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IL-4R α signalling in B cells and T cells play differential roles in acute and chronic atopic dermatitis

Martyna Scibiorek^{1,2}✉, Nontobeko Mthembu¹✉, Sandisiwe Mangali^{1,2}, Amkele Ngomti¹, Paul Ikwegbue¹, Frank Brombacher^{1,2,3} & Sabelo Hadebe¹✉

Atopic dermatitis (AD) is a common pruritic inflammatory skin disease with complex environmental and genetic predisposing factors. Primary skin barrier dysfunction and aberrant T helper 2 (TH2) responses to common allergens, together with increased serum IgE antibodies, characterise the disease. B and T cells are essential in the disease manifestation, however, the exact mechanism of how these cells is involved is unclear. Targeting interleukin 4 receptor alpha (IL-4R α), an IL-4/IL-13 signalling axis, with dupilumab shows efficacy in AD. We investigated the importance of IL-4R α signalling specifically on B and T cells during acute and chronic models of AD. We used House dust mite (HDM) and Ovalbumin (OVA) in chronic models and a low-calcemic analog of vitamin D (MC903) for acute models of AD. We used mb1^{cre}IL-4R α ^{-/lox}, iLCK^{cre}IL-4R α ^{-/lox}, LCK^{cre}IL-4R α ^{-/lox}, CD4^{cre}IL-4R α ^{-/lox}, Foxp3^{cre}IL-4R α ^{-/lox} and IL-4R α ^{-/lox} littermate controls. IL-4R α -responsive B cells were essential in serum IgE levels, but not in epidermal thickening in both chronic and acute models. IL-4R α -responsive T cells were essential in epidermal thickening in the pan-T cell, but not CD4 or CD8 T cells suggesting the importance of $\gamma\delta$ T cells during acute AD. Our results suggest that IL-4R α responsiveness on innate T cells regulates acute atopic dermatitis, while on B cells it regulates IgE.

Atopic dermatitis (AD) affects approximately 20% of children and up to 3% of adults worldwide¹. It is a reoccurring chronic skin disorder with acute flare-ups characterized by eczematous lesions of dry skin and pruritus². AD leads to a decrease in patients' health-related quality of life and reports a higher incidence of depression, anxiety, and suicide as well as pain, fatigue, and insomnia³⁻⁶.

Recent studies on AD show that both intrinsic (non-allergic) and extrinsic (allergic) factors can play a role in the pathogenesis of disease progression⁷. As the endotypes vary in immunoglobulin E (IgE) levels, the type 2 cytokines namely interleukin 4 and 13 (IL-4 and IL-13) remain elevated in both types of the disease, particularly in the lesional areas of the skin⁸. Dupilumab, an interleukin 4 receptor alpha (IL-4R α) antagonist was recently approved by FDA as a treatment for AD after it showed positive effects in patients with moderate to severe AD, with reduced blood type 2 signature, and reversed epidermal abnormalities⁹. This clinical trial provided strong evidence for IL-4/IL-13 as a key target in controlling debilitating AD disease.

IL-4R α , a heterodimeric subunit binds to IL-4 and IL-13 for downstream signalling on hematopoietic and non-hematopoietic cells¹⁰. IL-4 binds to either type I receptor (IL-4R α and γ C chain shared with IL-2, IL-7, IL-9 and IL-21) or type II receptor (IL-4R α and IL-13R α 1)¹¹. Both receptor complexes can activate phosphorylation of the signal transducer and activator of transcription (STAT6) pathway via Janus kinase¹². Type I IL-4 receptor complex can also activate the insulin receptor substrate (IRS)-2 pathway to affect the symptoms of allergic diseases¹³. Type II receptor complex has been shown to have a much higher affinity for IL-4 compared to type I complex¹². IL-13 is the main pathology driver and mediates its effects through the type II receptor complex (IL-4R α and IL-13R α 1)¹⁴. IL-13 is also capable of signalling independently of IL-4R α through IL-13R α 2, which was previously thought to be a decoy receptor¹⁵⁻¹⁸.

IL-13 was found to be important in regulating epidermal thickening, whereas IL-4 was essential for systemic inflammation and anaphylactic shock, but not epidermal thickening¹⁹. Subsequent studies using Ovalbumin and

¹Division of Immunology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa. ²Division of Immunology, International Centre for Genetic Engineering and Biotechnology (ICGEB), Institute of Infectious Diseases and Molecular Medicine (IDM), Health Science Faculty, University of Cape Town, Cape Town, South Africa. ³Wellcome Centre for Infectious Diseases Research in Africa (CIDRI-Africa), Institute of Infectious Diseases and Molecular Medicine (IDM), Faculty of Health Sciences, University of Cape Town, Cape Town 7925, South Africa. ✉email: mascibiorek@gmail.com; MTHNON042@myuct.ac.za; sabelo.hadebe@uct.ac.za

Aspergillus epicutaneous allergen sensitisation and other skin irritants such as oxazolone validated the critical role of type 1 and type II IL-4R α signalling requirement in atopic dermatitis¹⁶. The critical importance of IL-13 in epidermal thickening has been shown to be mediated by both IL-4R α /IL-13R α 1 and IL-13R α 2 with the latter being more essential in keratinocyte signalling^{15,16,20}. IL-4 through signalling via type I IL-4R (IL-4R α / γ C chain) is crucial for IL-4 production, IgE class switching, CCL24, and skin eosinophilia, whereas IL-13 via type II IL-4R (IL-4R α /IL-13R α 1) is essential for epidermal hyperplasia, TNF- α , CXCL1, and CCL11 production²⁰. Interestingly, IL-4R α signalling on CD4 T cells or macrophages was shown not to be essential in epidermal hyperplasia during *Anisakis* induced AD, despite these cell types being abundant in an inflamed skin¹⁹. Whether the requirement of signalling of IL-4R α in different adaptive cell types is critical in disease outcomes is unclear. Furthermore, allergens that can cause acute or chronic AD may influence the need for IL-4R α signalling in different adaptive cells.

We investigated the possible role of IL-4R α in different adaptive cell types using conditional knockout and acute (low-calcemic analog of vitamin D (MC903)) or chronic (House Dust Mite and Ovalbumin) allergens. We found that IL-4R α signalling on CD4/CD8 T cells was redundant in acute and chronic AD-induced allergens, whereas IL-4R α signalling on B cells was mainly important for regulating IgE in chronic HDM-induced AD. Interestingly, IL-4R α signalling on $\gamma\delta$ T cells was essential in epidermal thickening and IgE production in calciprol-induced acute AD.

Our findings show a crucial role for IL-4R α signalling on $\gamma\delta$ T cells in acute AD, but not in chronic AD models, while IL-4R α signalling on B cells is required for IgE production.

Results

Chronic HDM exposure does not induce epidermal thickening but induces IgE. We have previously shown that IL-4R α is essential in OVA and *Anisakis*-induced AD and in IL-13-mediated epidermal thickening^{16,19}. Since many cell types in the skin express IL-4R α , we set out to investigate whether different T cell subsets expressing IL-4R α would be essential in HDM-induced AD (Fig. 1A). We observed no major differences in inguinal lymph node CD4, CD8 and B cells frequencies and numbers between iLCK^{cre}IL-4R α ^{-lox}, LCK^{cre}IL-4R α ^{-lox}, CD4^{cre}IL-4R α ^{-lox} mice and their respective littermate controls (Supplementary Fig. 1a,b,d,e). We observed a significant increase in $\gamma\delta$ T cells in CD4^{cre}IL-4R α ^{-lox} and iLCK^{cre}IL-4R α ^{-lox} compared to their respective littermate controls (Supplementary Fig. 1c,e). We could also validate a decrease in IL-4R α expression in all T cell-specific subsets compared to littermate controls except for $\gamma\delta$ T cells in LCK^{cre}IL-4R α ^{-lox} mice as expected (Supplementary Fig. 1a–c). We compared epidermal thickening in iLCK^{cre}IL-4R α ^{-lox}, LCK^{cre}IL-4R α ^{-lox},

