

Innate IL-17A Enhances IL-33-Independent Skin Eosinophilia and IgE Response on Subcutaneous Papain Sensitization

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IL-33-activated group 2 innate lymphoid cells critically contribute to protease allergen-induced airway inflammation models. However, IL-33 is dispensable for a subcutaneous (s.c.) papain-induced skin inflammation model, suggesting distinct mechanisms between intranasal and s.c. sensitization. Here, we examined the role of IL-17A in the s.c. model. Papain-exposed skin produced IL-17A and an excess amount of a soluble decoy receptor for IL-33, with the latter being a possible reason for the independence of the s.c. model from IL-33. An IL-17A deficiency attenuated papain-induced skin eosinophilia and serum papain-specific IgE and IgG1 levels, whereas the s.c. administration of IL-17A with enzymatically inactive papain enhanced serum papain-specific IgE and IgG1 levels and T helper 2 development in draining lymph nodes in an IL-33-independent manner, suggesting IL-33-independent enhancement of papain-specific type 2 responses by IL-17A. The s.c. papain increased IL-17A⁺ $\gamma\delta$ T cells in draining lymph nodes, approximately half of which were V γ 4⁺, as the majority of IL-17A⁺ cells, and increased V γ 5⁺ and V γ 4⁺ $\gamma\delta$ T cells in the skin. Depletion of $\gamma\delta$ TCR⁺ cells reduced T helper cytokine production in antigen-restimulated draining lymph node cells. These results suggest a novel role for IL-17A as an enhancer of skin eosinophilia and serum antigen-specific IgE production and for $\gamma\delta$ T cells as an enhancer of T helper cell activation in the s.c. papain model.

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INTRODUCTION

The protease activity of environmental allergens is considered to be an important property by which allergens inflict allergenic insults. The house dust mite-derived group 1 major allergens, Der f 1 and Der p 1, are among the most clinically important allergens worldwide. They disrupt barrier function, stimulate airway epithelial cells and keratinocytes through their protease activity to produce proinflammatory cytokines, cleave cell surface and soluble molecules, and induce T helper (Th) 2 responses (Chevigné and Jacquet, 2018; Gough et al., 1999; Kato et al., 2005; Kikuchi et al., 2006; King et al., 1998; Nakamura et al., 2006; Takai and Ikeda, 2011; Wan et al., 1999). Although exposure to protease allergens

causes allergic symptoms in mice, the mechanisms by which early innate responses in exposed tissues lead to systemic Th2 sensitization remain unclear.

Papain, a papaya-derived occupational allergen, belongs to the same family of cysteine proteases as Der f 1 and Der p 1 (Barrett and Rawlings, 2001; Chua et al., 1988). Papain is commonly used as a model allergen to study protease-induced allergic diseases in murine models. In papain-induced allergic airway inflammation models, early innate responses critically contribute to adaptive Th2 responses (Halim et al., 2014; Kamijo et al., 2013). IL-33 from papain-damaged epithelium activates lung group 2 innate lymphoid cells (ILC2s) to produce IL-5, which induces early lung eosinophilia (Halim et al., 2012). The activated ILC2s also produce IL-13, inducing lung dendritic cells to migrate into draining lymph nodes (DLNs) to mediate Th2 differentiation (Halim et al., 2014). A recent study demonstrated that protease allergens, including papain, directly process and activate IL-33 by cleaving it within the protease-sensor domain (Cayrol et al., 2018). These findings emphasize the critical contribution of IL-33-activated ILC2s to adaptive Th2 sensitization in airway papain models. In contrast, we previously demonstrated that the single subcutaneous (s.c.) administration of papain-induced Th2 differentiation, skin eosinophilia, and serum IgE and IgG1 responses in a manner dependent on the protease activity of papain but independent of IL-33 (the s.c. papain model) (Kamijo et al., 2016b), suggesting distinct mechanisms of Th2 induction between the airways and the skin. In papain-exposed skin, factors other than IL-33 appear to substitute for the role of IL-33 in the airways.

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Abbreviations: DETC, dendritic epidermal T cell; DLN, draining lymph node; ILC2, group 2 innate lymphoid cell; OVA, ovalbumin; rIL, recombinant IL; s.c., subcutaneous; sST2, soluble ST2; Th, T helper

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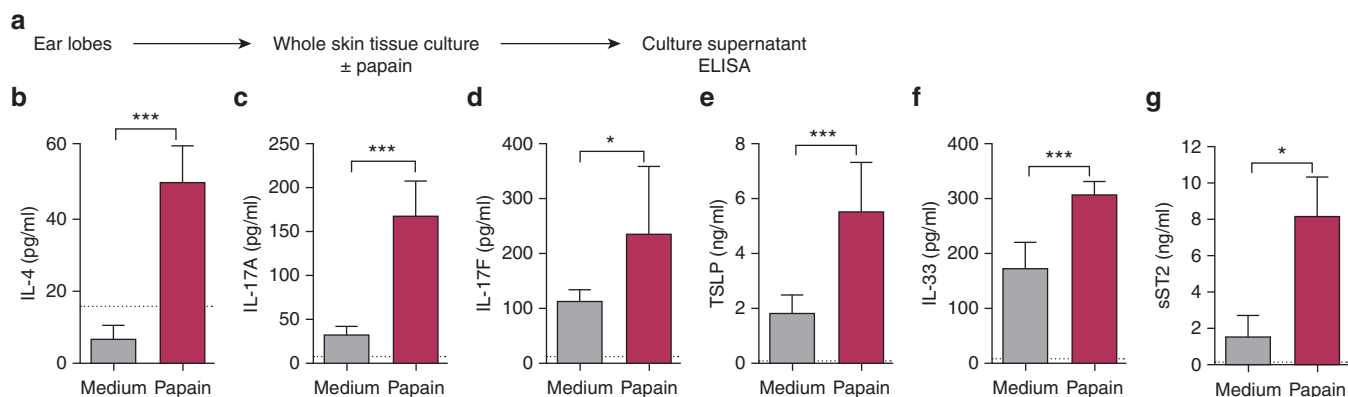


Figure 1. In vitro cytokine production from papain-stimulated skin tissue. (a) Experimental procedure. The ear lobes of C57BL/6J mice were cut, separated into ventral and dorsal sides, and incubated with or without papain (100 µg/ml) in vitro for 24 hours. Data indicate the means ± SD of nine (b, d), six (c, e, f), or three (g) ears. Data are representative of four (b, e), three (c, d, g), or two (f) independent experiments with similar results. Detection limits are indicated by dotted lines. * $P < 0.05$ and *** $P < 0.001$ by t -test. sST2, soluble ST2.

IL-17A is a proinflammatory cytokine produced by Th17 cells, group 3 innate lymphoid cells, NKT cells, and $\gamma\delta$ T cells (Veldhoen, 2017). Although IL-17A was originally associated with infectious immunity and autoimmunity, recent studies implicated it in allergic diseases such as asthma, atopic dermatitis, and contact hypersensitivity (Koga et al., 2008; Nakae et al., 2002; Oyoshi et al., 2009). Furthermore, IL-17A was shown to play a role in Th2 differentiation (Na et al., 2018; Nakajima et al., 2014). Thus, IL-17A may be a candidate mediator that contributes to Th2 differentiation and allergic skin inflammation in our s.c. papain model.

The $\gamma\delta$ T cells, one of the early innate producers of IL-17A, are divided into several different subsets depending on their tissue localization and effector function, and subset members are distinguished by V γ and V δ chains in their $\gamma\delta$ TCRs (Bonneville et al., 2010; Heilig and Tonegawa, 1986; Papotto et al., 2017). Although the involvement of $\gamma\delta$ T cells as an early innate source of IL-17A has been reported in skin inflammation models of psoriasis (Yoshiki et al., 2014), contact hypersensitivity (Nielsen et al., 2014), and atopic dermatitis (Nakajima et al., 2014), the roles of IL-17A and IL-17A⁺ $\gamma\delta$ T cells in protease allergen-induced allergic skin inflammation models remain unknown.

In this study, we demonstrated that papain-exposed skin tissue produced IL-17A and a soluble decoy receptor for IL-33 in excess of the amount of IL-33 present and examined the role of innate IL-17A in the pathogenesis of the s.c. papain model. We also investigated the accumulation of IL-17A-producing $\gamma\delta$ T cell subsets in papain-exposed skin and DLNs.

RESULTS

Cytokine production profile of papain-stimulated skin tissue

To analyze early cytokine responses in papain-exposed skin, we stimulated ear skin tissue with papain in vitro, which resulted in the production of IL-4, IL-17A, and IL-17F (Figure 1a–d) but not IL-13, or IFN- γ (Supplementary Figure S1). Although TSLP and IL-33 were produced (Figure 1e and f), IL-12p70 was not (Supplementary Figure S1). We detected approximately 30-fold and 10-fold

higher amounts of soluble ST2 (sST2), a soluble decoy receptor for IL-33, than IL-33 in skin tissue cultured with and without papain, respectively (Figure 1g). Among the papain-induced cytokines shown in Figure 1, we focused on IL-17 because the roles of IL-4 and TSLP have already been reported (Tang et al., 2010) and IL-33 was dispensable for the s.c. papain model (Kamijo et al., 2016b).

Opposite balance between IL-33 and sST2 in the skin and lungs

We analyzed and detected the expression of ST2L, a subunit of the IL-33 receptor, in the skin ILC2s (Supplementary Figure S2a and b). However, papain failed to upregulate the ILC2 activation marker (CD25) (Scanlon and McKenzie, 2012) regardless of the production of IL-33 (Figure 1f and Supplementary Figure S2c).

