Published in final edited form as:

J Allergy Clin Immunol. 2023 October ; 152(4): 907–915. doi:10.1016/j.jaci.2023.05.025.

IL-4 Receptor alpha blockade dampens allergic inflammation and upregulates IL-17A expression to promote *S. aureus* clearance in antigen sensitized mouse skin

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Abstract

Background: Skin colonization with *S. aureus* aggravates atopic dermatitis (AD) and exaggerates allergic skin inflammation in mice. IL-4Rα blockade is beneficial in AD and reduces *S. aureus* skin colonization through unknown mechanisms. The cytokine IL-17A restrains *S. aureus* growth.

Objective: Examine the effect of IL4Rα blockade on *S. aureus* colonization at sites of allergic skin inflammation in mice and determine the mechanism involved.

Methods: Balb/c mice were epicutaneously sensitized with ovalbumin (OVA). Immediately after, PSVue794-labeled *S. aureus* strain SF8300 or saline was applied and a single dose of anti-IL-4Rα blocking antibody, a mixture of anti-IL-4Rα and anti-IL-17A blocking antibodies, or IgG isotype controls were administered intradermally. *S. aureus* load was assessed two days later by *in vivo* imaging and enumeration of colony forming units (CFUs). Skin cellular infiltration was examined by flow cytometry, and gene expression by qPCR and transcriptome analysis.

Results: IL-4Rα blockade decreased allergic skin inflammation in OVA-sensitized skin, as well as in OVA-sensitized and *S. aureus*-exposed skin, evidenced by significantly decreased epidermal thickening and reduced dermal infiltration by eosinophils and mast cells. This was accompanied by increased cutaneous expression of *Il17a* and IL-17A-driven antimicrobial genes with no change in *Il4* and *Il13* expression. IL-4Rα blockade significantly decreased *S. aureus* load in OVA-sensitized and *S. aureus*-exposed skin. IL-17A blockade reversed the beneficial effect of

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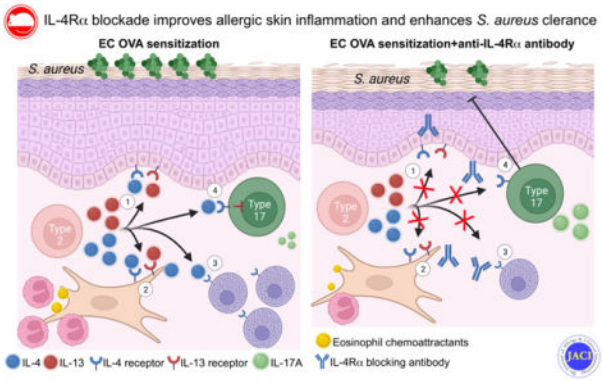
Disclosure of potential conflict of interest: Dr. Yui-Hsi Wang is an employee of Sanofi. The rest of the authors declare that they have no relevant conflicts of interest.

IL-4Rα blockade on *S. aureus* clearance. and reduced the cutaneous expression of IL-17A driven antimicrobial genes.

Conclusion: IL-4Rα blockade promotes *S. aureus* clearance from sites of allergic skin inflammation in part by enhancing IL-17A expression.

Graphical Abstract



The type 2 cytokines IL-4 and IL-13 produced by Th2 cells, ILC2s basophils and mast cells (MCs) in the skin play an important role in allergic skin inflammation and *S. aureus* colonization. Left panel: IL-4 and IL-13 promote epidermal hyperplasia (1), production of eosinophil chemoattractants by stromal cells (2), cutaneous mast cells expansion (3) and suppression of IL-17A production by Type 17 cells (TCR γδ cells, Th17 cells) which is critical for *S. aureus* clearance (4). Right panel: Local IL-4Rα blockade by monoclonal antibody to IL-4Rα, shared by the receptors for IL-4 and IL-13, alleviates allergic skin inflammation and enhances IL-17A production and thereby promotes *S. aureus* clearance.

Capsule Summary.

Local IL-4Rα blockade ameliorates allergic skin inflammation and enhances *S. aureus* clearance in mice in part by promoting local IL-17A expression.

Keywords

Atopic dermatitis; *S. aureus* ; Type 2 cytokines; IL-4Rα; IL-17A

Introduction.

Atopic dermatitis (AD) is characterized by a defective skin barrier function and a type 2 dominated local and systemic response to antigens encountered through the skin[1]. AD lesional skin is almost always colonized with *S. aureus*, and the *S. aureus* load correlates with disease severity[1, 2].

We previously demonstrated that epicutaneous (EC) sensitization with ovalbumin (OVA) elicits allergic skin inflammation that shares many features with AD skin lesions[3–6]. These include epidermal hyperplasia, infiltration with $CD4^+$ T cells and eosinophils, increased local levels of *Il4*, *Il13* and *Il17a* mRNA and delayed clearance of topically applied *S.*

aureus bacteria[3–7]. In addition, OVA sensitized mice develop a systemic response to OVA with OVA specific IgE antibodies and cytokine secretion by splenocytes in response to OVA stimulation *in vitro*. Genetic ablation experiments in mice have demonstrated that the Th2 cytokines IL-4 and IL-13 play an important role in the development of allergic skin inflammation elicited by EC sensitization and inhibit the clearance of *S. aureus* topically applied to antigen sensitized mouse skin[4, 7–9].