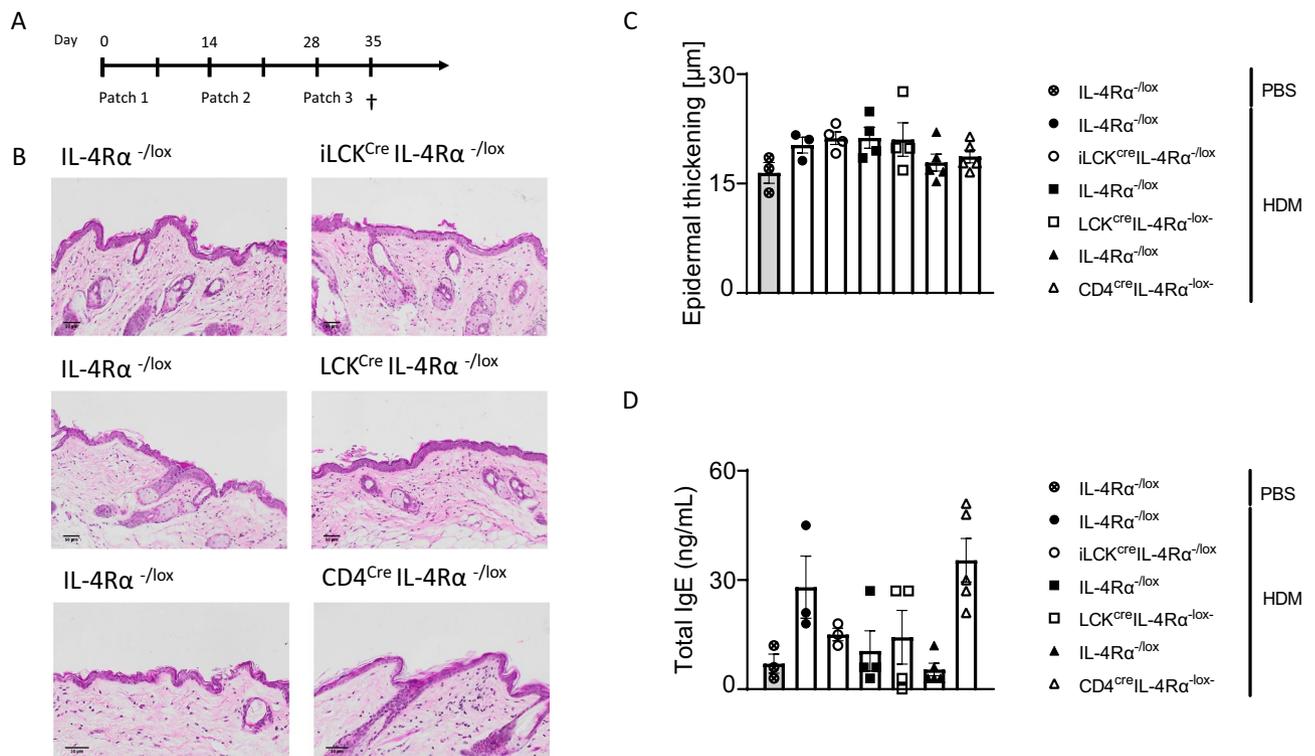


Figure 1. Epicutaneous sensitization with 10ug HDM induces insufficient skin inflammation. (A) Shaved mice (iLCK^{cre}IL-4R α ^{-lox}, CD4^{cre}IL-4R α ^{-lox}, LCK^{cre}IL-4R α ^{-lox} and their respective IL-4R α ^{-lox} littermate controls) were epicutaneously sensitised via patch containing PBS or HDM 100 μ g at day 0, 14 and 28 and blood and skin were collected at day 35. (B) Representative histology images of skin biopsies of ventral side from HDM or PBS treated mice, stained with H&E, scale bar 50 μ m. (C) Quantification of epidermal thickening using QuPath software. (D) Quantification of total IgE serum by ELISA. Shown is one representative experiment with mean \pm SD. Statistical analysis was performed using Student t-test Mann–Whitney where * $p < 0,05$, ** $p < 0,01$ between littermate IL-4R α ^{-lox} PBS group vs HDM challenged mice. $n = 3$ –5 mice per group.

CD4^{cre}IL-4R α ^{-/-lox} mice to their respective littermate controls IL-4R α ^{-/-lox} and IL-4R α ^{-/-lox} PBS control (Fig. 1B). We did not observe any significant epidermal thickening changes between iLCK^{cre}IL-4R α ^{-/-lox}, LCK^{cre}IL-4R α ^{-/-lox}, CD4^{cre}IL-4R α ^{-/-lox} mice compared to their respective littermate control treated with HDM (Fig. 1B,C). There was no significant difference in epidermal thickening between HDM and PBS control exposed mice. Tap stripping and shaving have been shown to induce epidermal thickening independent of allergen exposure²¹. We compared epidermal thickening in naïve untreated mouse strains and found no significant differences between naïve groups (Supplementary Fig. 2a,b). To validate whether three HDM exposure was sufficient to sensitise mice we measured IgE and found increased but not significant levels in almost all HDM-exposed mice compared to PBS-exposed control (Fig. 1D). We also measured dermal mast cells and basophils by flow cytometry and found increased numbers of these cells in iLCK^{cre}IL-4R α ^{-/-lox} mice treated with HDM when compared to their respective littermate control or PBS treated littermate control (Supplementary Fig. 2c,d). We observed similar findings in mice deficient in IL-4R α in T regs (Foxp3^{cre}IL-4R α ^{-/-lox}) compared to littermate control when treated with HDM or OVA, except for increased but not significant IgE (Supplementary Fig. 3b). All together these data suggested that topical allergen exposure induces IgE but is not sufficient for epidermal thickening.

IL-4R α -responsiveness in all T cells mediates MC903-induced AD inflammation. To understand whether IL-4R α would be essential in other forms of AD, such as the one induced by acute skin irritants, we compared iLCK^{cre}IL-4R α ^{-/-lox}, LCK^{cre}IL-4R α ^{-/-lox}, CD4^{cre}IL-4R α ^{-/-lox} mice to their respective littermate controls (Fig. 2A). MC903 an analogue of Vitamin D3 induces acute AD-like lesions and itch in mice when applied topically²². We optimised the concentration of MC903 to apply in our setting and set 45 μ M as sufficient and stable to induce AD-like symptoms (Supplementary Fig. 4a). We observed no significant differences in weight loss and epidermal thickness between the vehicle (EtOH) treated iLCK^{cre}IL-4R α ^{-/-lox}, LCK^{cre}IL-4R α ^{-/-lox}, CD4^{cre}IL-4R α ^{-/-lox} mice and their respective littermate controls (Fig. 2B–D, Supplementary Fig. 4b). We also observed no significant differences in weight loss (Fig. 2C,D) and epidermal thickness (Fig. 2E,F) between LCK^{cre}IL-4R α ^{-/-lox}, CD4^{cre}IL-4R α ^{-/-lox} mice compared to their respective littermate controls when treated with 45 μ M MC903. Interestingly, we observed a significant decrease in weight loss (Fig. 2B) starting at day 5 of treatment in IL-4R α ^{-/-lox} littermate control mice compared to iLCK^{cre}IL-4R α ^{-/-lox} which was accompanied by striking epidermal thickening (Fig. 2E,F). Total IgE levels in serum were similar in iLCK^{cre}IL-4R α ^{-/-lox} mice treated with MC903 when compared to the littermate control treated with the same substance (Fig. 2G). Cytokine, IL-17A which is upregulated during MC903 induced AD was also reduced slightly in iLCK^{cre}IL-4R α ^{-/-lox} compared to