To compare the expression profiles of IL-33 and sST2 in the skin and the lungs in vivo, we administered papain subcutaneously or intranasally and compared the mRNA expression levels of *IL-33*, *sST2*, and *ST2L*. Intranasal papain enhanced *IL-33* mRNA expression in the lungs (Figure 2a), whereas s.c. papain enhanced *sST2* mRNA expression in the skin, reaching an approximately 20-fold higher level than the lungs with intranasal papain (Figure 2b). The basal mRNA expression levels of *ST2L* were higher in the lungs than in the skin (Figure 2c).

The papain-stimulated lung explant culture released a more than 3-fold higher amount of IL-33 than the skin tissue culture (Figure 1f and Supplementary Figure S3a), and the papain-stimulated skin tissue culture released a 10-fold higher amount of sST2 than the lung explant culture (Figure 1g and Supplementary Figure S3b). These results indicate the preferential expression of sST2 over IL-33 in papain-exposed skin and IL-33 over sST2 in papain-exposed lungs.

IL-17A^{-/-} mice show reduced skin eosinophilia and papain-specific IgE and IgG1 production in response to s.c. papain

The mRNA expression of *IL-17A* was upregulated in the ear skin of s.c. papain-administered wild-type mice (Figure 3a). To examine the role of IL-17A, we subcutaneously administered papain to *IL-17A*^{-/-} mice. *IL-17A*^{-/-} mice showed less

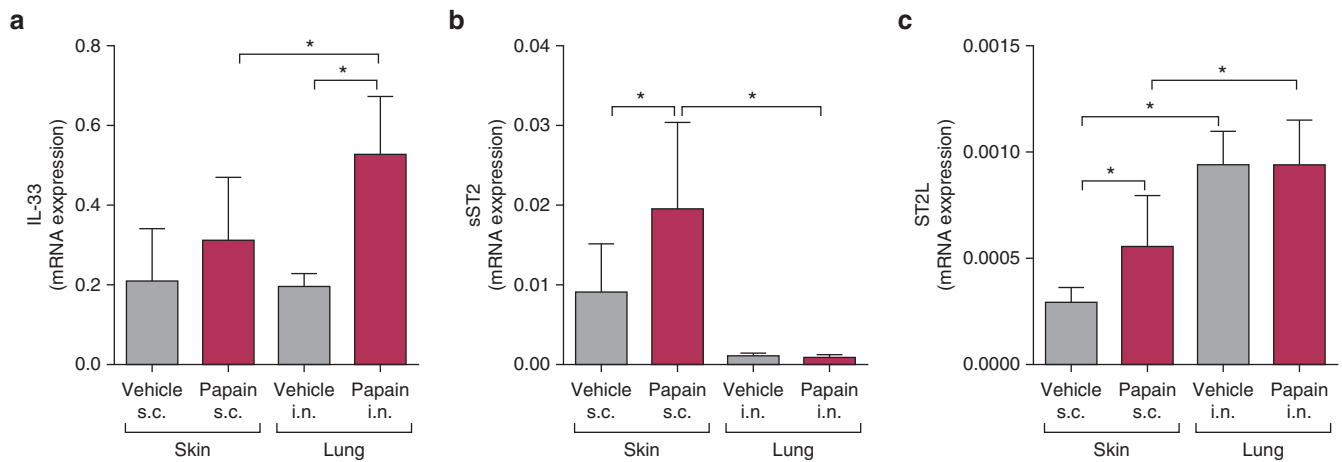


Figure 2. mRNA expression of *IL-33*, *sST2*, and *ST2L* in papain-exposed skin and lungs in vivo. Papain (20 μ g per mouse) was administered s.c. or i.n. into the ear lobes or lungs, respectively. After 24 hours, total RNA samples were extracted from the ear lobes or lungs and the mRNA expression levels were measured by real-time PCR. mRNA expression levels relative to those of *GAPDH* are shown. Data indicate the means $\pm$ SD of nine ear lobes or seven lungs from two independent experiments with similar results. * $P < 0.05$ by one-way ANOVA with Tukey's post hoc test. i.n., intranasal; s.c., subcutaneous; sST2, soluble ST2.

eosinophil infiltration and lower levels of papain-specific IgE and IgG1 than wild-type mice (Figure 3b–d and Supplementary Figure S4a). IL-17A receptors consist of a common IL-17RA paired with either IL-17RC or IL-17RD (Su et al., 2019; Toy et al., 2006). B cells, CD4⁺ T cells, and T follicular helper cells (Miyauchi et al., 2016) expressed IL-17RA (Supplementary Figure S5a and b) and were increased by s.c. papain (Supplementary Figure S5c). The s.c. papain preferentially increased GATA-3⁺ IL-4⁺ Th2 cells among CD4⁺ T cells (Figure 3e and Supplementary Figure S6).

IL-23 and TNF- α stimulate skin tissue to produce IL-17A and IL-17F

In addition to the cytokines shown in Figure 1, IL-1 β , IL-23, and TNF- α were released from the papain-stimulated skin tissue culture (Figure 4a). Thus, we stimulated skin tissue with recombinant IL (rIL)-1 β , rIL-23, or recombinant TNF- α and found the induction of IL-17A and IL-17F by IL-23 and TNF- α (Figure 4b). IL-23 induced the most significant IL-17A and IL-17F production. These results suggest that IL-23 and TNF- α , the production of which is triggered by papain, subsequently induced IL-17A and IL-17F production in the skin tissue.

The s.c. injection of rIL-17A with E64-papain enhances E64-papain-specific type 2 responses

To clarify the function of IL-17A in papain-exposed skin, we subcutaneously injected rIL-17A with E64-papain, a covalent complex between E-64 and papain, into the ear lobes of wild-type mice. The coadministration of rIL-17A and E64-papain increased the levels of total IgE and papain-specific IgE and IgG1 in serum, whereas E64-papain or rIL-17A alone did not (Figure 5a and Supplementary Figure S4b). It increased Th1, Th2, and Th17 cells in the DLNs upon polyclonal restimulation with phorbol 12-myristate 13-acetate and ionomycin (Supplementary Figure S7a) but exclusively enhanced Th2 cytokine responses upon E64-papain-specific restimulation (Figure 5b).

rIL-17A did not induce Th2 cytokines in skin tissue in vitro, suggesting that the effect of IL-17A was not due to Th2 cytokine production by skin-derived innate cells (Supplementary Figure S8a). The induction of papain-specific IgE and IgG1 by skin IL-17A was observed in *IL-33*^{−/−} mice, indicating that the enhancement of type 2 responses by skin IL-17A occurred independently of IL-33 (Supplementary Figure S8b).

Coadministration of rIL-17A with ovalbumin (OVA) induced OVA-specific IgE and IgG1 (Supplementary Figure S9a) and weaker or no enhancement of T-cell responses compared with coadministration with E64-papain (Supplementary Figures S7b and S9b).

The s.c. injection of papain induces the accumulation of IL-17A⁺ $\gamma\delta$ T cells in DLNs

We then examined the expansion of IL-17A-producing cells in the skin DLNs and found higher numbers of $\gamma\delta$ TCR⁺ IL-17A⁺ cells in papain-injected mice than in vehicle-injected mice (Figure 6a). In the DLNs of papain-injected mice, the majority of IL-17A⁺ cells were $\gamma\delta$ T cells ($64.5 \pm 14.6\%$ [mean $\pm$ SD], nine mice), and $22.8 \pm 7.5\%$ of all $\gamma\delta$ TCR⁺ cells were IL-17A⁺. At least three populations are known as IL-17A-producing $\gamma\delta$ T cell subsets in the skin: V γ 4⁺ or V γ 6⁺ $\gamma\delta$ T17 cells in the dermis and V γ 5⁺ dendritic epidermal T cells (DETCs) in the epidermis (nomenclature by Heilig and Tonegawa [1986]) (Cai et al., 2014; Li et al., 2017; MacLeod et al., 2013). The s.c. administration of papain increased the numbers of V γ 4⁺ and V γ 5⁺ cells (no V γ 6-specific antibody was available) in the DLNs and the skin (Figure 6b). We then subdivided $\gamma\delta$ TCR⁺ IL-17A⁺ cells in the skin DLNs into V γ 4⁺ IL-17A⁺ cells and V γ 5⁺ IL-17A⁺ cells (Figure 6c). The absolute number of V γ 4⁺ IL-17A⁺ cells in the DLNs was increased by the s.c. papain. V γ 5⁺ IL-17A⁺ cells did not expand in response to the s.c. papain in the DLNs.

