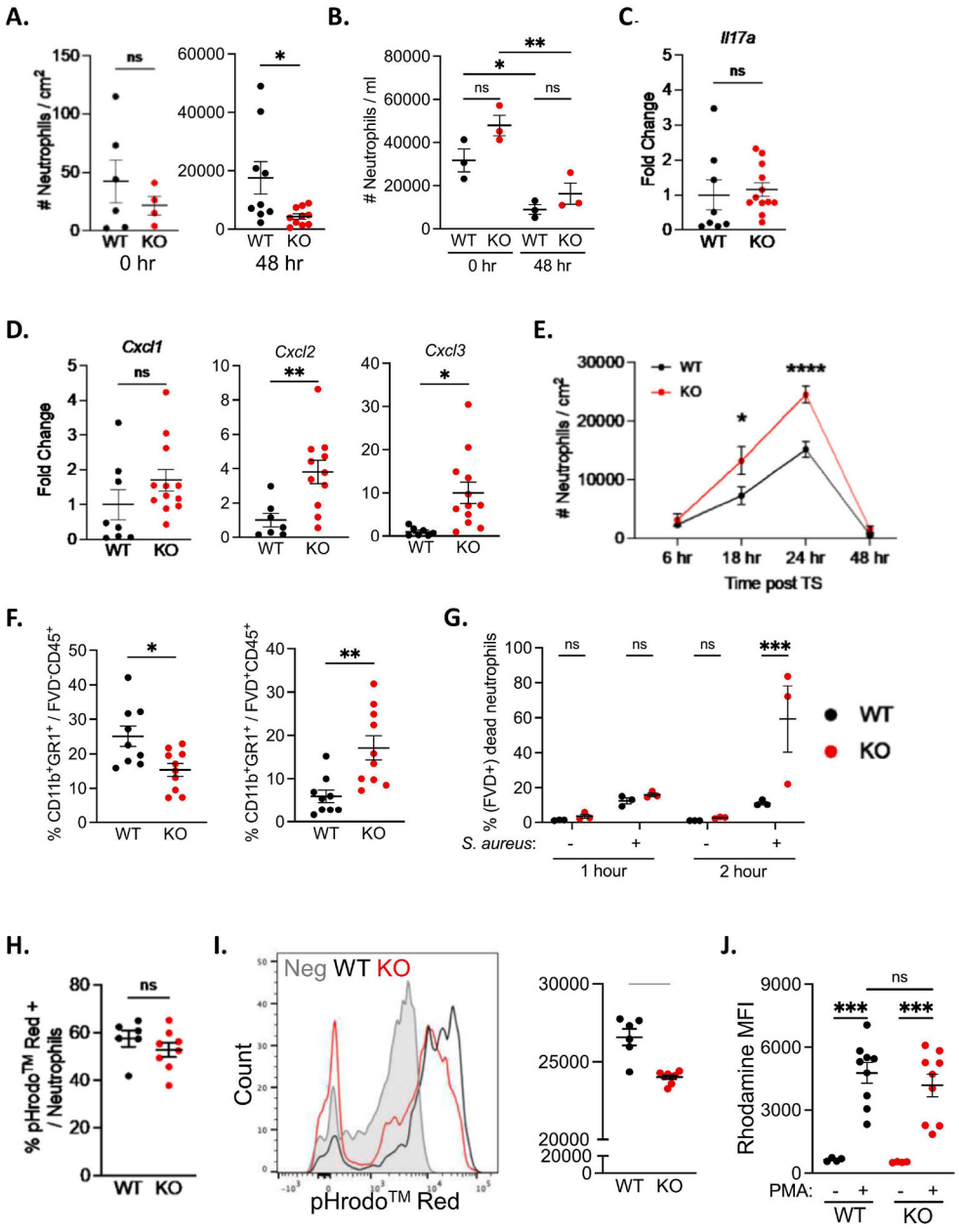

Figure 2. Decreased infiltration and viability of neutrophils in tape stripped and *S. aureus* exposed skin of *Dock8*$^{-/-}$ mice and reduced phagocytosis of DOCK8 deficient neutrophils.

A, B. Numbers of CD11b^{+}Gr1^{+} neutrophils isolated from 1 cm^{2} skin (**A**) and blood (**B**) at 0 hours and 48 hours after tape stripping and exposure to *S. aureus* in *Dock8*$^{-/-}$ (KO) mice and WT controls. **C, D.** Cutaneous expression of *Il17a* (**C**) and neutrophil attracting chemokines (**D**) 48 hours after tape stripping and exposure to *S. aureus* in KO and WT mice. **E.** Numbers of neutrophils isolated from the skin at various times post tape stripping in KO and WT mice. **F.** Percentages of FVD− live (left) and FVD+ dead (right) neutrophils in the skin 48 hours after tape stripping and exposure to *S. aureus* in KO and WT mice. **G.** Percentages of FVD+ dead purified BM neutrophils from KO and WT mice after 1 and 2 hours incubation with medium or *S. aureus*. **H, I.** Percentage of pHrodo red

positive neutrophils (**H**), representative histogram of pHrodo red fluorescence in neutrophils (**I**, left) and quantification of pHrodo red mean fluorescence intensity (MFI) (**I**, right) following incubation of purified BM neutrophils from KO and WT mice with pHrodo red *S. aureus* BioParticles. The negative control (Neg) in the histogram represents neutrophils incubated with pHrodo red *S. aureus* BioParticles on ice. **J.** Geometric mean fluorescence intensity (MFI) of rhodamine in purified BM neutrophils from KO and WT mice stimulated with medium or PMA. Data display mean ± SEM and are pooled from two independent experiments (A-F) or are representative of one experiment repeated at least twice (G-J). ns, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Student's T-test (A, C, D, F, H, I), one-way ANOVA (J), or two-way ANOVA (E, G).