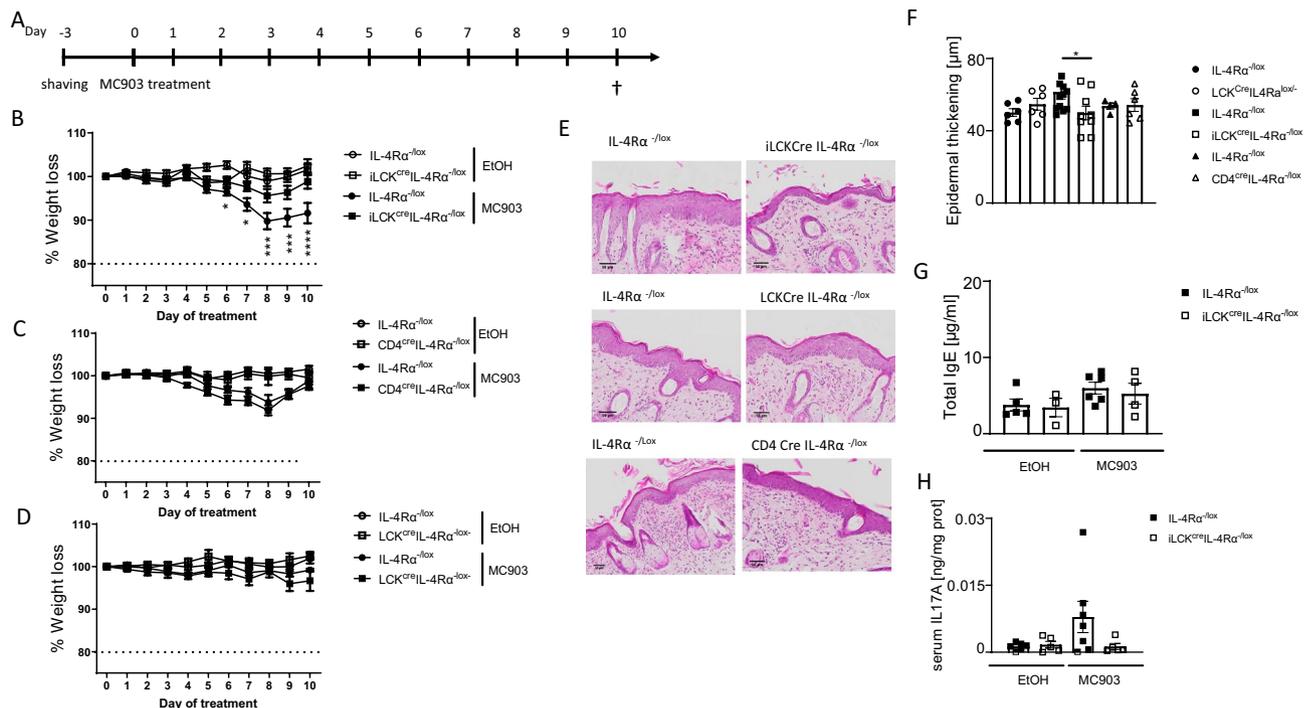


Figure 2. Pan T cell IL-4R α responsiveness is essential in acute MC903-induced epidermal thickening. (A) Mice were shaved at -3 days before treatment with Ethanol or 45 μ M MC903 for 9 consecutive days. (B) Weight loss in IL-4R α ^{-/-lox} and iLCK^{cre}IL-4R α ^{-/-lox} mice. (C) Weight loss in IL-4R α ^{-/-lox} and CD4^{cre}IL-4R α ^{-/-lox} mice. (D) Weight loss in IL-4R α ^{-/-lox} and LCK^{cre}IL-4R α ^{-/-lox} mice. (E) H&E-stained images showing epidermal thickening, scale bar 50 μ m. (F) Epidermal thickening using QuPath software, (G) Total IgE serum levels. (H) IL-17A serum levels. Shown is one representative experiment of 2 with mean \pm SD. Statistical analysis was performed using Two-way ANOVA with Bonferroni post-test (B–D) or Student t-test Mann–Whitney (F–H) where * $p < 0.05$, ** $p < 0.01$, *** $p > 0.001$, **** $p > 0.0001$ between IL-4R α ^{-/-lox} vs iLCK^{cre}IL-4R α ^{-/-lox} MC903 treated mice. $n = 5$ –8 mice per group.

littermate control (Fig. 2H). Type 2 cytokine, IL-4 in serum was significantly changed between $iLCK^{cre}IL-4R\alpha^{-/lox}$ compared to littermate control but not IL-13 (Supplementary Fig. 5a) These data suggested that the lack of IL-4Ra in all T cells protects against acute MC903-induced AD.

IL-4Ra-responsiveness in all T cells mediates cytokine production in acute AD. We then measured CD4 and CD8 T cells in iLN between $iLCK^{cre}IL-4R\alpha^{-/lox}$ and littermate control treated with vehicle or MC903. Although the number of CD4 T cells was increased in mice treated with MC903 compared to vehicle-treated mice, there was a significant difference between $iLCK^{cre}IL-4R\alpha^{-/lox}$ compared to the littermate control (Fig. 3A). This also translated to a significant reduction in IL-4Ra expression in $iLCK^{cre}IL-4R\alpha^{-/lox}$ compared to littermate control (Fig. 3B). We checked for intracellular production of cytokines by these CD4 T cells and found a significant reduction in CD4 T cells producing IL-5 and IL-13, but not IFN- γ or IL-17A in $iLCK^{cre}IL-4R\alpha^{-/lox}$ compared to littermate control (Fig. 3C). We observed similar reductions in iLN CD8 T cells (Fig. 3D), IL-4Ra expression (Fig. 3E) and CD8 T cells production producing IL-5, IL-13 and IL-17A in $iLCK^{cre}IL-4R\alpha^{-/lox}$ compared to littermate control (Fig. 3F). We observed higher numbers and frequencies of $\gamma\delta$ T cells in MC903-treated and vehicle-treated $iLCK^{cre}IL-4R\alpha^{-/lox}$ mice compared to littermate controls, like our observations in HDM-treated mice (Data not shown). Overall, these data pointed to a redundant role of IL-4Ra signalling in CD4 and CD8 T cells, but a requirement of this receptor in $\gamma\delta$ T cells as mice deficient of this receptor in all T cells were protected from acute AD.

IL-4Ra responsive B cells are not essential in chronic HDM AD but regulate IgE production. B cells secreting IgE have been shown to be essential in tumour surveillance in the skin^{21,23}. These B cells were shown to receive their IL-4 signal via $\gamma\delta$ T cells allowing IgE class switching by B cells²³. Given that we had shown indirectly a role for IL-4Ra in $\gamma\delta$ T cells in acute MC903-induced AD, we wondered whether IL-4Ra responsiveness by B cells would be important in both chronic and acute models of AD. We epicutaneously sensitised $mb1^{cre}IL-4R\alpha^{-/lox}$, $IL-4R\alpha^{-/-}$ and littermate controls $IL-4R\alpha^{-/lox}$ mice to HDM with $IL-4R\alpha^{-/lox}$ mice exposed to PBS serving as controls (Fig. 4A). We found reduced epidermal thickening in $mb1^{cre}IL-4R\alpha^{-/lox}$ and $IL-4R\alpha^{-/-}$ mice sensitised to HDM when compared to littermate controls sensitised to HDM although this did not reach significance (Fig. 4B,C). We then measured cellular infiltrate in the iLNs and found significantly higher total B cells and frequencies in littermate control mice sensitised to HDM when compared to $mb1^{cre}IL-4R\alpha^{-/lox}$, $IL-4R\alpha^{-/-}$ mice and PBS control mice (Fig. 4D,E). To validate the deletion of IL-4Ra on B cells, we showed a significant reduction in IL-4Ra in $mb1^{cre}IL-4R\alpha^{-/lox}$ mice compared to littermate controls and no expression of IL-4Ra in $IL-4R\alpha^{-/-}$ mice as expected (Fig. 4F). We then measured total IgE in serum and found significantly

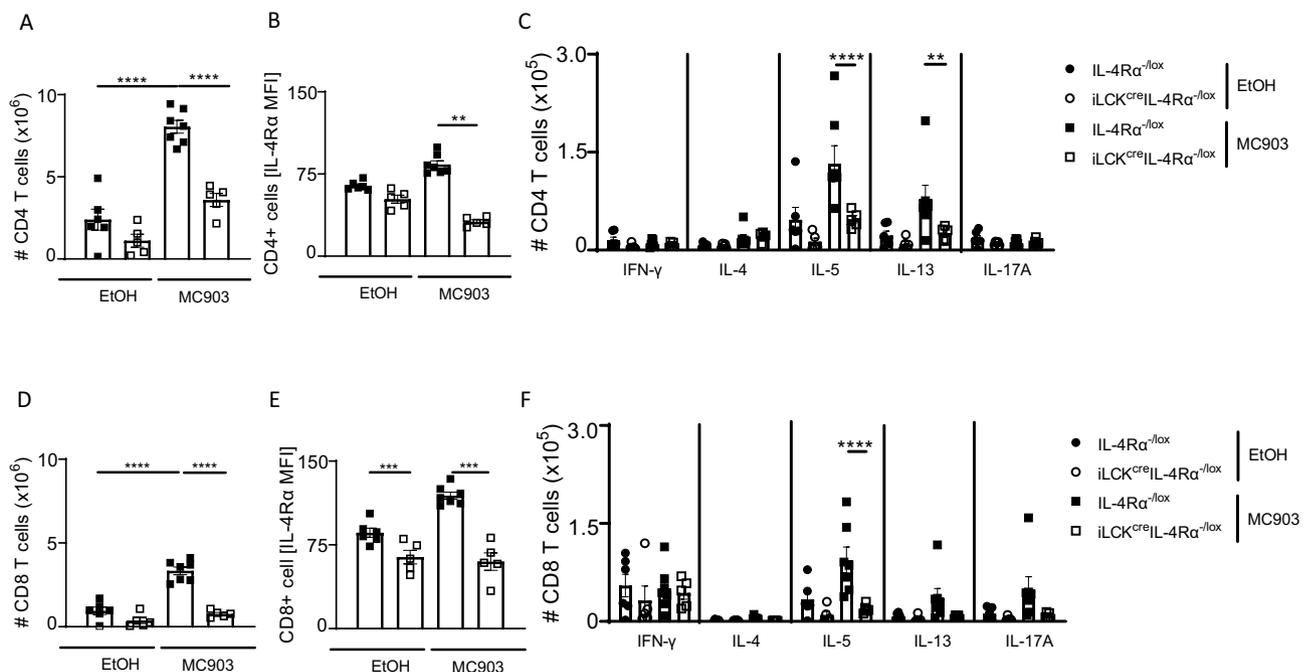


Figure 3. Pan T cell IL-4Ra responsiveness is essential in acute MC903-induced atopic dermatitis. (A) CD4 T cell numbers in inguinal lymph nodes in $IL-4R\alpha^{-/lox}$ littermate and $iLCK^{cre}IL-4R\alpha^{-/lox}$ treated with EtOH and MC903. (B) IL-4Ra expression in CD4 T cells. (C) CD4 T cell cytokine production (IFN- γ , IL-4, IL-5, IL-13 and IL-17A). (D) CD8 T cells numbers in iLNs. (E) IL-4Ra expression in CD8 T cells. (F) CD8 T cell cytokine production (IFN-g, IL-4, IL-5, IL-13 and IL-17A). Shown are 2 pooled experiments with mean \pm SEM. Statistical analysis was performed using the Mann–Whitney Student t-test where *p < 0,05, **p < 0,01, ***p < 0,001, ****p < 0,0001 between knockout and its respective littermate $IL-4R\alpha^{-/lox}$ control in either EtOH group or MC903 treated mice. n = 5–8 mice per group.