To evaluate the contribution of $\gamma\delta$ T cells to the s.c. papain model, we administered the depleting anti- $\gamma\delta$ TCR mAb (clone: GL3). GL3 decreased $\gamma\delta$ TCR⁺ cells, V γ 4⁺ cells (Supplementary Figure S10), and the production of Th

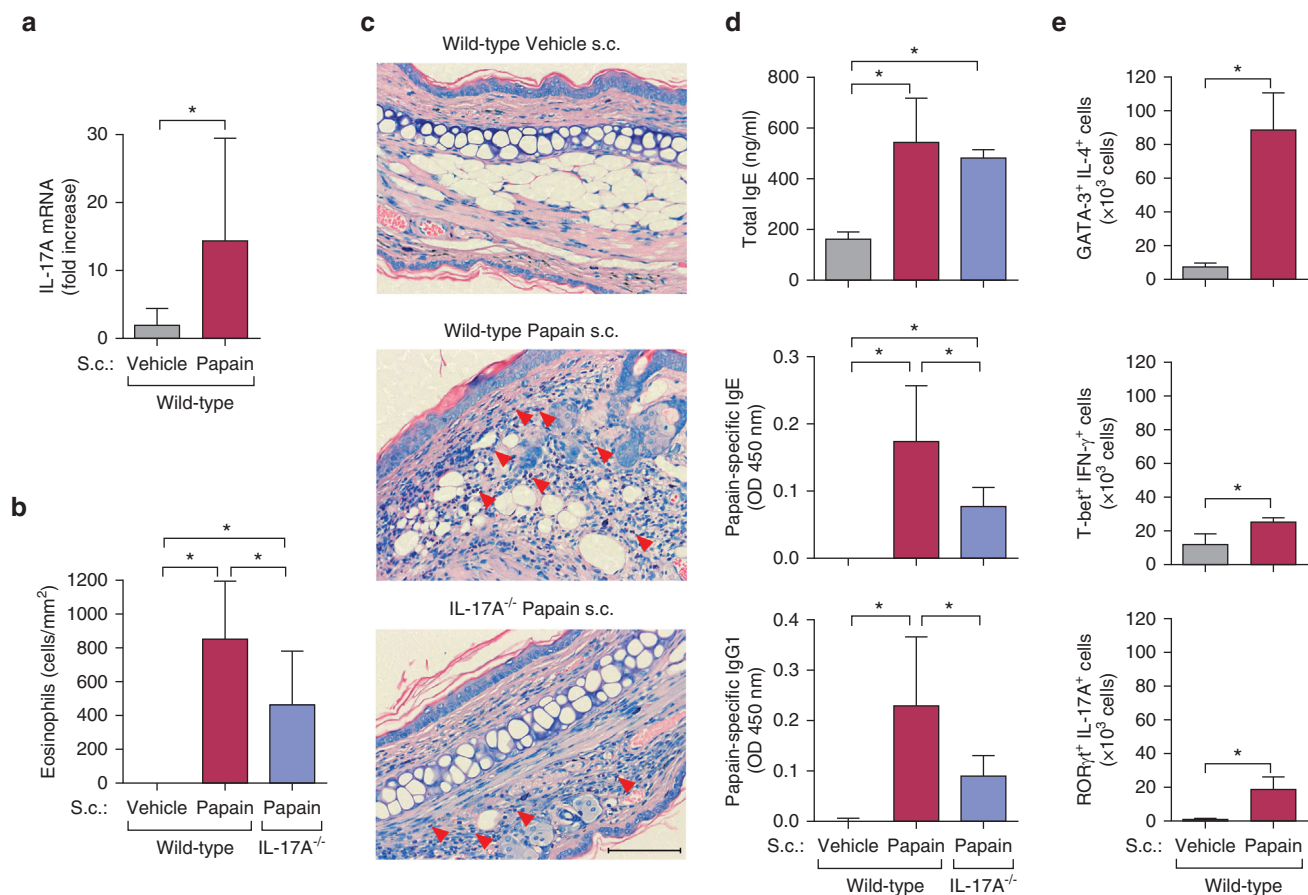


Figure 3. An IL-17A deficiency attenuated papain-induced allergic skin inflammation. Papain (10 μ g per ear) was subcutaneously administered to both ears of wild-type or IL-17A^{-/-} mice on day 0. (a) IL-17A mRNA expression in wild-type skin tissue 4 hours after s.c. injection of papain. (b) The number of eosinophils in the Giemsa-stained skin tissue sections were counted under microscopy. (c) Histology. Giemsa-stained paraffin sections of ear lobes isolated on day 11. Eosinophils are indicated with red arrowheads. Bar = 100 μ m. (d) Antibody responses. Sera were collected on day 14 (total IgE) or day 9 (papain-specific IgE and IgG1). (e) Numbers of Th2, Th1, and Th17 cells in the DLNs on day 10. Before analysis, DLN cells were stimulated with PMA and ionomycin for 6 hours. Data indicate the means $\pm$ SD of replicates, the numbers of which are indicated in Supplementary Materials and Methods. Data are representative of (a–d) three or (e) two independent experiments with similar results. * P < 0.05 by (a–d) one-way ANOVA with Tukey's post hoc test or (e) the t -test. DLN, draining lymph node; OD, optical density; PMA, phorbol 12-myristate 13-acetate; s.c., subcutaneous; Th, T helper.

cytokines by antigen-restimulated DLN cells but not serum antibody responses (Supplementary Figure S11).

These results suggested that s.c. administration of papain expands IL-17A-producing $\gamma\delta$ T cells, including V γ 4⁺ IL-17A⁺ cells, in the DLNs and that $\gamma\delta$ T cells contribute to Th cell responses in the DLNs.

The s.c. injection of papain induces the activation of DETCs in the epidermis

Papain injection expanded V γ 5⁺ cells in the skin (Figure 6b). Thus, we investigated the activation of V γ 5⁺ DETCs (Figure 6d). Papain rendered DETCs with a round-shaped morphology in vivo (Figure 6d) and in vitro (Supplementary Figure S12), indicating their activated state (Nielsen et al., 2014). Consistent with the round-shaped morphology, papain-activated DETCs showed lower side scatter, indicating their simpler cellular structure (Supplementary Figure S13a). DETCs are epidermal-resident $\gamma\delta$ T cells distinct from Langerhans cells (Bergstresser et al., 1983). Epidermal Thy1.2⁺ cells were $\gamma\delta$ TCR⁺ (Supplementary Figure S13b) and distinct from major histocompatibility complex class II-positive Langerhans cells (Supplementary Figure S12d).

DISCUSSION

Th2 sensitization to allergens plays a central role in chronic allergic diseases. However, the mechanisms by which the initial exposure of naive individuals to allergens leads to Th2 sensitization remain unclear. In the natural progression of allergic diseases, atopic dermatitis often predates the development of food allergies, asthma, or allergic rhinitis. This observation, known as the atopic march, suggests that skin with a defective barrier function or eczema is the site at which allergic sensitization begins (Dharmage et al., 2014; Spergel and Paller, 2003). Thus, it is important to understand how innate responses to early local allergen exposure in the skin tissue lead to systemic Th2 sensitization. In this study, we demonstrated papain-induced IL-17A expression in skin tissue in vitro (Figure 1c) and in vivo (Figure 3a), increase of IL-17A⁺ $\gamma\delta$ T cells, including V γ 4⁺ IL-17A⁺ cells, in the DLNs by the s.c. papain (Figure 6a and c), and the contribution of IL-17A to papain-induced skin eosinophilia, papain-specific Th2 responses in the DLNs, and serum papain-specific IgE and IgG1 responses (Figures 3 and 5). The administration of anti- $\gamma\delta$ TCR mAb reduced Th cytokine production by the antigen-restimulated DLN cells

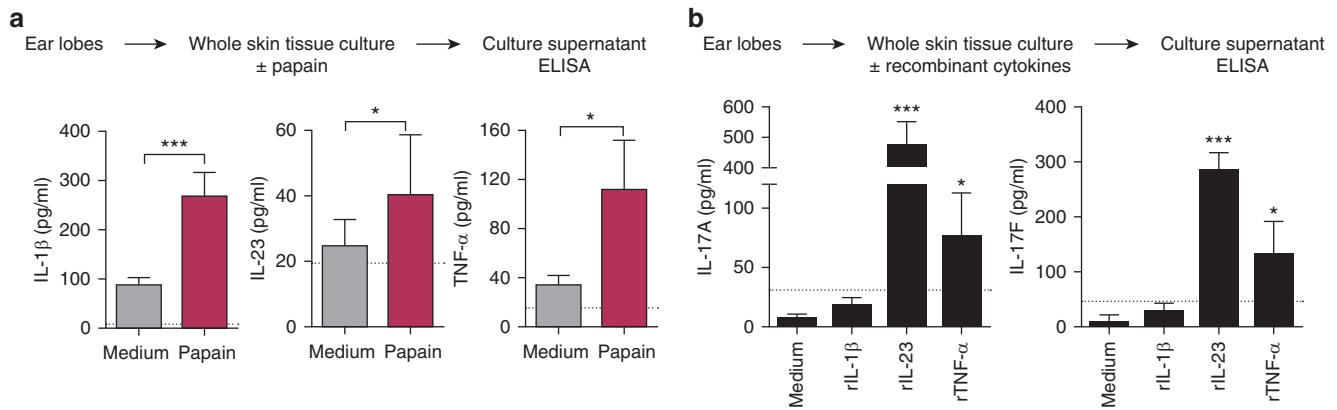


Figure 4. Proinflammatory cytokines induced IL-17A and IL-17F production from in vitro-cultured skin tissue. The ear lobes were cut, separated into ventral and dorsal sides, and incubated with or without (a) papain (100 μ g/ml) or (b) rIL-1 β (100 ng/ml), rIL-23 (100 ng/ml), or rTNF- α (200 ng/ml) in vitro for 24 hours. Data indicate the means $\pm$ SD of (a) six (IL-1 β), nine (IL-23), three (TNF- α), or (b) three ears. Data are representative of (a) four (IL-1 β), two (IL-23 and TNF- α) or (b) three independent experiments with similar results. Detection limits are indicated by dotted lines. * P < 0.05 and *** P < 0.001 by t -test versus the medium group. rIL, recombinant IL; rTNF- α , recombinant TNF- α .

(Supplementary Figure S11a). Collectively, these results suggest the contribution of skin-derived IL-17A and $\gamma\delta$ T cells to the transition from initial innate responses to late phase Th2-mediated responses in the model.