The cytokine IL-17A plays an important role in protecting against bacterial infections, including those of skin, by recruiting neutrophils and promoting the production of antimicrobial genes[10, 11]. Patients and mice with defects in IL-17A, IL-17A receptor chains or IL-17A signaling are susceptible to mucosal and skin infections caused by *S aureus*[10, 12]. IL-17A production by TCRγδ cells is critical for *S. aureus* clearance from the skin, and is inhibited by IL-4[10, 13]. lL-4 also inhibits the induction of antimicrobial peptides in keratinocytes by IL-17A[12]. IL-4 and IL-13 inhibit the local and systemic IL-17A response in allergic skin inflammation[14]. Of interest, the use of the IL-4Rα blocker Dupilumab has been linked to the emergence of Th17 type diseases in some patients[15].

The IL-4Rα chain is shared by the Th2 cytokines IL-4 and IL-13. Systemic IL-4Rα blockade in AD patients improves the signs and symptoms of the disease, increases microbial diversity, and reduces the abundance of *S. aureus* in the skin[16–18]. The mechanism of this reduction is not well understood. We made use of a monoclonal antibody against the murine IL-4Rα chain to investigate whether and how IL-4Rα blockade dampens allergic skin inflammation and promotes the clearance of topically applied *S. aureus* in mice.

Methods.

Mice.

BALB/c mice were purchased from Charles River Laboratory. All mice were kept in a pathogen-free environment and fed an OVA-free diet. All procedures were performed in accordance with the Animal Care and Use Committee of the Children's Hospital Boston.

S. aureus preparation and quantification.

S. aureus inoculum was prepared as described previously[13, 19]. To enumerate the bacterial load in vivo, *S. aureus* was labeled with PSVue794 reagent kit (LI-COR) and quantified as described previously[7, 13]. To enumerate the bacterial load from the skin, two 8 mm^2 skin biopsies were obtained and analyzed as previously described[13, 19].

Epicutaneous (EC) sensitization, antibody treatments and *S. aureus* application.

Female mice 6–8-weeks old were epicutaneously sensitized for 8 days as described previously[4]. Briefly, Mice were anesthetized, and their back skin was shaved and tape-stripped with a film dressing (TegadermTM, 3M) followed by the application of 200 μg OVA (Sigma-Aldrich) or saline every other day. On day 9, 100 μg of anti-IL-4Rα monoclonal antibody (clone mIL4R-M1, BD Biosciences), or IgG isotype control were intradermally injected in OVA sensitized skin with or without immediate application of 10^8 CFU *S. aureus* using a cotton swab. Alternatively anti-IL-4Rα monoclonal antibody

plus anti-IL-17A antibody (clone 17F3, Bioxcell) or anti-IL-4Rα monoclonal antibody plus IgG isotype control were intradermally injected in OVA sensitized skin with or without immediate application of 10^8 CFU *S. aureus*. Analyses were done at D11.

Histology and measurement of epidermal thickness.

Skin specimens were fixed in 4% paraformaldehyde embedded in paraffin and H&E stained and analyzed as previously described[4, 20].

Skin cell preparation, and flow cytometry.

$1cm^2$ skin pieces from EC sensitized skin were obtained and the cell isolation was performed as previously described[4, 6]. For flow cytometry, skin cells were preincubated with FcγR-specific blocking mAb (2.4G2) and washed before staining with the following monoclonal antibodies (mAbs): CD3 (17A2), CD45 (30F11), Gr1 (RB6–8C5) from eBioscience, CD11b (M1/70) and CD117 (2B8) from Biolegend and anti-Siglec-F (E50–2440) and anti-IgE (R35–72) from BD Biosciences. Cells were analyzed by flow cytometry using an LSRFortessa machine (BD Biosciences). The data were analyzed with FlowJo software.

mRNA expression analyses.

Total skin RNA extraction and measurement of cytokines were performed and analyzed as previously described[3, 4]. PCR reactions were run on ABI Prism 7300 (Applied Biosystems) sequence detection system platform. Taqman primers and probes were obtained from Life technologies. The housekeeping gene β_2-microglobulin was used as an internal control. Relative mRNA expression was quantified using the $2^{-\Delta\Delta Ct}$ method.

Transcriptomic analysis.

Total skin RNA was isolated as described above, followed by cDNA synthesis using the SuperScript VILO cDNA Synthesis Kit (ThermoFisher Scientific). The Ion AmpliSeq Transcriptome Mouse Gene Expression Kit was used to prepare bar-coded libraries and sequenced by using an Ion S5 next-generation sequencer. The AmpliSeqRNA plug-in (ThermoFisher Scientific) was used to calculate differential gene expression analysis. Pathway analysis was performed by using Ingenuity Pathway Analysis (Qiagen) and Gene Set Enrichment Analysis (Broad Institute and University of California San Diego) on genes with at least a 1.5-fold difference between the conditions ($p<.05$).