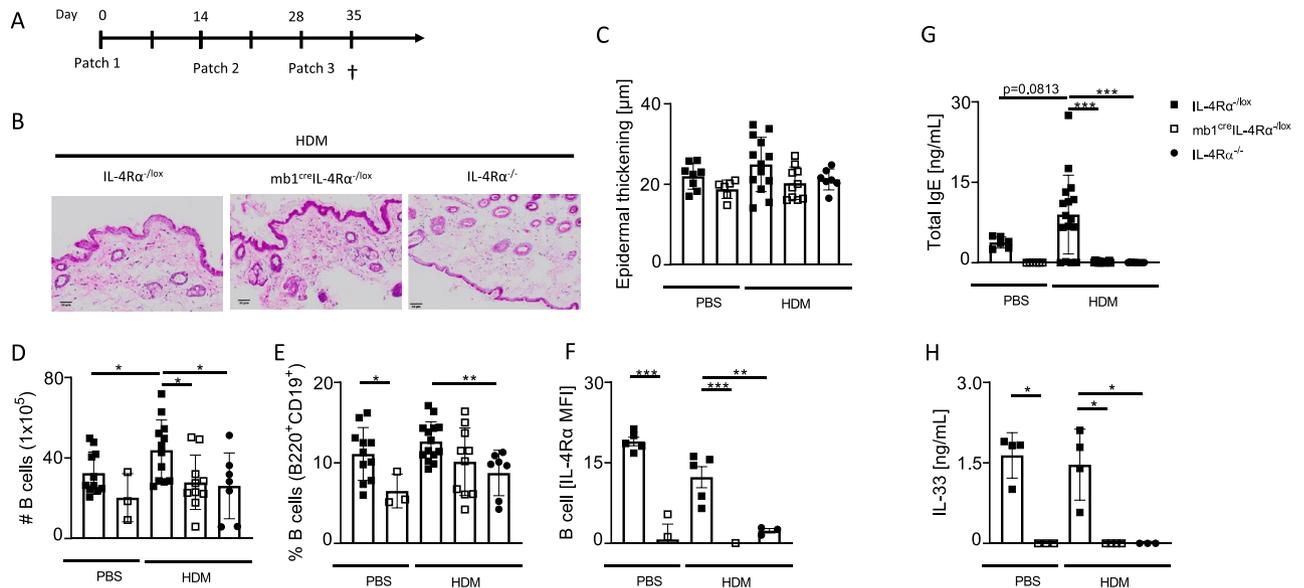


Figure 4. Epicutaneous sensitization with HDM induces insufficient skin inflammation, but IgE-dependent IL-4Ra expression in B cells. Shaved mice ($mb1^{cre}IL-4Ra^{-/lox}$, $IL-4Ra^{-/lox}$ littermate controls and $IL-4Ra^{-/-}$) were epicutaneously sensitised as in Fig. 1. (A) H&E-stained images showing epidermal thickening, scale bar 50 μm . (B) Epidermal thickening using QuPath software. (C) Total B cell numbers in the iLNs. (D) Frequency of B cells (Live + singlets, lymphocytes, $CD19^{+}BB20^{+}MHCII^{+}$) in the iLNs. (E) IL-4Ra expression in B cells. (F) Total IgE serum levels. (G) IL-33 serum levels. Shown are 2 pooled experiments with mean \pm SEM. Statistical analysis was performed using Student t-test Mann-Whitney where * $p < 0,05$, ** $p < 0,01$, *** $p < 0.001$ between littermate $IL-4Ra^{-/lox}$ vs $mb1^{cre}IL-4Ra^{-/lox}$ or $IL-4Ra^{-/-}$ mice. $n = 3-12$ mice per group.

increased IgE in littermate controls sensitised to HDM when compared to $mb1^{cre}IL-4Ra^{-/lox}$ and $IL-4Ra^{-/-}$ sensitised to HDM (Fig. 4G). We observed increased but not significantly changed IL-33 secretion in both PBS and HDM sensitised littermate control mice, which was absent in both $mb1^{cre}IL-4Ra^{-/lox}$ and $IL-4Ra^{-/-}$ mice (Fig. 4H). This suggested an IL-4Ra-dependent and allergen-independent IL-33 secretion. Interestingly, in the skin, we did not observe any changes in mRNA expression of *il-33* or any other type 2 or type 17 transcripts (Supplementary Fig. 6a).

IL-4Ra responsive B cells are not essential in acute AD-induced skin inflammation but regulate IgE production. To further understand the dynamics of IL-4Ra responsive B cells in acute AD, we treated $mb1^{cre}IL-4Ra^{-/lox}$ and $IL-4Ra^{-/lox}$ littermate controls with a skin irritant MC903 (Fig. 5A). We monitored weight loss over 10 days and found MC903 mice to lose weight at similar levels starting at day 5. Mice treated with vehicle (EtOH) did not lose weight during this time (Fig. 5B). We measured total serum IgE and found significantly increased IgE in littermate control mice exposed to MC903 compared to $mb1^{cre}IL-4Ra^{-/lox}$ mice or mice lacking IL-4Ra in all cells or mice deficient in B cells (Fig. 5C). We then measured epidermal thickening on day 10 and found increased but not significantly different thickness between $mb1^{cre}IL-4Ra^{-/lox}$ and littermate control treated with MC903 (Fig. 5D,E). Similar results were observed in vehicle-treated mice except for low levels of epidermal thickening was observed (Fig. 5D,E). There was a decreased but not significant type 2 and type 17 mRNA expression in the skin between $mb1^{cre}IL-4Ra^{-/lox}$ and littermate control treated with MC903 (Supplementary Fig. 6b). Overall, these results suggested that IgE production was dependent on IL-4Ra expressing B cells.

IL-4Ra responsive B cells are required for germinal centre (GC) formation and class switching in acute AD-induced skin inflammation. We further investigated the importance of IL-4Ra signalling on B cells in class switching during skin irritant-induced acute AD. We measured the frequencies of B cell populations in the iLN. We observed no significant changes in follicular (FO) or marginal zone (MZ) B cells between $mb1^{cre}IL-4Ra^{-/lox}$ mice and littermate control mice in both vehicles and MC903 treated mice (Fig. 6A). We measured frequencies and number of GCs ($FAS^{+}GL7^{+}$) in iLN and found significantly reduced GCs frequencies and numbers in $mb1^{cre}IL-4Ra^{-/lox}$ mice compared to littermate controls exposed to MC903 (Fig. 6B,C). The frequency and number of IgG1 and IgE expressing B cells were also significantly reduced in $mb1^{cre}IL-4Ra^{-/lox}$ mice compared to littermate controls (Fig. 6D,E), which corroborated our earlier findings of reduced total IgE in these mice.