We considered the early IL-17A sources in our s.c. papain model to be innate immune cells such as group 3 innate lymphoid cells, NKT cells, and IL-17A-producing $\gamma\delta$ T cells, including V γ 4 $^{+}$ $\gamma\delta$ T17 cells and V γ 5 $^{+}$ DETCs (Cai et al.,

2014; Kim et al., 2020; Li et al., 2017; MacLeod et al., 2013; Papotto et al., 2017; Veldhoen, 2017), because a single s.c. injection of papain did not induce Th17 differentiation in the skin DLNs (Kamijo et al., 2016b), whereas the repeated epicutaneous administration of papain did (Ochi et al., 2017; Shimura et al., 2016). We found an increased number of $\gamma\delta$ TCR $^{+}$ IL-17A $^{+}$ cells, approximately 50% of which were V γ 4 $^{+}$ IL-17A $^{+}$ cells, in the skin DLNs, the

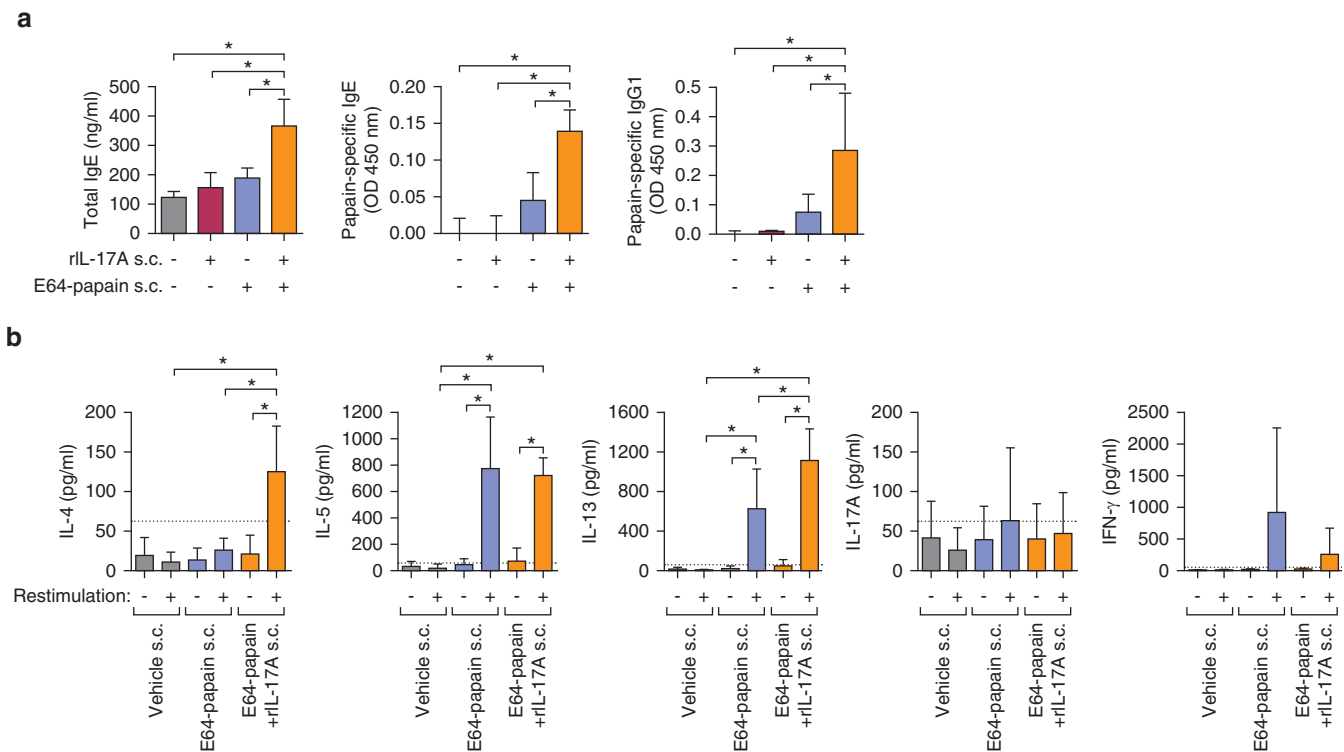


Figure 5. Administration of rIL-17A and inactive papain enhanced serum papain-specific IgE and IgG1 production and DLN Th2 responses. (a) Antibody responses. rIL-17A (1 μ g per ear) and E64-papain (5 μ g per ear) were administered on day 0. Sera were collected on day 14 (total IgE and papain-specific IgE) or day 10 (papain-specific IgG1). (b) Th cytokine responses. rIL-17A (1 μ g per ear) was s.c. coadministered with E64-papain (10 μ g per ear) to both ears on days 0 and 10. DLN cells were collected on day 11 and restimulated with E64-papain for 72 hours. Detection limits are indicated by dotted lines. Data indicate the means $\pm$ SD of (a) five or (b) six mice from (a) three or (b) two independent experiments with similar results. * P < 0.05 by one-way ANOVA with Tukey's post hoc test. DLN, draining lymph node; rIL-17A, recombinant IL-17A; s.c., subcutaneous; Th, T helper.

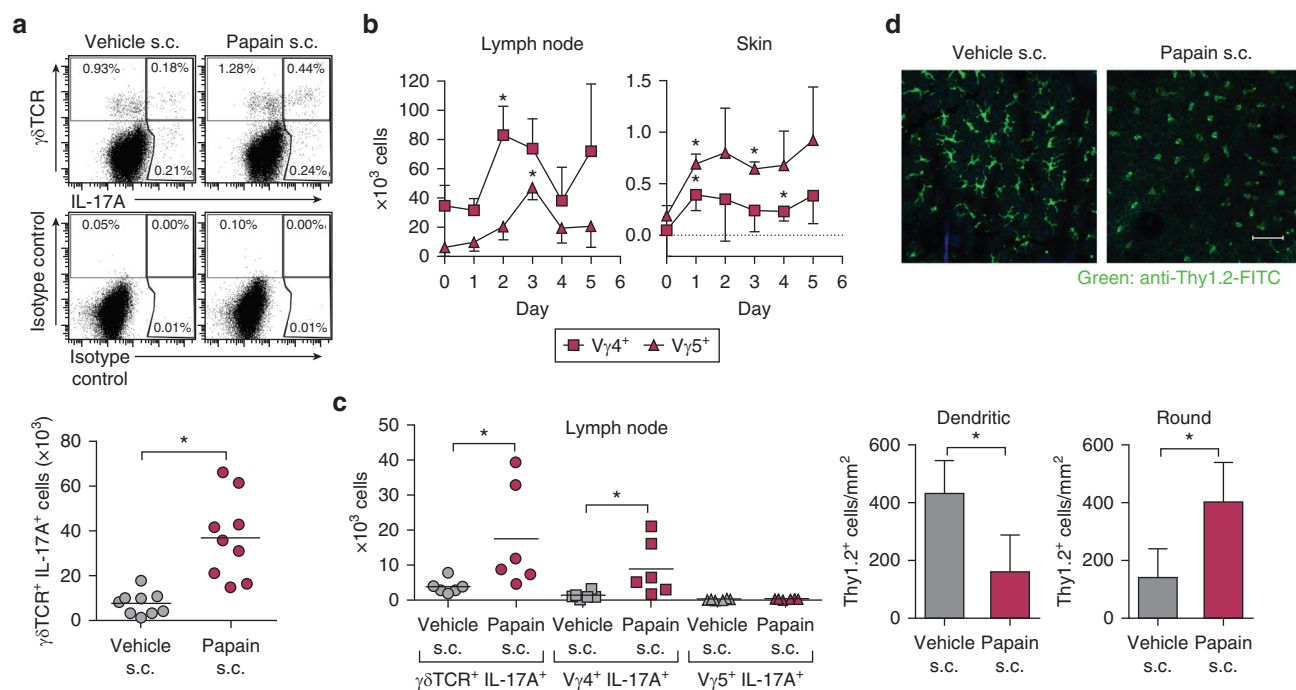


Figure 6. The s.c. administration of papain induced the expansion of $\gamma\delta$ TCR⁺ IL-17A⁺ cells in DLNs and activation of DETCs in the epidermis. Papain (10 μ g per ear) was s.c. administered to both ears of mice. (a) Frequency and numbers of $\gamma\delta$ TCR⁺ IL-17A⁺ cells in DLNs 24 hours after papain administration. (b) Time course of V γ 4⁺ and V γ 5⁺ cell numbers in DLNs and ear skin in papain-administered mice. (c) Numbers of V γ 4⁺ IL-17A⁺ and V γ 5⁺ IL-17A⁺ cells in DLNs 48 hours after administration. Before analysis, DLN cells were stimulated with PMA and ionomycin for 6 hours in (a) and (c). (d) Confocal microscopy and numbers of epidermal Thy1.2⁺ cells with a dendritic or round-shaped morphology in the ear epidermal sheets prepared 24 hours after papain administration. Bar = 50 μ m. Data indicate the means $\pm$ SD of replicates, the numbers of which are indicated in [Supplementary Materials and Methods](#). * $P < 0.05$ by *t*-test. DETC, dendritic epidermal T cell; DLN, draining lymph node; PMA, phorbol 12-myristate 13-acetate; s.c., subcutaneous.

accumulation of V γ 4⁺ $\gamma\delta$ T17 cells and V γ 5⁺ DETCs in the DLNs and the skin tissue, and the activation of DETCs in the s.c. papain-treated mice (Figure 6). An anti- $\gamma\delta$ TCR mAb, GL3, reduced Th cytokine responses in the DLNs (Supplementary Figure S11). GL3 has been used for depletion of $\gamma\delta$ T cells and has shown similar effects with TCR δ -deficient mice (Coffelt et al., 2015). A previous study reported that GL3 can induce the internalization of $\gamma\delta$ TCR (Koenecke et al., 2009). The function of papain-activated DETCs remains to be elucidated because papain did not increase V γ 5⁺ IL-17A⁺ cells in the DLNs (Figure 6c), and BALB/c, a strain with fewer DETCs in the ear skin (Lee et al., 2002), developed s.c. papain-induced responses (Kamijo et al., 2016b).