Statistical analysis.

Statistical significance was determined by the two-tailed Student's t test. A p value <0.05 was considered statistically significant.

Results

Local IL-4Ra blockade reduces allergic skin inflammation elicited by EC sensitization with OVA.

We previously reported that EC sensitization with OVA causes epidermal thickening, accumulation of T cells, mast cell and eosinophils, and local upregulation of *Il4*, *Il13*, and *Il17a*, but not *ifng* expression[3–6]. To examine the effect of IL-4Rα blockade on allergic skin inflammation BALB/c mice were EC sensitized with OVA for 8 days. The following day, 100 μg rat anti-mouse anti-IL-4Rα blocking antibody or IgG2a isotype control was administered intradermally (*i.d.*) into OVA sensitized skin and the skin was examined 2 days later as illustrated in Fig. 1A.

Administration of anti-IL-4Rα significantly reduced epidermal hyperplasia (Fig. 1B, C), as well as skin infiltration by eosinophils and mast cells, but not $CD4^+$ T cells, compared to administration of isotype control (Fig. 1D–F). It also significantly increased infiltration by neutrophils (Fig, 1G). The decrease in skin eosinophils is consistent with the role of Th2 cytokines in driving eotaxin expression in the skin[21] and is supported by the finding that IL-4Rα blockade caused a decreased in *Ccl24* expression in OVA sensitized skin (Fig. E1). The decrease in skin mast cells is consistent with the role of IL-4 as a promoter of mast cell proliferation and survival *in vitro* and *in vivo*[22, 23] and is supported by the finding that mast cells were decreased in OVA sensitized skin from $Mcpt5cre^{Tg/0}Il4ra^{flox/-}$ mice, which lack IL-4Rα specifically in mast cells, compared to $Il4ra^{flox/-}$ controls (Fig. E2).

Cutaneous expression of *Il4*, *Il13* and *Ifng* was comparable in mice injected *i.d.* with anti-IL-4Rα or isotype control (Fig. 1H). However, IL-4Rα blockade caused a significant increase in the cutaneous expression of *Il17a* compared to control (Fig. 1H). IL-4Rα blockade caused no significant change in the expression of the IL-17 family members *Il17b*, *Il17c*, *Il17d* and *Il17f*. *Il22* expression was not detectable in OVA sensitized skin (data not shown). The higher expression of *Il17a* is consistent with the higher expression of *Il17a* in OVA sensitized skin of $Il4^{-/-}/Il13^{-/-}$ mice[7, 14] and likely underlies the increased neutrophil infiltration in OVA sensitized skin caused by IL-4Rα blockade. IL-4 inhibits TNFα expression in macrophages[24]. IL-4Rα blockade caused a significant increase in the cutaneous expression of *Tnf* compared to control (Fig. 1H). In contrast to its effects in OVA sensitized skin, IL-4Rα blockade had no significant effects on the expression of cytokine genes, including, *Il17a* and *Tnfa*, or on neutrophil accumulation in shaved intact skin (Fig. E3).

EC sensitization with OVA drives the production of OVA-specific IgE antibodies and the secretion of IL-4, IL-13, IL-17A, and IFNγ by splenocytes restimulated with OVA *in vitro*[3, 4, 20]. There were no significant differences in total or OVA-specific serum IgE levels or secretion of IL-4, IL-13, IL-17A, and IFNγ cytokines by OVA stimulated splenocytes between mice EC sensitized with OVA that received anti-IL-4Rα antibody compared to isotype control (Fig. E4 A–C). The lack of effect of IL-4Rα blockade on systemic Th2 responses in our model is consistent with the IL-4 independence of already established Th2 responses and suggests that IL-4Rα blockade needs to happen earlier and/or extend longer to detect an effect on these responses.

Our results indicate that local IL-4Rα blockade ameliorates Th2 dominated allergic skin inflammation induced by EC sensitization with OVA but does not impact the systemic immune response to the antigen. These results suggest IL-4Rα blockade in the skin may be sufficient for the improvement in skin inflammation in AD patients treated with Dupilumab[16, 18].

Local IL-4Rα blockade modulates global gene expression at sites of allergic skin inflammation.