Discussion

The importance of TH2-targeted therapeutics has been vastly studied in the context of atopic dermatitis^{20,24-29}. The recently recommended treatment is systemic administration of monoclonal antibody targeting IL-4Ra—dupilumab, in conjunction with topical glucocorticosteroids³⁰. Such a regime is highly effective in alleviating AD

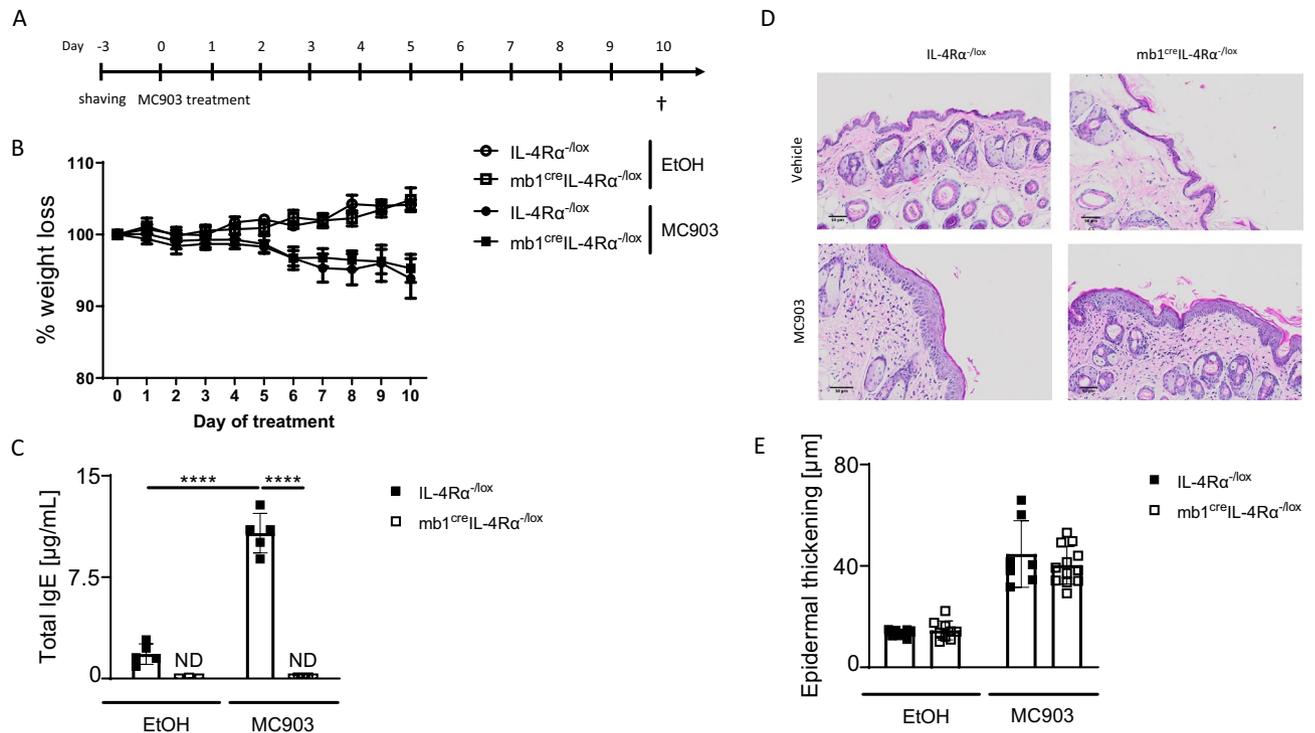


Figure 5. IL-4R α responsiveness in B cells is not essential in acute MC903-induced epidermal thickening but is required for IgE production. **(A)** Mice were shaved 3 days prior to topical treatment with either Ethanol or 45 μ M MC903 for 9 consecutive days. **(B)** Weight loss in IL-4R α ^{-/-lox} and mb1^{cre}IL-4R α ^{-/-lox} treated with EtOH and MC903. **(C)** Total IgE serum levels. **(D)** H&E-stained images showing epidermal thickening, scale bar 50 μ m. **(E)** Quantification of epidermal thickening using QuPath software. Shown are pooled 2 independent experiments with mean \pm SEM. Statistical analysis was performed using the Student t-test Mann–Whitney where **** $p > 0.0001$ between littermate IL-4R α ^{-/-lox} group vs iLCK^{cre}IL-4R α ^{-/-lox} group treated with EtOH or MC903. $n = 5$ –10 mice per group.

clinical symptoms, and a reduction in IL-22 or eotaxin, as well as total IgE. However, clinical studies show an increase in IL-4 and IL-13 upon the treatment as a resultant side effect^{31,32}. The most often reported side effect is conjunctivitis and blood eosinophilia. These studies suggest that despite high efficacy, the treatment could still be improved. Here, we used acute and chronic models of AD to understand the cell-specific requirements of IL-4R α in adaptive cells, mainly CD4, CD8 and B cells. We found that IL-4R α expressed by innate $\gamma\delta$ T cells was essential in MC903-induced acute AD, whereas IL-4R α expressed by all T cells were redundant in chronic HDM or OVA-induced AD. We also found that IL-4R α expressed by B cells was essential in chronic HDM-induced AD and regulated IgE.

Previously our group showed that the reduction of IL-4R α expression on LCK^{cre} IL-4R α ^{-/-lox} mouse strain contributes to the reduction of IL-5 and IL-4 but not IL-13 in the *Anisakis*-induced model of AD¹⁹. We further showed that in absence of IL-13 but not IL-4, we can reduce the effect of *Anisakis* application like epidermal hyperplasia and cellular infiltration. Bitton et al. described a mechanism of protection from oxazolone-induced AD murine model by inhibition of IL-13 detection via IL-13R α 1²⁰. Our group has also shown an IL-4R α -independent function of IL-13 in OVA-induced AD¹⁶. Here, we showed that IL-4R α responsiveness in T cells was redundant in chronic HDM models as mice lacking IL-4R α in CD4 alone, CD4 and CD8 or CD4, CD8 and $\gamma\delta$ T cells. We also did not observe any changes in IgE levels. Previous studies have shown that the lack of GATA3 in Foxp3 T regs led to the poor accumulation of T regs in lymphoid tissues and autoimmunity³³. T regs have been shown to be essential in skin homeostasis where they are crucial in promoting hair follicle stem cell regeneration³⁴. In our study, deficiency of IL-4R α in Foxp3 T reg cells did not lead to adverse skin inflammation in chronic AD models but led to an increase in total IgE. These findings suggest that although local skin inflammation is not impacted by a deficiency in IL-4R α in T reg cells, it may have an impact on systemic IgE dysregulation. It is possible that HDM and OVA allergens were not sufficient to induce a keratinocyte mechanical injury required for skin sensitisation as previously observed^{35–38}.

In acute AD models, we found that mice lacking IL-4R α in pan T cells were protected from MC903, shown by a transient loss in weight, reduced epidermal thickening and reduced type 2 cytokine production by CD4 and CD8 T cells. This contrasted with mice lacking IL-4R α only in CD4 and CD8 T cells, which were susceptible to similar levels to littermate controls. This suggested that the IL-4R α protective effect observed in pan T cell IL-4R α -deficient mice might be $\gamma\delta$ T cell-derived, and these cells could potentially influence B cell function.

B cells can be both IL-4-responsive due to the presence of the IL-4 receptor on the cell surface, as well as secrete the cytokine, so the deletion of the receptor can have both autocrine and paracrine consequences³⁹. The