Our preliminary results showed that the early-phase inflammation (days 1–3) mainly consisted of neutrophils (data not shown), whereas eosinophils became more dominant (Figure 3b and c; day 11) in the late phase (days 7–15) of the s.c. papain model (Kamijo et al., 2016b). The IL-17A deficiency attenuated eosinophil infiltration and papain-specific IgE and IgG1 production in the late phase (Figure 3b–d), indicating that skin IL-17A affects not only the neutrophil-dominant early phase but also the Th2-mediated late phase of the s.c. papain model. IL-17A acts as an enhancer of papain-specific Th2 responses (Figures 3e and 5b). Two recent studies reported the contribution of IL-17A to Th2 differentiation, showing that IL-17A added to the Th2-skewing culture of naive CD4⁺ T cells increased the differentiation of IL-4⁺ cells in vitro (Nakajima et al., 2014) and that the double deficiency of IL-17A and IL-17F reduced Th2

responses in vivo (Na et al., 2018). Our results suggest the contribution of skin IL-17A (Figures 1c, 3a, and 5) and $\gamma\delta$ T-cell-derived IL-17A in the DLNs (Figure 6 and Supplementary Figures S5 and S11a) to the s.c. papain model. It remains to be elucidated whether skin IL-17A or IL17A in the DLNs contribute most to the model, or whether they contribute equally.

The s.c. papain increased IL-17RA⁺ CD4⁺ T cells (Supplementary Figure S5), the majority of which were Th2 cells (Figures 3e and S6), and IL-17RA⁺ T follicular helper cells (Supplementary Figure S5), being consistent with the induction of papain-specific IgE and IgG1 responses (Figure 3d). B cells also expressed IL-17RA and expanded concurrently with IL-17A⁺ $\gamma\delta$ T cells (Figure 6c and Supplementary Figure S5), suggesting a hypothetical direct B-cell activation by IL-17A, which may explain the OVA-specific antibody responses without detectable Th responses (Supplementary Figure S9). However, it needs to be verified with further analysis because IL-17RA is a shared component of receptors for IL-17C (Song et al., 2011) and IL-17E (Rickel et al., 2008).

IL-1 and IL-23 are inducers of IL-17A production from $\gamma\delta$ T cells in various disease models (Coffelt et al., 2015; Muschaweckh et al., 2017; Yoshiki et al., 2014). For example, IL-1 triggers IL-17A from skin $\gamma\delta$ T cells and Th17 cells in *Staphylococcus aureus* infection models (Liu et al., 2017; Nakagawa et al., 2017). We detected IL-1 β , IL-23, and TNF- α in the supernatant of the papain-stimulated skin tissue culture (Figure 4a). Among them, IL-23 induced the most robust IL-17A and IL-17F production in the skin tissue

culture (Figure 4b). The TNF- α -induced production of skin IL-17A and IL-17F may occur through an indirect pathway involving IL-23 derived from TNF- α -activated dendritic cells, Langerhans cells, and/or keratinocytes.

Papain-stimulated skin tissue produced IL-4 (Figure 1b) possibly from basophils known for IL-4 production in response to proteases in vivo (Sokol et al., 2008) and in vitro (Kamijo et al., 2016a, 2013). The induction of IL-5, IL-13, and ILC2 activation was absent (Supplementary Figures S1 and S2) regardless of the IL-33 production (Figure 1f). The papain-induced production of sST2 in an excess amount (Figures 1g and 2b) may be responsible for the absence of ILC2 activation in papain-exposed skin (Supplementary Figure S2). We detected higher levels of the gene expression for *IL-33* and *ST2L* and lower levels for *sST2* in papain-exposed lung tissue than in papain-exposed skin tissue in vivo (Figure 2), and the lung explant culture released higher amounts of IL-33 and lower amounts of sST2 than the skin tissue culture (Figure 1f and g and Supplementary Figure S3). These results are consistent with the robust production of IL-5 and IL-13 by IL-33-activated lung ILC2s in papain airway inflammation models (Halim et al., 2012; Kamijo et al., 2013).

We propose the following hypothetical mechanism on the basis of the results obtained in this study: papain initially induces IL-23 and TNF- α in exposed skin, which then activate dermal V γ 4⁺ $\gamma\delta$ T17 cells to produce IL-17A in the skin tissue, resulting in early neutrophil infiltration. $\gamma\delta$ T cells accumulate not only in the skin but also in the DLNs to increase IL-17A-producing $\gamma\delta$ T cells, including V γ 4⁺ $\gamma\delta$ T17 cells. As shown by precedent studies, skin dendritic cells, which are matured in the presence of papain-induced IL-4 and TSLP, migrate into the DLNs to present papain to naive CD4⁺ T cells in a manner favorable for Th2 differentiation (Gao et al., 2013; Kumamoto et al., 2013; Tang et al., 2010). IL-17A derived from the increased number of $\gamma\delta$ T17 cells further enhances the ongoing Th2 differentiation in the DLNs (Nakajima et al., 2014), which then contributes to skin eosinophilia and the IgE response in the late phase. However, the contribution of other mediators needs to be considered because the attenuation of the responses in *IL-17A*^{-/-} mice was partial.

In conclusion, the results of this study suggest a novel role for skin IL-17A and IL-17A⁺ $\gamma\delta$ T cells in the DLNs as enhancers of Th2 sensitization to protease allergens administered subcutaneously. In addition, we found the release of excess amount of sST2 from skin tissue with or even without exposure to papain, which is a possible reason for the independence of s.c. or epicutaneous sensitization models from the IL-33-ST2L pathway (Kamijo et al., 2016b; Ochi et al., 2017; Shimura et al., 2016) in contrast to papain-induced IL-33-dependent models of allergic airway inflammation (Halim et al., 2012; Kamijo et al., 2013; Maruyama et al., 2019; Nishioka et al., 2018; Oboki et al., 2010).

MATERIALS AND METHODS

Detailed materials and methods are provided in the [Supplementary Materials and Methods](#).

Mice

The 7- to 10-week-old female C57BL/6 were purchased from Charles River Laboratories Japan (Yokohama, Japan) and Sankyo Labo Service (Ibaraki, Japan). *IL-17A*^{-/-} mice (C57BL/6) background) and *IL-33*^{-/-} mice (C57BL/6N background) were provided courtesy of Dr Susumu Nakae (Nakae et al., 2002; Oboki et al., 2010) and Dr Hirohisa Saito (Oboki et al., 2010). Mice were maintained in a specific pathogen-free animal facility at Juntendo University, Tokyo, Japan, and used in accordance with the guidelines of the institutional committee on animal experiments.

In vitro ear skin tissue culture

Ear lobes were isolated, separated into the ventral and dorsal sides, and submerged dermis side-down in the culture media in the presence or absence of papain or cytokines. After 24 hours, the culture supernatant was collected for cytokine ELISA.

The s.c. administration

Papain, E64-papain, OVA, or rIL-17A was subcutaneously injected into the ear skin. Sera were collected for antibody ELISA. Paraffin-embedded sections of the ear skin specimens were stained with Giemsa. DLN cells were restimulated with E64-papain or OVA. Total RNA in the skin was extracted for qPCR. DLN cells and skin cells were isolated for flow cytometric analysis.

Antigen restimulation of the skin DLN cells

Cervical lymph node cells were cultured in the presence of E64-papain or OVA (30 μ g/ml) in culture media using 96-well plates at 37 °C. After 72 hours, the culture supernatant was collected for the measurement of cytokines by ELISA.

Flow cytometry

Cell surface antigens were stained with target-specific antibodies. In the intracellular staining, cells were cultured with phorbol 12-myristate 13-acetate, ionomycin, and BD Golgi plug (BD Biosciences, Franklin Lakes, NJ) and permeabilized with BD Cytofix/Cytoperm reagents (BD Biosciences). Acquisition and analysis were performed using a FACSVerse cell sorter (BD Biosciences) and FlowJo software (BD Biosciences).

Intranasal administration of papain

Mice were lightly anesthetized with the inhalation of isoflurane and allowed to inhale papain (20 μ g/50 μ l per mouse) following alternate application to both nares with a pipette. Lung tissues excised 24 hours later were subjected to RNA extraction and gene expression analysis by qPCR.

Preparation of epidermal sheets

Papain was subcutaneously administered to both ears. Ear lobes were isolated, separated into ventral and dorsal pieces, and incubated in 0.5 M ammonium thiocyanate (Sigma-Aldrich, Saint Louis, MO) at 37 °C for 30 minutes. Epidermal sheets were peeled and stained with target-specific antibodies. Stained epidermal sheets were then washed and subjected to confocal microscopy.

Statistical analysis

Statistical analysis was performed as indicated in the legends. A value of *P* < 0.05 was considered to be statistically significant.