We investigated the effect of local IL-4Rα blockade on skin gene expression, by comparing global gene expression in OVA sensitized skin *i.d.* injected with anti-IL-4Rα or isotype control. IL-4Rα blockade differentially regulated the expression of 118 genes by a more than 1.5-fold difference ($p<0.05$) compared to isotype control. 78 of these genes were upregulated and 40 were downregulated (Table E1). Ingenuity Pathway Analysis (IPA) revealed IL-4, IL-13 and STAT6 as upstream regulators of gene expression changes in OVA sensitized skin *i.d.* injected with anti-IL-4Rα antibody. These changes included the upregulation of innate immunity genes (*Cd14*, *Tlr1* and *Cybb*) and interferon induced genes (*Ifi44l*, *Ifi203* and *Mx1*) (Fig. 1I and Table E2). IL-4Rα blockade caused no significant upregulation of anti-microbial peptide (AMP) genes known to be targets of suppression by IL-4 and IL-13 including *Defb* and *S100* family genes as well as *Camp*, and *Lcn2* (Fig. E5A)[25, 26]. Moreover, no significant upregulation in genes related to skin barrier function, including *Flg, Lor and Ivl*, genes known to be suppressed by IL-4 and IL-13, was observed following IL-4Rα blockade (Fig. E5B). IPA analysis also detected in OVA sensitized skin *i.d.* injected with anti-IL-4Rα antibody upregulation of defense response genes upregulated by IL-17A (*Cd14*, *Ccl2*, *Socs3*) and genes upregulated by TNFα (*Arsi, Cd247, Cd28, FceR1g, Gas5, Gbp4, Itgb7, Mcx1, Pla1a and Wnt3a*) several of which are involved in host defense (Fig. 1J and Table E2). These results are consistent with the role of IL-4 and IL-13 in suppressing the defense response against microorganisms in AD lesional skin[27–31]. Importantly, they suggest that local IL-4Rα blockade could enhance microbial clearance from sites of allergic skin inflammation.

Local IL-4Rα blockade enhances *S. aureus* clearance from sites of allergic skin inflammation.

To investigate whether local IL-4Rα blockade promotes *S. aureus* clearance from sites of allergic skin inflammation, mice were EC sensitized with OVA for 8 days followed by *i.d* injection on day 9 of anti-IL-4Rα antibody or IgG isotype control, immediate application of 10^8 CFUs of PSVue 794 labeled *S. aureus* strain SF8300 and analysis of the skin two days later as illustrated in Fig. 2A. *S. aureus* load was examined by *in vivo* whole animal imaging, as well as by measuring the numbers of colony forming units (CFUs) in skin homogenates plated on Chromagar. *In vivo* imaging revealed a decrease in PSVue 794 fluorescence in mice *i.d* injected with anti-IL-4Rα antibody compared with those *i.d* injected with isotype control (Fig. 2B). Moreover, significantly lower numbers of CFUs (~ 5 fold less) were recovered from homogenates of skin *i.d* injected with anti-IL-4Rα antibody compared to controls (Fig. 2C).These results indicate that local IL-4Rα blockade enhances the clearance of *S. aureus* from sites of allergic inflammation. In contrast to its effects in

OVA sensitized skin, IL-4Rα blockade had no significant effect on *S. aureus* clearance from shaved intact skin (Fig. E6).

IL-4Rα blockade in OVA-sensitized and *S. aureus*-exposed skin, like IL-4Rα blockade in OVA-sensitized skin, resulted in significantly reduced epidermal thickness, and decreased dermal infiltration by eosinophils and mast cells, but not $CD4^+$ cells (Fig. 2D–H). IL-4Rα blockade had no significant effect on the robust accumulation of neutrophils in OVA-sensitized and *S. aureus*-exposed skin. IL-4Rα also significantly increased the expression of *Il17a*, *Il17c* and *Tnf*, but not *Il4*, *Il13*, or *Ifng*, in IL-4Rα blockade in OVA-sensitized and *S. aureus*-exposed skin compared to treatment with isotype control (Fig. 2J). This result suggests that IL-4Rα blockade relieves IL-4 inhibition of the protective IL-17A response of TCR γδ T cells to *S. aureus* colonization[13]. The failure of IL-4Rα blockade to result in increased neutrophil infiltration in OVA sensitized and *S. aureus* exposed skin *S. aureus* load despite increased *Il17a* expression is apparently paradoxical. However, efficient *S. aureus* clearance may have led to neutrophils being rapidly cleared from the site. Further, *S. aureus* causes the release of cytokines and molecules that attract neutrophils independent of IL-17A. These include IL-1, IL-6, LTB4 and activated complement components[32]. The decreased *S. aureus* load would have resulted in a decrease of these neutrophil attractants.

IL-17A mediates the enhancement of *S. aureus* clearance from sites of allergic skin inflammation by IL-4Rα blockade.

Since local IL-4Rα blockade increased *Il17a* expression in OVA sensitized skin, we examined whether IL-17A mediates the enhancement by IL-4Rα blockade of *S. aureus* clearance from sites of allergic skin inflammation. Mice were EC sensitized with OVA for 8 days followed by *i.d* injection on day 9 of anti-IL-4Rα antibody together with anti-IL-17A antibody, anti-IL-4Rα antibody together with IgG isotype control, or anti-IL-17A together with IgG isotype control, then immediate application of 10^8 CFUs of *S. aureus* strain SF8300 and analysis of the skin on Day 11 as illustrated in Fig. 3A. Significantly higher numbers of CFUs (~4 fold more) were recovered from homogenates of *i.d* injected skin with the combination of anti-IL-4Rα and anti-IL-17A compared with anti-IL-4Rα and IgG isotype control (Fig. 3B). Significantly less CFUs were recovered with neutralization of IL-17A alone compared to dual neutralization of IL-4 and IL-17A, suggesting that not all *S. aureus* promoting caused by IL-4Rα blockade in OVA sensitized skin is due to IL-17A. These results demonstrate that increased IL-17A expression plays an important role in the enhancement of *S. aureus* clearance from sites of allergic skin inflammation by IL-4Rα blockade.