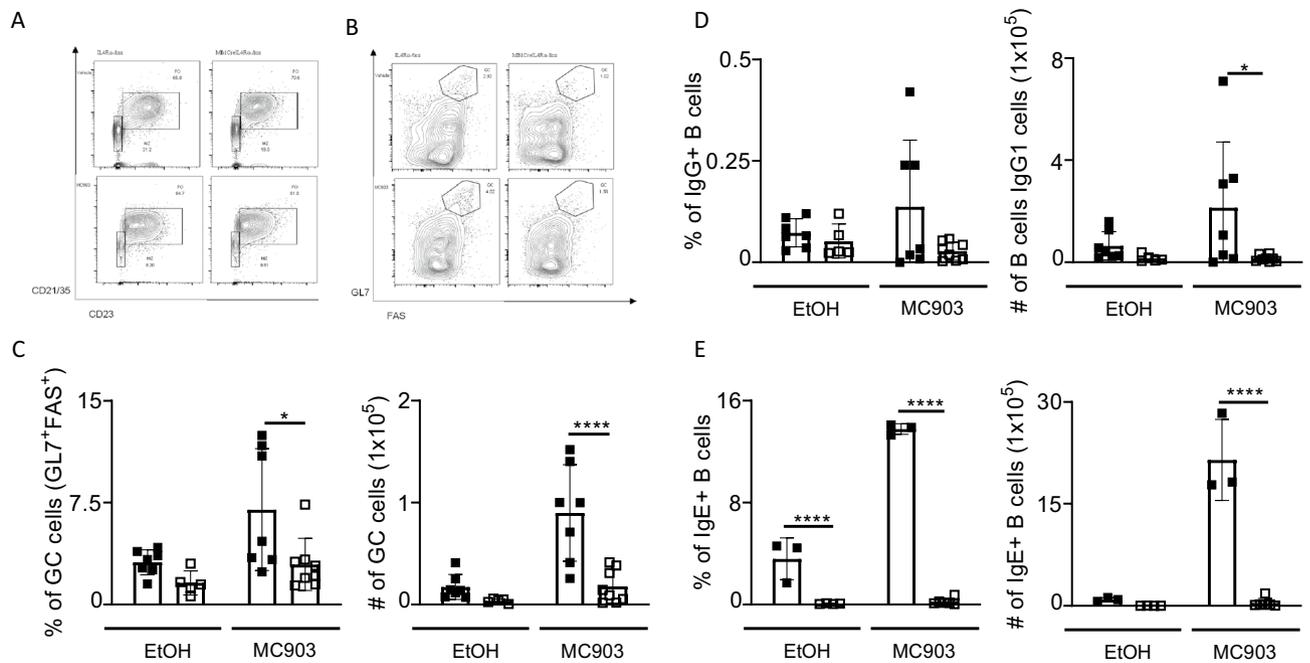


Figure 6. IL-4Ra responsiveness in B cells essential in GC and class switching during acute MC903-induced atopic dermatitis. **(A)** Representative FACS plots of follicular (FO) and marginal zone (MZ) B cells in inguinal lymph nodes between IL-4Ra^{-lox} and mb1^{cre}IL-4Ra^{-lox} treated with EtOH and MC903. **(B)** Representative FACS plots of Germinal Centre (GCs) B cells in inguinal lymph nodes. **(C)** Frequency and the total number of GC B cells. **(D)** Frequency and the total number of IgG1 + B cells. **(E)** Frequency and the total number of IgE + B cells. Shown are 2 pooled experiments with mean \pm SEM. Statistical analysis was performed using Student t-test Mann–Whitney where * $p < 0,05$, ** $p < 0,01$ between littermate IL-4Ra^{-lox} EtOH group vs MC903 treated mice. $n = 5\text{--}8$ mice per group.

effect of IL-4/IL-13 binding on IL-4Ra is required for B cell maturation, formation of GCs, B-T cell interaction and adequate antibody class switched IgE production^{40,41}. Interestingly, in the skin $\gamma\delta$ T cells have been shown to be essential in producing IL-4 production and influencing B cell class switching to IgE, which is essential in skin tumour surveillance^{23,42}. We found that mice deficient in IL-4Ra on B cells had little impact on epidermal skin thickening but were required in IgE production, Tfh cells and GC formation. This is consistent with our previous studies in allergic asthma where the absence of IL-4Ra on B cells led to reduced GC numbers, Tfh and IgE production⁴³. Despite IgE being essential in skin prick testing and in mast cell and basophil activation, its role in epidermal hypertrophy is limited⁴⁴. Mice deficient of IgE can develop epidermal hyperplasia when sensitised to OVA⁴⁴. Our data is consistent with these findings where we show that IgE production dependent on IL-4Ra responsive B cells is redundant in epidermal hyperplasia. Cell-specific factors that influence AD are not known and IL-4Ra, a key factor in AD, is expressed by many cell types. IL-4Ra expressed by $\gamma\delta$ T cells is essential in the pathogenesis of acute AD, while IL-4Ra expressed by other T cells is not important. IL-4Ra expressed by B cells is important in IgE production in both acute and chronic AD.

Overall, we showed mild allergic sensitization due to the lack of induction of epithelial damage by mechanical tape-stripping in OVA and HDM models. Other studies on AD have shown various combinations of HDM strain *Dermatophagoides farinae* to be efficient in the induction of AD-like lesions when boosted with skin irritants such as MC903, or capsaicin or with bacterial toxins like *Staphylococcal enterotoxin B* from *S. aureus*^{45–47}. We were confident that our epicutaneous sensitisation model with HDM primed skin resident cells, as we were able to observe atopic march characterised by increased allergic airway inflammation which was dependent on IL-4Ra expression by B cells (Data not shown).

In human AD, IL-4/IL-13 signalling is central in the pathogenesis of the disease, with increased colonisation of the skin with *S. aureus*⁴⁸, where both cytokines favour *S. aureus* adhesion and keratinocyte killing⁴⁸. At baseline, in skin lesion areas, there is less microbial diversity with a dominant *S. aureus*. After 16-week treatment with dupilumab, *S. aureus* is decreased in skin lesion areas and the diversity of other microbial skin commensals is increased^{9,48}. In human clinical trials, it is unclear whether there is an increased abundance of commensal microbiota that could be limiting *S. aureus*. The long-term implications of reducing pro-tissue repair mechanisms by cytokines IL-4 and IL-13 are currently unknown. Early *S. epidermis* skin colonisation has a long-term effect on T-cell priming, non-classical MHC I activation and the activation of mucosal-associated invariant T-cells⁴⁹.

Conclusions

In this article, we show that therapeutic targeting of IL-4R α expressing cells, particularly those of adaptive immunity may need clarification based on allergen and chronicity of the disease where acute cases characterised by itch may benefit from targeting innate T cells and chronic cases may benefit from targeting B cells and IgE secretion. Personalised therapeutics aimed at TH2 diseases require a clear understanding of the role of each cell type.

Material and methods

Ethical declarations for animal experiments. Animal procedures were performed according to strict recommendations by the South African Veterinary Council and were approved by the University of Cape Town Animal Ethics Committee (Reference number 017/004, 021/006). All authors complied with the ARRIVE guidelines and institutional guidelines on the use of animals in research.

Mice. All mice used in this study were generated at the University of Cape Town Animal Research Facility. Original cre strains of mice were purchased from Jackson Laboratories and IL-4R $\alpha^{lox/lox}$ was generated in house⁵⁰. To generate mice deficient of IL-4R α only on B cells (mb1^{cre}IL-4R $\alpha^{-/lox}$), CD4 T cells only (CD4^{cre}IL-4R $\alpha^{-/lox}$), CD4/CD8 T cells only (LCK^{cre}IL-4R $\alpha^{-/lox}$), T regs (Foxp3^{cre}IL-4R $\alpha^{-/lox}$) or pan-T cells (iLCK^{cre}IL-4R $\alpha^{-/lox}$) we intercrossed homozygous mb1^{cre} mice⁵¹ or CD4^{cre} mice⁵² or LCK^{cre} mice⁵³ or iLCK^{cre} mice or Foxp3^{cre} mice⁵⁴ with homozygous IL-4R $\alpha^{lox/lox}$ mice⁵⁰ to generate hemizygous mb1^{cre}IL-4R $\alpha^{-/lox}$ ⁵⁵, CD4^{cre}IL-4R $\alpha^{-/lox}$ ⁵³, LCK^{cre}IL-4R $\alpha^{-/lox}$ ⁵³, iLCK^{cre}IL-4R $\alpha^{-/lox}$ or Foxp3^{cre}IL-4R $\alpha^{-/lox}$ ^{54,56}. Hemizygous littermates (IL-4R $\alpha^{-/lox}$) expressing a single functional IL-4R α allele was used as a wild-type control in all experiments. In some instances, mice lacking B cell (μ MT^{-/-})⁵⁷ in Balb/c background were used as controls. Mice were housed in independently ventilated cages under specific pathogen-free conditions at the University of Cape Town Animal Facility. All mice were used at eight to 10 weeks of age.

Chronic atopic dermatitis models. Mice were anesthetized with ketamine (Anaket-V; Centaur Labs, Johannesburg, South Africa) and xylazine (Rompun; Bayer, Isando, South Africa) mix and shaved on ventral side 1 × 1 cm with single-blade disposable razor (BIC, South Africa). Mice were treated with 100 μ g of House Dust Mite (Stellergens Greer Laboratories, Lenoir, USA) or 200 μ g ovalbumin (OVA, grade V; Sigma-Aldrich, South Africa) diluted in 200 μ L of Phosphate-Buffered Saline (PBS, Thermofisher, South Africa) or with PBS as control on a sterile band aid (Elastoplast, Baiersdorf, South Africa) patch which was removed after 24 h. Treatments were done on day 0, 14 and 28 and mice were killed on day 35.

Acute atopic dermatitis model. Mice were shaved using a single-blade disposable razor (BIC, South Africa) on the ventral side 1 × 1 cm, 3 days prior to the start of the experiment. Mice were treated with 100 μ L of 45 μ M calcineurin inhibitor, MC903 (Tocris Bioscience, United Kingdom) in 100% ethanol (EtOH, Thermofisher, South Africa) or with 100% EtOH as a control vehicle for 10 consecutive days. Mice were weighed daily to monitor weight loss and general welfare. Mice were killed on day 8 or 11 and organs were collected for further analysis.

Sample collection and processing of skin tissue. Mice were euthanized using by halothane (Piramal Healthcare Limited, India) inhalation, blood collected via cardiac puncture and ventral side of the skin shaved. Patch size of the skin (19 mm × 10 mm) was cut out and placed in 4% formalin for histology analysis. Inguinal lymph nodes were collected into non-supplemented RPMI-1640 Medium (Thermofisher, South Africa) and processed as single-cell suspensions before counting in trypan blue.