Data availability statement

The datasets generated and/or analyzed during this study are available from the corresponding author on reasonable request.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: SK, SN, TT; Formal Analysis: SK, TT; Funding Acquisition: SK, KO, TT; Investigation: SK, MH, MS, TT; Methodology: SK, TT; Project Administration: SK, TT; Resources: SK, SN, TT; Supervision: SK, HO, KO, TT; Validation: SK, TT; Visualization: SK, TT; Writing - Original Draft Preparation: SK, TT; Writing - Review and Editing: SK, MH, MS, SN, HO, KO, TT

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2020.05.088>.

REFERENCES

- Barrett AJ, Rawlings ND. Evolutionary lines of cysteine peptidases. *Biol Chem* 2001;382:727–33.
- Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW. Thy-1 antigen-bearing dendritic cells populate murine epidermis. *J Invest Dermatol* 1983;81:286–8.
- Bonneville M, O'Brien RL, Born WK. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat Rev Immunol* 2010;10:467–78.
- Cai Y, Xue F, Fleming C, Yang J, Ding C, Ma Y, et al. Differential developmental requirement and peripheral regulation for dermal Vγ4 and Vγ6T17 cells in health and inflammation. *Nat Commun* 2014;5:3986.
- Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, et al. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. *Nat Immunol* 2018;19:375–85.
- Chevigné A, Jacquet A. Emerging roles of the protease allergen Der p 1 in house dust mite-induced airway inflammation. *J Allergy Clin Immunol* 2018;142:398–400.
- Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, et al. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. homology with cysteine proteases. *J Exp Med* 1988;167:175–82.
- Coffelt SB, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau CS, et al. IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* 2015;522:345–8.
- Dharmage SC, Lowe AJ, Matheson MC, Burgess JA, Allen KJ, Abramson MJ. Atopic dermatitis and the atopic march revisited. *Allergy* 2014;69:17–27.
- Gao Y, Nish SA, Jiang R, Hou L, Licona-Limón P, Weinstein JS, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* 2013;39:722–32.
- Gough L, Schulz O, Sewell HF, Shakib F. The cysteine protease activity of the major dust mite allergen Der p 1 selectively enhances the immunoglobulin E antibody response. *J Exp Med* 1999;190:1897–902.
- Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012;36:451–63.
- Halim TY, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* 2014;40:425–35.
- Heilig JS, Tonegawa S. Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature* 1986;322:836–40.
- Kamijo S, Nunomura S, Ra C, Kanaguchi Y, Suzuki Y, Ogawa H, et al. Innate basophil IL-4 responses against allergens, endotoxin, and cytokines require the Fc receptor γ-chain. *J Allergy Clin Immunol* 2016a;137:1613–5.e2.
- Kamijo S, Suzuki M, Hara M, Shimura S, Ochi H, Maruyama N, et al. Subcutaneous allergic sensitization to protease allergen is dependent on mast cells but not IL-33: distinct mechanisms between subcutaneous and intranasal routes. *J Immunol* 2016b;196:3559–69.
- Kamijo S, Takeda H, Tokura T, Suzuki M, Inui K, Hara M, et al. IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation. *J Immunol* 2013;190:4489–99.
- Kato T, Takai T, Mitsuishi K, Okumura K, Ogawa H. Cystatin A inhibits IL-8 production by keratinocytes stimulated with Der p 1 and Der f 1: biochemical skin barrier against mite cysteine proteases. *J Allergy Clin Immunol* 2005;116:169–76.
- Kikuchi Y, Takai T, Kuhara T, Ota M, Kato T, Hatanaka H, et al. Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p1 to sensitization toward IgE and IgG responses. *J Immunol* 2006;177:1609–17.
- Kim MH, Jin SP, Jang S, Choi JY, Chung DH, Lee DH, et al. IL-17A-producing innate lymphoid cells promote skin inflammation by inducing IL-33-driven type 2 immune responses. *J Invest Dermatol* 2020;140:827–37.e9.
- King C, Brennan S, Thompson PJ, Stewart GA. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J Immunol* 1998;161:3645–51.
- Koenecke C, Chennupati V, Schmitz S, Malissen B, Förster R, Prinz I. In vivo application of mAb directed against the gammadelta TCR does not deplete but generates “invisible” gammadelta T cells. *Eur J Immunol* 2009;39:372–9.
- Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 2008;128:2625–30.
- Kumamoto Y, Linehan M, Weinstein JS, Laidlaw BJ, Craft JE, Iwasaki A. CD301b⁺ dermal dendritic cells drive T helper 2 cell-mediated immunity. *Immunity* 2013;39:733–43.
- Lee HC, Tomiyama K, Ye SK, Kawai K, Ikuta K. Seeding of dendritic epidermal T cells in the neonatal skin is reduced in 129 strain of mice. *Immunol Lett* 2002;81:211–6.
- Li Y, Huang Z, Yan R, Liu M, Bai Y, Liang G, et al. Vγ4 γδ T cells provide an early source of IL-17A and accelerate skin graft rejection. *J Invest Dermatol* 2017;137:2513–22.
- Liu H, Archer NK, Dillen CA, Wang Y, Ashbaugh AG, Ortines RV, et al. Staphylococcus aureus epicutaneous exposure drives skin inflammation via IL-36-mediated T cell responses. *Cell Host Microbe* 2017;22:653–6.e5.
- MacLeod AS, Hemmers S, Garipo O, Chabod M, Mowen K, Witherden DA, et al. Dendritic epidermal T cells regulate skin antimicrobial barrier function. *J Clin Invest* 2013;123:4364–74.
- Maruyama N, Takai T, Kamijo S, Suchiva P, Ohba M, Takeshige T, et al. Cyclooxygenase inhibition in mice heightens adaptive- and innate-type responses against inhaled protease allergen and IL-33. *Allergy* 2019;74:2237–40.
- Miyauchi K, Sugimoto-Ishige A, Harada Y, Adachi Y, Usami Y, Kaji T, et al. Protective neutralizing influenza antibody response in the absence of T follicular helper cells. *Nat Immunol* 2016;17:1447–58.
- Muschawek A, Petermann F, Korn T. IL-1β and IL-23 promote extrathymic commitment of CD27⁺CD122⁺ γδ T cells to γδT17 cells. *J Immunol* 2017;199:2668–79.
- Na H, Lim H, Choi G, Kim BK, Kim SH, Chang YS, et al. Concomitant suppression of Th2 and Th17 cell responses in allergic asthma by targeting retinoic acid receptor-related orphan receptor γt. *J Allergy Clin Immunol* 2018;141:2061–73.
- Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing

- suppression of allergic cellular and humoral responses. *Immunity* 2002;17:375–87.
- Nakagawa S, Matsumoto M, Katayama Y, Oguma R, Wakabayashi S, Nygaard T, et al. Staphylococcus aureus virulent PSM α peptides induce keratinocyte alarmin release to orchestrate IL-17-dependent skin inflammation. *Cell Host Microbe* 2017;22:667–667.e5.
- Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Nakamizo S, Moniaga CS, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. *J Invest Dermatol* 2014;134:2122–30.
- Nakamura T, Hirasawa Y, Takai T, Mitsuishi K, Okuda M, Kato T, et al. Reduction of skin barrier function by proteolytic activity of a recombinant house dust mite major allergen Der f 1. *J Invest Dermatol* 2006;126:2719–23.
- Nielsen MM, Lovato P, MacLeod AS, Witherden DA, Skov L, Dyring-Andersen B, et al. IL-1 β -dependent activation of dendritic epidermal T cells in contact hypersensitivity. *J Immunol* 2014;192:2975–83.
- Nishioka I, Takai T, Maruyama N, Kamijo S, Suchiva P, Suzuki M, et al. Airway inflammation after epicutaneous sensitization of mice requires protease activity of low-dose allergen inhalation. *J Allergy Clin Immunol* 2018;141:2271–3.e7.
- Oboki K, Ohno T, Kajiura N, Arae K, Morita H, Ishii A, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* 2010;107:18581–6.
- Ochi H, Takai T, Shimura S, Maruyama N, Nishioka I, Kamijo S, et al. Skin treatment with detergent promotes protease allergen-dependent epicutaneous sensitization in a manner different from tape stripping in mice. *J Invest Dermatol* 2017;137:1578–82.
- Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *J Allergy Clin Immunol* 2009;124:485–93.e1.
- Papotto PH, Ribot JC, Silva-Santos B. IL-17 $^{+}$ $\gamma\delta$ T cells as kick-starters of inflammation. *Nat Immunol* 2017;18:604–11.
- Rickel EA, Siegel LA, Yoon BR, Rottman JB, Kugler DG, Swart DA, et al. Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J Immunol* 2008;181:4299–310.
- Scanlon ST, McKenzie AN. Type 2 innate lymphoid cells: new players in asthma and allergy. *Curr Opin Immunol* 2012;24:707–12.
- Shimura S, Takai T, Iida H, Maruyama N, Ochi H, Kamijo S, et al. Epicutaneous allergic sensitization by cooperation between allergen protease activity and mechanical skin barrier damage in mice. *J Invest Dermatol* 2016;136:1408–17.
- Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat Immunol* 2008;9:310–8.
- Song X, Zhu S, Shi P, Liu Y, Shi Y, Levin SD, et al. IL-17RE is the functional receptor for IL-17C and mediates mucosal immunity to infection with intestinal pathogens. *Nat Immunol* 2011;12:1151–8.
- Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 2003;112(Suppl. 6):S118–27.
- Su Y, Huang J, Zhao X, Lu H, Wang W, Yang XO, et al. Interleukin-17 receptor D constitutes an alternative receptor for interleukin-17A important in psoriasis-like skin inflammation. *Sci Immunol* 2019;4:eaau9657.
- Takai T, Ikeda S. Barrier dysfunction caused by environmental proteases in the pathogenesis of allergic diseases. *Allergol Int* 2011;60:25–35.
- Tang H, Cao W, Kasturi SP, Ravindran R, Nakaya HI, Kundu K, et al. The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. *Nat Immunol* 2010;11:608–17.
- Toy D, Kugler D, Wolfson M, Vanden Bos T, Gurgel J, Derry J, et al. Cutting edge: interleukin 17 signals through a heteromeric receptor complex. *J Immunol* 2006;177:36–9.
- Veldhoen M. Interleukin 17 is a chief orchestrator of immunity. *Nat Immunol* 2017;18:612–21.
- Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 1999;104:123–33.
- Yoshiki R, Kabashima K, Honda T, Nakamizo S, Sawada Y, Sugita K, et al. IL-23 from Langerhans cells is required for the development of imiquimod-induced psoriasis-like dermatitis by induction of IL-17A-producing $\gamma\delta$ T cells. *J Invest Dermatol* 2014;134:1912–21.