We had previously shown that IL-13 and IL-17A individually contribute to the increase in epidermal thickness in OVA sensitized skin[4, 20]. We observed no significant change in the epidermal thickness of OVA sensitized and *S. aureus* exposed skin when both IL-4Rα and IL-17A were blocked compared to IL-17A blockade alone or IL-4Rα blockade alone (Fig. 3C, D). The increased *S. aureus* skin colonization by the double blockade as shown in Fig. 3B may have masked any potential synergy between Th2 cytokines and IL-17A in driving epidermal thickness.

Dual IL-4Rα and IL-17A blockade had no further effect on skin infiltration by eosinophils, mast cells, and $CD4^+$ T cells (data not shown) or expression of *Il4, Il3 Il17a* and *Tnf* compared to IL-4Rα blockade alone (Fig 3E), indicating that the upregulation of *Il17a* and *Tnf* by IL-4Rα blockade remained unaffected by IL-17A blockade. Dual IL-4Rα and IL-17A blockade differentially regulated the expression of 170 genes in OVA sensitized skin by a difference of more than 1.5-fold ($p<0.05$) compared to IL-4Rα blockade alone. 32 of these genes were upregulated and 138 were downregulated (Table E3). Importantly, the expression of several IL-17A driven antimicrobial genes (*Ccl7, Ccl8, Fos, Klk11, Lox, Mmp3, Nr4a2, Plgs2, Saa3, Sele*, and *Timp1*) was downregulated in OVA sensitized skin co-injected with anti-IL-17A and anti-IL-4Rα compared to OVA sensitized skin injected with anti-IL-4Rα and IgG isotype control (Fig. 3F and Table E4). The expression of nine of these 11 genes also is known to be driven by TNF α; however, as IL-17A blockade had no significant effect on *Tnf* expression, their downregulation is likely a direct result of interrupting IL-17A signaling.

Discussion.

We demonstrate that IL-4Rα blockade decreased allergic skin inflammation in OVA-sensitized, as well as in OVA-sensitized and *S. aureus*-exposed mouse skin and importantly enhanced S. aureus clearance from OVA sensitized skin sites. IL-4Rα blockade was accompanied by increased cutaneous expression of *Il17a*, and IL-17A contributed to the improved clearance of *S. aureus* from sites of allergic skin inflammation.

The dose of anti-IL-4Rα blocking antibody we administered intradermally (*i.d.*) into OVA sensitized skin (~ 5 mg/kg for a 20 g mouse) is in the range of the dose of Dupilumab injected subcutaneously to treat adult patients with AD (~3.75 mg/kg every 2 weeks). The local delivery of the IL-4Rα blockade in our model may explain its rapid effect on allergic skin inflammation, an effect that is delayed in AD patients treated to with Dupilumab.

Our results show no significant effect of IL-4Rα blockade on the expression of genes coding for antimicrobial peptides or proteins important forsk in barrier function. This is at odds with the downregulation by type 2 cytokines of the expression of these genes in human keratinocytes[33–35]. This difference could be explained by the fact that different tissues were analyzed (whole skin in our study vs keratinocytes in the human studies) and/or may reflect species differences.

We observed no significant effect of IL-4Rα blockade on systemic Th2 responses in our model, including serum levels of total and OVA-specific IgE and Th2 cytokine secretion by OVA stimulated splenocytes. This is in contrast with the observed decrease in serum IgE levels and of circulating $CD4^+IL\text{-}4^+$ and $CD4^+IL\text{-}13^+$ Th2 cells in AD patients treated with Dupilumab[16, 36, 37]. This difference suggests that IL-4Rα blockade needs to happen earlier or/and last longer in order to detect an effect on these responses. Furthermore, IL-4Rα blockade in our model had no effect on skin infiltration by $CD4^+$ T cells or expression of *Il4* and *Il13*. This is in contrast with the decrease in $CD4^+$ T cells and reduced *Il13* expression in AD lesional skin of patients treated with Dupilumab[38]. These differences may be explained by the fact that IL-4Rα blockade was only for only 2 days in our model

whereas in AD the skin was examined weeks after repetitive administration of Dupilumab. The results suggest in our model, IL4Rα blockade has no effect on differentiated Th2 cells consistent with their IL-4 independence. In contrast, longer term $IL4R_{\alpha}$ blockade in Dupilumab treated patients, could affect the polarization of naïve T cells into Th2 cells and thereby the recruitment of newly generated Th2 cells to the skin.

While our data demonstrates an important role for the upregulation of IL-17A by IL-4Rα blockade in the enhanced clearance of *S. aureus* from sites of allergic skin inflammation, other factors may also play a role. These may include reversal of the inhibitory effect of IL-4 and IL-13 on the induction of antimicrobial peptides in keratinocytes[7, 13, 26] and induction of antimicrobial genes by the elevated TNF_{α} levels at sites of allergic skin inflammation treated with anti-IL-4Rα.