Flow cytometry. Inguinal lymph node single cells were seeded 2 × 10⁶ cells per well in 96-well U-bottom shaped plate (Thermo Scientific, USA) and were stained with surface cell markers in FACS buffer (1% BSA, 2% inactivated rat serum, 1% Fc block). Antibodies used in these experiments included, phycoerythrobilin (PE)- conjugated anti-Siglec-F (clone, E50-2440), anti-CD124 (IL-4R α , clone, M-1), anti-CD44 (clone, KM114), FITC- conjugated anti-Gr-1 (clone, RB6-8C5), CD45 (clone, 30-F11), PerCP Cy5.5- conjugated anti-Ly6C (clone, AL-21), -CD45.1 (clone, A20), Allophycocyanin (APC)- conjugated anti-CD11c (clone, HL3), anti-FoxP3 (clone, MF23), V450 conjugated anti-CD11b (clone, M1/70), anti-CD62L (clone, MEL-14), anti-IgG1 (clone, A110-1), AlexaFlour 700- conjugated anti-CD3 ϵ (clone, 145-2C11), V500- anti-CD4 (clone, RM4-5) and anti- B220 (clone, RA3-6B2), APC-Cy7-conjugated anti-CD19 (clone, 1D3) and anti-CD8 (clone, 53-6.7), BV786 conjugated anti-IgE (clone, R35-72) and anti-IL-33R (ST2) (clone, U29-93), biotin-CD25 (clone, 7D4) were purchased from BD Pharmingen (San Diego, CA). PE-Cyanine7 anti-F4/80 (clone, BM8), AlexaFlour 700- conjugated anti-MHC II (clone, M5/114) and Live/dead Fixable Yellow stain (Qdot605 dead cell exclusion dye) were purchased from eBiosciences. Biotin-labelled antibodies were detected by Texas Red conjugated PE (BD Biosciences).

Intracellular staining. For intracellular cytokine staining, cells were restimulated with phorbol myristate acetate (Sigma-Aldrich) (50 ng/mL), ionomycin (Sigma-Aldrich) (250 ng/mL), and monensin (Sigma-Aldrich) (200 mM in IMDM/10% FCS) for 5 h at 37 °C then fixed in 2% PFA, permeabilised with Foxp3 transcriptional factor staining buffer kit (eBioscience) before intracellular staining with appropriate cytokine antibodies, FITC anti IL-4 (clone, 11B11), PE anti-IL-5 (clone, TRFK5), PE-Cy7 anti-IL-13 (clone, eBio13A), PerCP Cy5.5 anti-IL-17A (clone, TC11-18H10), and acquisition through LSR Fortessa machine (BD Immunocytometry system, San Jose, CA, USA) and data was analysed using Flowjo software (Treestar, Ashland, OR, USA).

Histology. Formalin-fixed (4%) skin biopsies from mice were stained with haematoxylin and eosin (H&E). Image acquisition was done using VS120 Virtual Slide Microscope (Olympus Global, USA) 20× magnification and quantification of epidermal thickening was performed using QuPath.Ink open software.

Antibody ELISAs. For antibody ELISAs, the plate was coated with using 5 µg/ml HDM for specific IgGs. Total IgE in serum was measured using anti-mouse IgE (BD Biosciences, 553413) to coat, mouse IgE (κ, anti-TNP, BD Biosciences, 557079) as standard and biotin anti-mouse IgE (BD Biosciences, 553419) as secondary antibody.

In vitro cytokine ELISAs. For in vitro cytokine production analysis, single cell suspensions were prepared from inguinal lymph nodes of treated and control mice. Cells (2×10^5 cells, in 200 µL) were incubated for 5 days in IMDM/10% FCS (Delta Bioproducts, Kempton Park, South Africa) in 96-well plates. Cells were either stimulated with HDM (30 µg/mL), OVA (30 µg/mL) or anti-CD3 (10 µg/mL) and supernatants were collected after a 5-day incubation period. Concentrations of IL-4, IL-5 (BD Biosciences) and IL-13 (R&D Systems, Minneapolis, Minn), were measured using ELISA assays according to the manufacturer's protocol.

Statistical analysis. P-values were calculated in GraphPad Prism 6 (GraphPad Software, Inc) by using a nonparametric Mann–Whitney Student's t-test or Two-way ANOVA with Bonferroni's post-test for multiple comparisons, and results are presented as the standard error of the mean (SEM) or mean of standard deviation (SD). Differences were considered significant if P was < 0.05.

Data availability

All data generated or analysed during this study are included in this published article.

Received: 7 September 2022; Accepted: 19 December 2022

Published online: 04 January 2023

References

- Nutten, S. Atopic dermatitis: Global epidemiology and risk factors. *Ann. Nutr. Metab.* **66**(suppl 1), 8–16 (2015).
- Cork, M. J., Danby, S. G. & Ogg, G. S. Atopic dermatitis epidemiology and unmet need in the United Kingdom. *J. Dermatol. Treat.* <https://doi.org/10.1080/09546634.2019.1655137> (2019).
- Silverberg, J. I., Garg, N. K., Paller, A. S., Fishbein, A. B. & Zee, P. C. Sleep disturbances in adults with eczema are associated with impaired overall health: A US population-based study. *J. Invest. Dermatol.* **135**, 56–66 (2015).
- Drucker, A. M., Wang, A. R. & Qureshi, A. A. Research gaps in quality of life and economic burden of atopic dermatitis: The National Eczema Association burden of disease audit. *JAMA Dermatol.* **152**, 873–874 (2016).
- Yu, S. H. & Silverberg, J. I. Association between atopic dermatitis and depression in US adults. *J. Invest. Dermatol.* **135**, 3183–3186. <https://doi.org/10.1038/jid.2015.337> (2015).
- Dieris-Hirche, J. *et al.* Suicidal Ideation in adult patients with atopic dermatitis: A German cross-sectional study. *Acta Derm. Venereol.* **97**, 1189–1195 (2017).
- Bieber, T. *et al.* Clinical phenotypes and endophenotypes of atopic dermatitis: Where are we, and where should we go?. *J. Allergy Clin. Immunol.* **139**, S58–S64 (2017).
- Nomura, T., Wu, J., Kabashima, K. & Guttman-Yassky, E. Endophenotypic variations of atopic dermatitis by age, race, and ethnicity. *J. Allergy Clin. Immunol. Pract.* **8**, 1840–1852 (2020).
- Guttman-Yassky, E. *et al.* Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* **143**, 155–172 (2019).
- Maes, T., Joos, G. F. & Brusselle, G. G. Targeting interleukin-4 in asthma: Lost in translation?. *Am. J. Respir. Cell Mol. Biol.* **47**, 261–270. <https://doi.org/10.1165/rcmb.2012-0080TR> (2012).
- Nelms, K., Keegan, A. D., Zamorano, J., Ryan, J. J. & Paul, W. E. The IL-4 receptor: Signaling mechanisms and biologic functions. *Annu. Rev. Immunol.* **17**, 701–738 (1999).
- LaPorte, S. L. *et al.* Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* **132**, 259–272 (2008).
- Gour, N. & Wills-Karp, M. IL-4 and IL-13 signaling in allergic airway disease. *Cytokine* **75**, 68–78 (2015).
- Brombacher, F. The role of interleukin-13 in infectious diseases and allergy. *BioEssays* **22**, 646–656 (2000).
- Sivaprasad, U. *et al.* IL-13Ra2 has a protective role in a mouse model of cutaneous inflammation. *J. Immunol.* **185**, 6802–6808 (2010).
- Hoving, J. C., Nieuwenhuizen, N. E. E., Schäfer, G., Katz, A. A. & Brombacher, F. IL-13 signals independent of IL-4 receptor-alpha chain to drive ovalbumin-induced dermatitis. *J. Invest. Dermatol.* **136**, 1286–1290 (2016).
- Grünig, G. *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* **282**, 2261–2263 (1998).
- Fichtner-Feigl, S., Strober, W., Kawakami, K., Puri, R. K. & Kitani, A. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. *Nat. Med.* **12**, 99–106 (2006).
- Nieuwenhuizen, N., Herbert, D. R., Brombacher, F. & Lopata, A. L. Differential requirements for interleukin (IL)-4 and IL-13 in protein contact dermatitis induced by Anisakis. *Allergy Eur. J. Allergy Clin. Immunol.* **64**, 1309–1318 (2009).
- Bitton, A. *et al.* A key role for IL-13 signaling via the type 2 IL-4 receptor in experimental atopic dermatitis. *Sci. Immunol.* **5**, 2938 (2020).
- Dallessandri, T., Crawford, G., Hayes, M., Castro Seoane, R. & Strid, J. IL-13 from intraepithelial lymphocytes regulates tissue homeostasis and protects against carcinogenesis in the skin. *Nat. Commun.* **7**, 12080 (2016).
- Kim, B. S. *et al.* Basophils promote innate lymphoid cell responses in inflamed skin. *J. Immunol.* **193**, 3717–3725 (2014).
- Crawford, G. *et al.* Epithelial damage and tissue γδ T cells promote a unique tumor-protective IgE response. *Nat. Immunol.* **19**, 859–870 (2018).
- Miyake, Y., Tanaka, K. & Arakawa, M. Case-control study of eczema in relation to IL4Ralpha genetic polymorphisms in Japanese women: The Kyushu Okinawa Maternal and Child Health Study. *Scand. J. Immunol.* **77**, 413–418 (2013).
- Richgels, P. K., Yamani, A., Choungnet, C. A. & Lewkowich, I. P. Maternal house dust mite exposure during pregnancy enhances severity of house dust mite-induced asthma in murine offspring. *J. Allergy Clin. Immunol.* **140**, 1404–1415.e9 (2017).
- Stott, B. *et al.* Human IL-31 is induced by IL-4 and promotes TH2-driven inflammation. *J. Allergy Clin. Immunol.* **132**, 446–54e5 (2013).