SUPPLEMENTARY MATERIALS

SUPPLEMENTARY DISCUSSION

Possible mechanisms for the IL-17A-mediated enhancement of the responses in the subcutaneous papain model

The subcutaneous (s.c.) papain administration induced skin IL-17A gene expression (Figure 3a: 4 hours) and IL-17A⁺ cells in draining lymph nodes (DLNs) (Figure 6a: 24 hours; Figure 6c: 48 hours) in the early phase, whereas it did not induce T helper (Th)17 cells in the DLNs in the late phase (day 10) (Kamijo et al., 2016). This study showed that s.c. recombinant IL (rIL)-17A enhanced papain-specific type 2 responses (Figure 5 and Supplementary Figure S4b), and Nakajima et al. (2014) reported enhancing effect of IL-17A on the in vitro culture of naive CD4⁺ T cells in a Th2-skewing condition. The s.c. papain-induced skin eosinophilic inflammation and serum papain-specific IgE and IgG1 production (Figure 3 and our previous report [Kamijo et al., 2016]) along with an increase of T follicular helper cells (Supplementary Figure S5) and Th2-dominant CD4⁺ T-cell development in the DLNs (Figure 3e) were observed in the late phase (after 1 week or more). Thus, we considered that the innate IL-17A enhanced the papain-induced Th2 responses, contributing to the papain-specific IgE and IgG1 production and skin eosinophilia in the s.c. papain model, although we cannot exclude the possibility of Th2-independent mechanisms such as direct activation of B cells by innate IL-17A because coadministration of rIL-17A and ovalbumin (OVA) resulted in OVA-specific IgE and IgG1 production in the absence of detectable levels of Th2 responses (Supplementary Figures S7b and S9), and B cells expressed a subunit of the receptors for IL-17A (Supplementary Figure S5). Detailed mechanisms by which IL-17A enhances type 2 responses are yet to be elucidated.

Effects of treatment with the depleting anti- $\gamma\delta$ TCR mAb

Intraperitoneal administration of the depleting anti- $\gamma\delta$ TCR mAb (GL3) decreased the production of Th cytokines by antigen-restimulated DLN cells (Supplementary Figure S11a), suggesting that $\gamma\delta$ T cells contribute to Th cell responses in DLNs. It did not affect serum papain-specific IgE and IgG1 responses at the experimental setting (Supplementary Figure S11b), which might be because of the limitation of the methodology, such as incomplete depletion and/or incomplete blocking of the function of $\gamma\delta$ T cells or contribution of non- $\gamma\delta$ T cell IL-17 producer, group 3 innate lymphoid cells for example (Kim et al., 2020). Although we confirmed the efficacy of depletion by GL3 using another clone (UC7-13D5) of anti- $\gamma\delta$ TCR antibody for detection of $\gamma\delta$ T cells (Supplementary Figure S10), it might partially reflect the internalization of $\gamma\delta$ TCR by GL3 (Koenecke et al., 2009).

SUPPLEMENTARY MATERIALS AND METHODS

In vitro stimulation of ear skin tissue

Ear lobes were isolated from naive mice and separated into the ventral and dorsal sides. Isolated ear skin tissue was submerged dermis side-down in the culture media in the presence or absence of papain (Calbiochem, San Diego, CA), rIL-17, rIL-1 β , rIL-23, or recombinant TNF- α (BioLegend, San Diego, CA), the concentrations of which are indicated in the

Figure legends. After 24 hours, the culture supernatant was collected for the measurement of cytokines by ELISA.

The s.c. administration of antigens and rIL-17A

Papain, E-64-treated papain (E64-papain) (E-64, Peptide Institute, Osaka, Japan) (Kamijo et al., 2013; Varughese et al., 1989), endotoxin-free OVA (Hyglos GmbH, Munich, Germany), or rIL-17A was subcutaneously injected into the ear skin. The amounts administered are indicated in the Figure legends. Serum samples were collected for the measurement of antibodies by ELISA. Paraffin-embedded sections of the ear skin specimens were stained with Giemsa for histological analysis. DLN cells were isolated for in vitro restimulation with E64-papain or OVA. Total RNA in the skin was extracted for qPCR. The DLN cells and the skin cells were isolated for flow cytometric analysis.

Antigen restimulation of skin DLN cells

Cervical lymph nodes were aseptically recovered. Single-cell suspensions were prepared by gently teasing apart the tissue in culture media (RPMI-1640 medium [Sigma-Aldrich, Saint Louis, MO] supplemented with 2 mM L-glutamine, 10% [v/v] heat-inactivated fetal bovine serum, 10 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, 0.05 mM 2-mercaptoethanol, and antibiotics), that is, the homogenization of DLNs by sandwiching and crushing them between two glass slides and passage through EASYstrainer (70- μ m pore size, Greiner Bio-One, Tokyo, Japan). After centrifugation at 300g at 4 °C for 5 minutes, cells were resuspended in ammonium-chloride-potassium buffer at room temperature (2 ml, 2 minutes) to deplete red blood cells. DLN cells (5×10^5 cells/200 μ l per well) were cultured in the presence of E64-papain (30 μ g/ml) or endotoxin-free OVA (30 μ g/ml) in culture media using flat-bottomed 96-well cell culture plates at 37 °C. After 72 hours, the culture supernatant was collected for the measurement of cytokines by ELISA.

In vitro culture of a lung explant

A lung explant was prepared and cultured as described by Halim et al. (2012) with minor modifications. In brief, mice were anesthetized with an intraperitoneal injection of pentobarbital (Somnopentyl; Kyoritsu, Tokyo, Japan). Lungs were instilled with 1.5 ml of 1% low-melting point agar (Agarose XP, Nippon Gene, Tokyo, Japan) in PBS at 37 °C using an 18G catheter and cooled on ice. Lungs were sliced by scalpels into ~1 mm thick sections, and two slices of the lung explant were cultured for 24 hours in 300 μ l of culture media in the presence or absence of papain (100 μ g/ml) in 24-well cell culture plates. Culture supernatants were subjected to measurements of soluble ST2 (sST2) and IL-33 by ELISA.

Intraperitoneal administration of a depleting anti-mouse $\gamma\delta$ TCR antibody

In the depletion of $\gamma\delta$ TCR⁺ cells, 100 μ g/200 μ l PBS per mouse of an anti-mouse $\gamma\delta$ TCR mAb (clone: GL3, BioLegend) or hamster IgG (BioLegend) was administered intraperitoneally to wild-type C57BL/6J mice on days -3, 0, 3, 7, and 13. Depletion was confirmed by flow cytometry on peripheral blood samples on day 12 using the anti-mouse $\gamma\delta$ TCR mAb (clone: UC7-13D5, BioLegend).

Flow cytometry and antibodies

In the detection of IL-17RA⁺ B cells, IL-17RA⁺ CD4⁺ T cells, IL-17RA⁺ T follicular helper (Tfh) cells, $\gamma\delta$ TCR⁺ cells and Thy1.2⁺ cells, whole-skin cells or DLN cells were counted, and the cell surface was stained with FITC-conjugated anti-mouse CD19 (clone: 1D3, TONBO, Kobe, Japan), PE-Cy7-conjugated anti-mouse CD3 (clone: 145-2C11, BioLegend), PerCP-Cy5.5-conjugated anti-mouse CD4 (clone: RM4-5, BD Biosciences, Franklin Lakes, NJ), allophycocyanin (APC)/Cy7-conjugated anti-mouse CD4 (clone: GK1.5, BioLegend), FITC-conjugated anti-mouse PD-1 (clone: 29F.1A12, BioLegend), biotinylated anti-mouse CXCR5 (clone: L138D7, BioLegend), and streptavidin-APC (BioLegend); PE-conjugated anti-mouse IL-17RA (clone: PAJ-17R, Invitrogen, Carlsbad, CA) or PE-conjugated rat IgG2a isotype control (Thermo Fisher, Waltham, MA); FITC-conjugated anti-mouse Thy1.2 (clone: 53-2.1, Thermo Fisher), FITC-conjugated anti-mouse V γ 2 (V γ 4 in nomenclature of Heilig and Tonegawa [1986], clone: UC3-10A6, BioLegend), PE-conjugated anti-mouse V γ 3 (V γ 5 in nomenclature of Heilig and Tonegawa [1986], clone: 536, BioLegend), PE-conjugated anti-mouse CD45 (clone: 30-F11, BioLegend), PerCP-conjugated anti-mouse CD45 (clone: 30-F11, Thermo Fisher), APC-conjugated anti-mouse $\gamma\delta$ TCR (clone: GL3, BioLegend), and APC-conjugated anti-mouse CD3e (clone: 145-2C11, TONBO). In the intracellular staining, cells were stimulated with phorbol 12-myristate 13-acetate (50 ng/ml) and ionomycin (1 μ g/ml) in the presence of BD Golgi plug (BD Biosciences) for 6 hours. After surface staining, washing, fixation, and permeabilization with BD Cytfix/Cytoperm reagents (BD Biosciences), intracellular IL-4, IL-17A, IFN- γ , GATA-3, ROR γ t, and T-bet were stained with PE-conjugated anti-mouse IL-4 (clone: 11B11, BD Biosciences) or PE-conjugated rat IgG1 isotype control (BioLegend), FITC- or APC-conjugated anti-mouse IL-17A (clone: eBio17B7, Thermo Fisher) or a FITC- or APC-conjugated rat IgG2a isotype control (BD Biosciences), FITC-conjugated anti-mouse IFN- γ (clone: XMGI.2, BD Biosciences) or FITC-conjugated rat IgG1 isotype control (BD Biosciences), eFluor 660-conjugated anti-mouse GATA-3 (clone: TWAJ, Invitrogen) or eFluor 660-conjugated rat IgG2b isotype control (Thermo Fisher), PerCP-eFluor 710-conjugated anti-mouse ROR γ t (clone: B2D, Invitrogen) or PerCP-eFluor 710-conjugated rat IgG1, and PE-conjugated anti-human or mouse T-bet (clone: 4B10, BioLegend) or PE-conjugated mouse IgG1 isotype control (BioLegend). Acquisition and analysis were performed using a FACSCalibur or FACSVerse cell sorter (BD Biosciences) and Cell Quest or FlowJo software (BD Biosciences).