S. aureus skin colonization can trigger allergic skin inflammation as well as exacerbate it. Restraining the growth of *S. aureus* in lesional skin sites may contribute to the beneficial effect of IL-4Rα blockade in AD. We demonstrate that in addition to dampening allergic skin inflammation, IL-4Rα blockade upregulates cutaneous *Il17a* expression and enhances *S. aureus* clearance from antigen sensitized mouse skin. This is mediated in large part by IL-17A. Upregulation of IL-17A expression may strongly contribute to the beneficial effect of IL-4Rα blockade on *S. aureus* clearance in AD reported in the accompanying paper by Simpson *et al.*

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

The work was supported by NIH/NIAID grants U19AI117673, 5T32AI007512-32, and Sanofi Awards program (to J-ML-C and RSG).

Abbreviations.

AD	Atopic dermatitis
EC	epicutaneous
OVA	ovalbumin
i.d.	intradermal
IPA	Ingenuity Pathway Analysis
MRSA	Methicillin-resistant Staphylococcus aureus
CFUs	colony forming units

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Key Messages.

- Local IL-4Rα blockade improves allergic skin inflammation and enhances local IL-17A expression in mice.
- Local IL-4Rα blockade enhances *S. aureus* clearance from sites of allergic skin inflammation.
- The beneficial effect of local IL-4Rα blockade on *S. aureus* clearance from sites allergic skin inflammation is mediated in part by IL-17A.

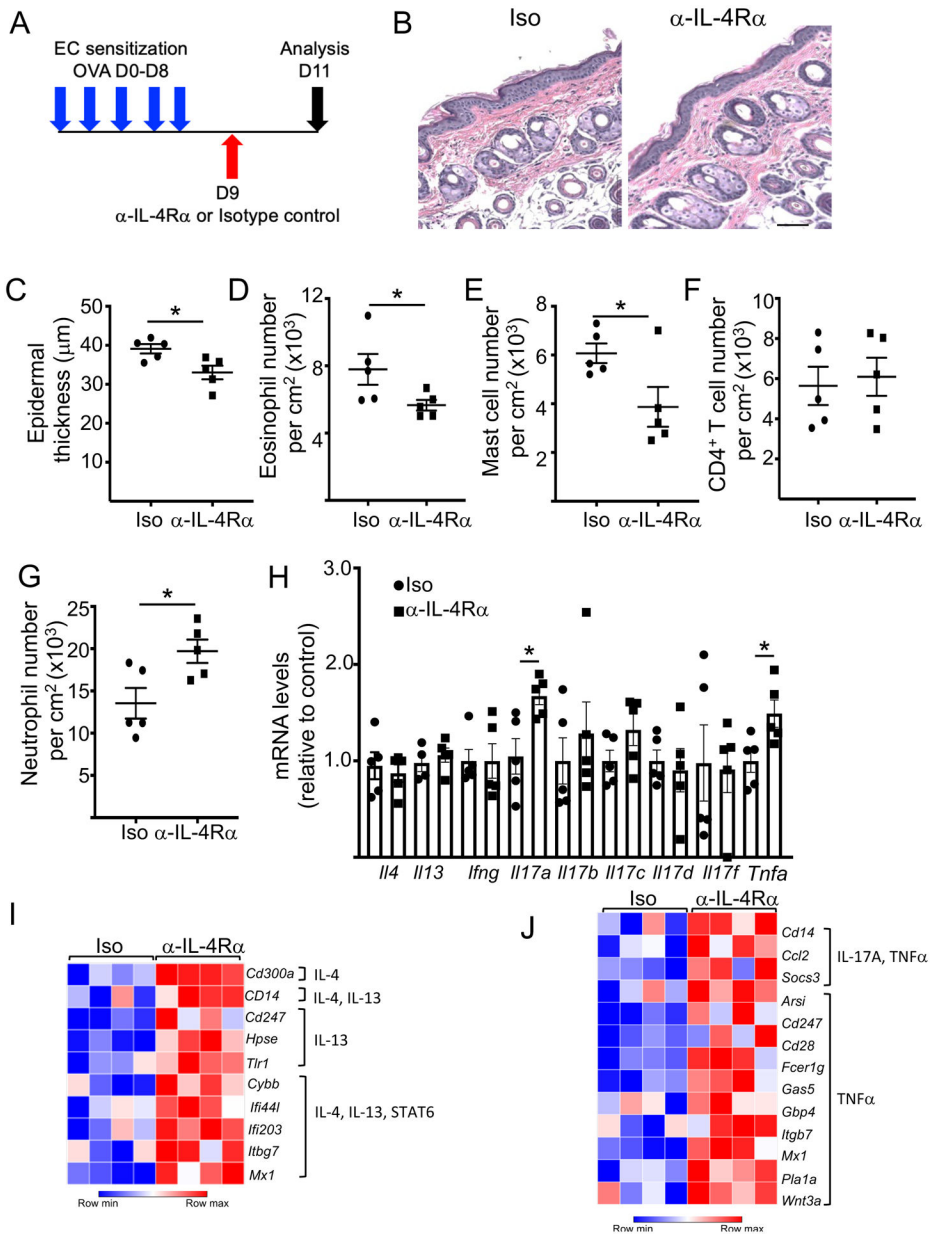

Figure 1. Local IL-4Rα blockade reduces allergic skin inflammation induced by EC sensitization with OVA.