27. Caniatti, M. C., Marchioro, A. A., Guilherme, A. L. & Tsuneto, L. T. Association of cytokines in individuals sensitive and insensitive to dust mites in a Brazilian population. *PLoS ONE* **9**, e107921 (2014).
28. Kumar, S., Jeong, Y., Ashraf, M. U. & Bae, Y. S. Dendritic cell-mediated Th2 immunity and immune disorders. *Int. J. Mol. Sci.* **20**, 2159 (2019).
29. Deleanu, D. & Nedelea, I. Biological therapies for atopic dermatitis: An update. *Exp. Ther. Med.* **17**, 1061–1067 (2019).
30. Wollenberg, A. *et al.* Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: Part 1. *J. Eur. Acad. Dermatol. Venereol.* **32**, 657–682 (2018).
31. Ariëns, L. F. M. *et al.* Dupilumab is very effective in a large cohort of difficult-to-treat adult atopic dermatitis patients: First clinical and biomarker results from the BioDay registry. *Allergy* **75**, 116–126 (2020).
32. Nettis, E. *et al.* Use of dupilumab for 543 adult patients with moderate-to-severe atopic dermatitis: A multicenter, retrospective study. *J. Investig. Allergol. Clin. Immunol.* <https://doi.org/10.18176/jiaci.0641> (2020).
33. Wohlfert, E. A. *et al.* GATA3 controls Foxp3+ regulatory T cell fate during inflammation in mice. *J. Clin. Invest.* **121**, 4503–4515 (2011).
34. Ali, N. *et al.* Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell* **169**, 1119–1129.e11 (2017).
35. Wang, G. *et al.* Repeated epicutaneous exposures to ovalbumin progressively induce atopic dermatitis-like skin lesions in mice. *Clin. Exp. Allergy* **37**, 151–161 (2007).
36. Jin, H. *et al.* IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. *J. Clin. Invest.* **119**, 47–60 (2009).
37. Kim, J., Kim, B. E. & Leung, D. Y. M. Pathophysiology of atopic dermatitis: Clinical implications. *Allergy Asthma Proc.* **40**, 84–92 (2019).
38. Matsuoka, H. *et al.* A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite *Dermatophagoides farinae*. *Allergy* **58**, 139–145 (2003).
39. Hurdal, R. *et al.* IL-4-producing B cells regulate T helper cell dichotomy in type 1- and type 2-controlled diseases. *Proc. Natl. Acad. Sci. USA* **114**, E8430–E8439 (2017).
40. Carballido, J. M. *et al.* IL-4 induces human B cell maturation and IgE synthesis in SCID-hu mice. Inhibition of ongoing IgE production by in vivo treatment with an IL-4/IL-13 receptor antagonist. *J. Immunol.* **155**, 4162–4170 (1995).
41. Guo, B. & Rothstein, T. L. IL-4 upregulates Igα and Igβ protein, resulting in augmented IgM maturation and B cell receptor-triggered B cell activation. *J. Immunol.* **191**, 670–677 (2013).
42. Strid, J., Sobolev, O., Zafirova, B., Polic, B. & Hayday, A. The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy. *Science (1979)* **334**, 1293–1297 (2011).
43. Hadebe, S. *et al.* Deletion of IL-4Ra signaling on B cells limits hyperresponsiveness depending on antigen load. *J. Allergy Clin. Immunol.* **148**, 99–109.e5 (2021).
44. Spergel, J. M., Mizoguchi, E., Oettgen, H., Bhan, A. K. & Geha, R. S. Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. *J. Clin. Invest.* **103**, 1103–1111 (1999).
45. Yun, J.-W. *et al.* TRPV1 antagonist can suppress the atopic dermatitis-like symptoms by accelerating skin barrier recovery. *J. Dermatol. Sci.* **62**, 8–15 (2011).
46. Serhan, N. *et al.* House dust mites activate nociceptor-mast cell clusters to drive type 2 skin inflammation. *Nat. Immunol.* **20**, 1435–1443 (2019).
47. Mishra, S. K. *et al.* Periostin activation of integrin receptors on sensory neurons induces allergic itch. *Cell Rep.* **31**, 107472 (2020).
48. Callewaert, C. *et al.* IL-4Ra blockade by dupilumab decreases *Staphylococcus aureus* colonization and increases microbial diversity in atopic dermatitis. *J. Investig. Dermatol.* **140**, 191–202.e7 (2020).
49. Constantiniides, M. G. *et al.* MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science (1979)* **366**, eaax6624 (2019).
50. Herbert, D. R. *et al.* Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* **20**, 623–635 (2004).
51. Hobeika, E. *et al.* Testing gene function early in the B cell lineage in mb1-cre mice. *Proc. Natl. Acad. Sci.* **103**, 13789–13794 (2006).
52. Dewals, B. *et al.* IL-4Rα responsiveness of non-CD4 T cells contributes to resistance in schistosoma mansoni infection in pan-T cell-specific IL-4Rα-deficient mice. *Am. J. Pathol.* **175**, 706–716 (2009).
53. Kirstein, F., Nieuwenhuizen, N. E., Jayakumar, J., Horsnell, W. G. C. & Brombacher, F. Role of IL-4 receptor α-positive CD4+ T cells in chronic airway hyperresponsiveness. *J. Allergy Clin. Immunol.* <https://doi.org/10.1016/j.jaci.2015.10.036> (2015).
54. Abdel Aziz, N., Nono, J. K., Mpotje, T. & Brombacher, F. The Foxp3+ regulatory T-cell population requires IL-4Ra signaling to control inflammation during helminth infections. *PLoS Biol.* **16**, e2005850 (2018).
55. Hoving, J. C. *et al.* B cells that produce immunoglobulin E mediate colitis in BALB/c mice. *Gastroenterology* **142**, 96–108 (2012).
56. Khumalo, J., Kirstein, F., Hadebe, S. & Brombacher, F. IL-4Ra signalling in CD4+CD25+FoxP3+ T regulatory cells restrain airway inflammation via limiting local tissue IL-33. *JCI Insight* <https://doi.org/10.1172/jci.insight.136206> (2020).
57. Kitamura, D., Roes, J., Kühn, R. & Rajewsky, K. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature* **350**, 423 (1991).

Acknowledgements

We thank the UCT Research Animal Facility for maintaining mice, and Munadia Ansari for genotyping mice. We are grateful to Lizette Fick and Raygaana Jacobs for their excellent histology services and for confocal microscopy core facilities. We are grateful to Ronnie Dreyer for the excellent cell sorting and Flow Cytometry Core Facility. We thank FlowJo for proving free service to Africa.

Author contributions

Conceived and supervised study: S.H., F.B. Performed the experiments: M.S., N.M., S.M., A.N., P.I. Analysed the data: M.S., N.M., S.M., A.N., P.I., S.H. Wrote the paper: S.H. All authors discussed the results and commented on the manuscript.

Funding

This work was supported by ICGEB, Cape Town Component, Medical Research Council (MRC) South Africa as well as support by the South African National Research Foundation (NRF) Research Chair initiative (SARChI) and Wellcome Trust CIDRI-Africa (203135Z/16/Z) to FB. SH is supported by NRF Thuthuka Grant (117721), NRF Competitive Support for Unrated Researcher (138072), MRC South Africa under a Self-initiated grant. MS was supported by ICGEB Arturo Falaschi PhD Fellowship NM was supported by South African MRC PhD Fellowship. AN was supported by NRF MSc Scholarship. This research was funded in whole, or in part, by the

Wellcome Trust [Grant number 203135Z/16/Z]. For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-022-26637-6>.

Correspondence and requests for materials should be addressed to M.S., N.M. or S.H.

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