A. Experimental protocol. **B-J.** Representative H&E staining (B) epidermal thickness (C), number of eosinophils (D), mast cells (E), $CD4^+$ T cells (F) and neutrophils (G), and mRNA levels of *cytokines* expressed relative to the mean of isotype injected WT controls (H). **I-J.** Heatmap of Ingenuity pathway analysis of genes differentially expressed (change > 1.5 fold, $p < 0.05$) in OVA-sensitized skin intradermally injected with anti-IL-4Rα antibody or isotype control. * $p<0.05$.

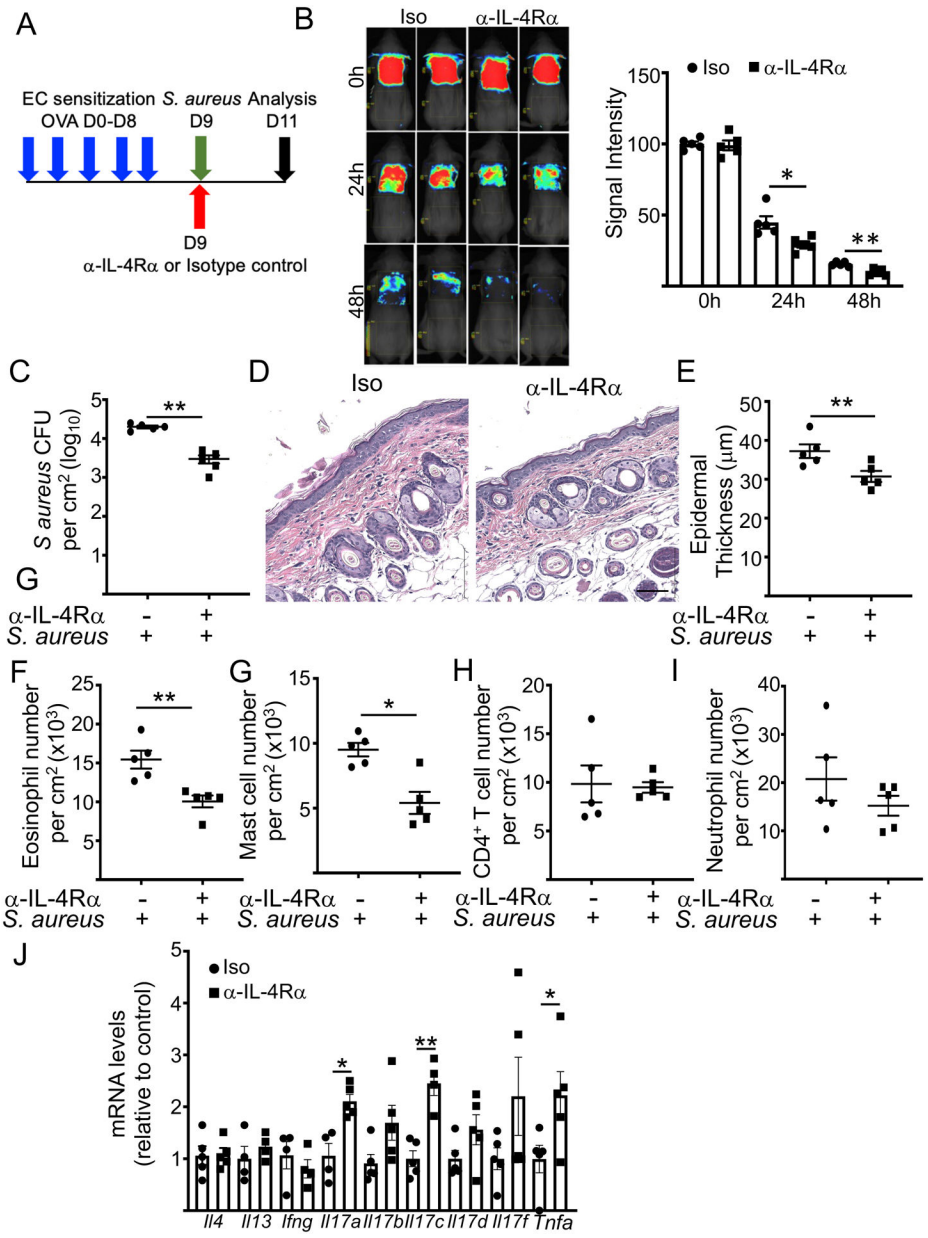

Figure 2. Local IL-4Ra blockade enhances *S. aureus* clearance from skin EC sensitized with OVA.

A. Experimental protocol. **B-C.** Representative *in vivo* imaging of *S. aureus* fluorescence (B) and quantitation of *S. aureus* CFUs in skin homogenates (C) in OVA-sensitized skin exposed to *S. aureus* and intradermally injected with anti-IL-4Rα antibody or IgG2a isotype control. **D-J.** Representative H&E staining (D) epidermal thickness (E), number of eosinophils (F), mast cells (G), CD4+ T cells (H) and neutrophils (I), and mRNA levels of *cytokines* expressed relative to the mean of isotype injected WT controls (J). *p<0.05 and **p<0.005.

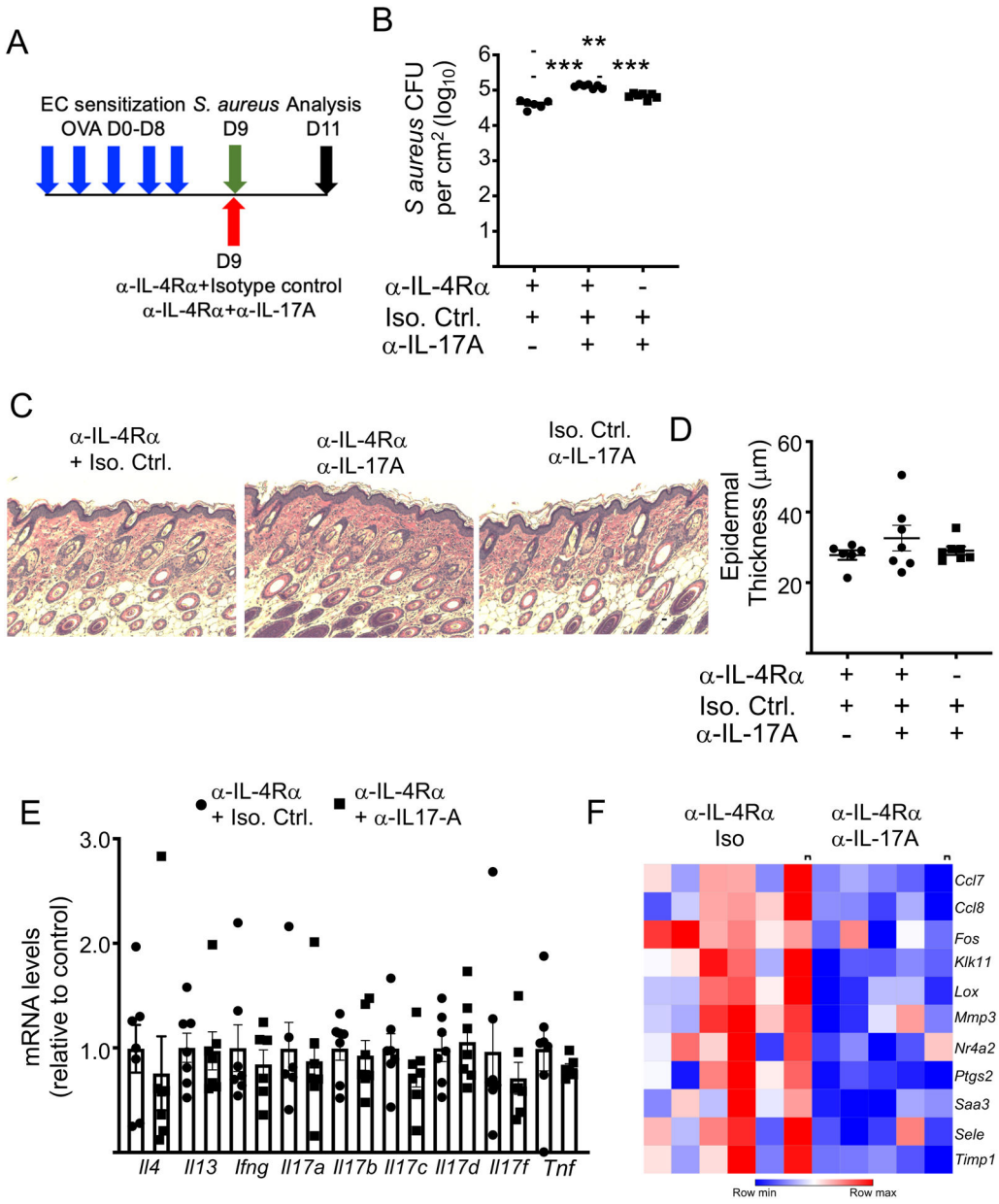

Figure 3. Local IL-17A blockade impairs clearance of *S. aureus* induced by IL-4Ra blockade in EC sensitized skin with OVA.

A. Experimental protocol. **B-D.** Quantitation of *S. aureus* CFUs in skin homogenates(B), representative H&E staining (C) and epidermal thickness (D) in OVA-sensitized skin exposed to *S. aureus* and intradermally injected with anti-IL-4Rα antibody anti-IL-17A antibody or both with the appropriate IgG isotype control. **E.** mRNA levels of cytokines expressed relative to the mean of isotype injected WT controls (E). **F.** Heatmap of Ingenuity pathway analysis of genes differentially expressed (change > 1.5 fold, $p<0.05$) in OVA-sensitized skin exposed to *S. aureus* and intradermally injected with anti-IL-4Rα antibody and anti-IL17A antibody or isotype control. **$p<0.005$ and ***$p<0.001$