Lineage[−]CD25⁺CD44⁺ innate lymphoid cells in the ear skin were analyzed as follows: Single-cell suspensions were prepared from ear lobes digested with collagenase type 2 (1.6 mg/ml; Worthington Biochemical, Lakewood, NJ), hyaluronidase (1.2 mg/ml; Sigma-Aldrich), and DNase I (100 μ g/ml; Roche Life Science, Indianapolis, IN) at 37 °C for 60 minutes with gentle shaking. Whole-skin cells were stained and analyzed using the following antibodies: PE-Cy7-conjugated anti-mouse lineage markers (B220 [clone RA3-6B2], CD3 [clone 145-2C11], CD11b [clone M1/70], Ter119 [clone TER-119], CD16/CD32 [clone 2.4G2] [BD], CD14 [clone Sa2-8], CD49b [clone DX5] [Thermo Fisher],

CD11c [clone N418], and Fc ϵ RI α [clone MAR-1]), APC-conjugated anti-mouse CD25 (clone PC61), FITC-conjugated anti-mouse and human CD44 (clone IM7) (BioLegend), PE-conjugated anti-mouse c-Kit (clone 2B8), PE-conjugated anti-mouse CD127 (clone A7R34) (Thermo Fisher), PE-conjugated anti-mouse CD45 (clone 30-F11) (BioLegend), PE-conjugated anti-mouse Sca-1 (clone E13-161.7), PE-conjugated anti-mouse Thy1.2 (clone 30-H12) (BD Bioscience), biotinylated anti-mouse T1/ST2 (clone DJ8) (MD Bio-products, Zürich, Switzerland), and streptavidin-PE (BD Biosciences).

Preparation of epidermal sheets

In vivo experiments, papain (10 μ g per ear) was subcutaneously administered to both ears of mice. In vitro experiments, ear lobes were isolated, separated into ventral and dorsal pieces, and stimulated in vitro with papain for 1 hour. Ear lobes were incubated in 0.5 M ammonium thiocyanate (Sigma-Aldrich) at 37 °C for 30 minutes. Epidermal sheets were peeled with forceps and transferred to the wells of 24-well cell culture plates containing anti-mouse CD16/CD32 (clone: 2.4G2, TONBO). After being incubated at room temperature for 30 minutes, epidermal sheets were washed and stained with FITC- or PE-conjugated anti-mouse Thy1.2 mAb (clone: 53-2.1, Thermo Fisher) at room temperature for 1 hour. Then, stained epidermal sheets were washed 6 times and mounted onto slide glasses for confocal microscopic analysis. In some experiments, ear epidermal sheets were stained with FITC-conjugated anti-mouse I-A/I-E mAb (clone: M5/114.15.2, Thermo Fisher) for the detection of Langerhans cells.

Microscopic analysis

Microscopic images were acquired using LSM700 microscope equipped with an AxioCam MRc camera (Carl Zeiss, Jena, Germany). Confocal microscopic images were acquired using Zen software (Carl Zeiss). The acquisition and analysis of microscopic images other than confocal images were performed using AxioVision software (Carl Zeiss). All microscopic analysis was performed at room temperature.

qPCR

Ear lobes or lungs were excised and homogenized in TRI Reagent (Molecular Research Center, Cincinnati, OH) using TissueLyser II (Qiagen, Hilden, Germany). Total RNA was extracted from homogenized skin or lung tissue specimens using an RNeasy Plus Micro Kit (Qiagen). First-strand cDNA was synthesized from total RNA using a ReverTra Ace qPCR RT Master Mix (TOYOBO, Osaka, Japan). Real-time qPCR was performed with the TaqMan method using a StepOne-Plus real-time PCR system (Applied Biosystems, Foster City, CA). The mRNA levels of the target genes were shown as values relative to the gene expression levels of the endogenous control (*GAPDH*). The following probes were purchased from Applied Biosystems: *IL-33* (Mm00505403_m1), *sST2* (Mm04176272_s1), *ST2L* (Mm01233982_m1), *IL-17A* (Mm00439618_m1), and *GAPDH* (Pre-Developed TaqMan Assay Reagents Mouse *GAPDH*).

Cytokine ELISA

Cytokine concentrations were measured with ELISA kits (Quantikine or DuoSet, R&D Systems, Minneapolis, MN) except for the measurement of sST2. The following procedure

was employed to measure sST2: sST2 was captured on plates coated with 2.5 µg/ml of purified anti-mouse ST2 (clone: #245714; R&D Systems) at room temperature for 15 hours, and then blocked with ImmunoBlock (DS Pharma, Osaka, Japan) at room temperature for 60 minutes. An incubation with culture supernatants was conducted at room temperature for 2 hours. The plates were developed with 0.4 µg/ml of biotin-conjugated goat polyclonal anti-mouse ST2 (at room temperature for 2 hours; R&D Systems) plus horseradish peroxidase (HRP)-conjugated streptavidin (at room temperature for 30 minutes, BD Biosciences). Culture supernatants and detection antibodies were diluted with PBS containing 0.05% Tween 20 and 5% ImmunoBlock. The BD OptEIA 3,3',5,5' tetramethylbenzidine Substrate Reagent Set (BD Biosciences) was used as a substrate at room temperature for 20 minutes in the dark, and color development was stopped using 2N sulphuric acid solution.

ELISA for serum total IgE and papain- or OVA-specific antibodies

Serum IgE was captured on plates coated with 2 µg/ml of purified anti-mouse IgE (clone: R35-72; BD Biosciences) for total IgE or 10 µg/ml of papain for papain-specific IgE, IgG1, IgG2c, and IgM or 1 mg/ml of OVA (Sigma-Aldrich, grade V) for OVA-specific IgE, IgG1, IgG2c, and IgM at 4 °C for 15 hours and blocked with ImmunoBlock (DS Pharma) at 37 °C for 90 minutes. Serum dilutions were 1:200 to detect IgE, IgG2c, and IgM and 1:5,000 for IgG1 except for [Supplementary Figures S4, S9a, and S11b](#), in which serum dilutions were 1:200 for total IgE, papain- or OVA-specific IgG2c and IgM, 1:50 for papain- or OVA-specific IgE, and 1:2,500 for papain- or OVA-specific IgG1. An incubation with serum was conducted at 37 °C for 1.5 hours for total IgE and papain- or OVA-specific IgG1, IgG2c, and IgM and at 4 °C for 15 hours for papain- or OVA-specific IgE. Plates were developed with HRP-conjugated anti-mouse IgE (at 37 °C for 1.5 hours, clone: LO-ME2; Technopharm Biotechnology, Paris, France), biotin-conjugated anti-mouse IgE (at room temperature for 1 hour, clone: R35-118; BD) or biotin-conjugated anti-mouse IgM (at 37 °C for 1.5 hours, goat polyclonal; Southern Biotech, Birmingham, AL) plus HRP-conjugated streptavidin (at room temperature for 30 minutes, BD Biosciences), or HRP-conjugated anti-mouse IgG1 (at 37 °C for 1.5 hours, clone: X56; BD Biosciences) or HRP-conjugated anti-mouse IgG2a and IgG2c (at 37 °C for 1.5 hours, clone: R19-15; BD) for the detection of total IgE, papain- or OVA-specific IgE, papain- or OVA-specific IgM, papain- or OVA-specific IgG1, or papain- or OVA-specific IgG2c, respectively. Sera and detection antibodies were diluted with PBS containing 0.05% Tween 20 and 5% ImmunoBlock. The BD OptEIA 3,3',5,5' tetramethylbenzidine Substrate Reagent Set (BD Biosciences) was used as a

substrate at room temperature for 20 minutes in the dark, and color development was stopped using 2N sulphuric acid solution. In the figures showing antigen-specific antibodies, the average optical density of the vehicle group was set to zero.

Nomenclature of the murine Vγ gene

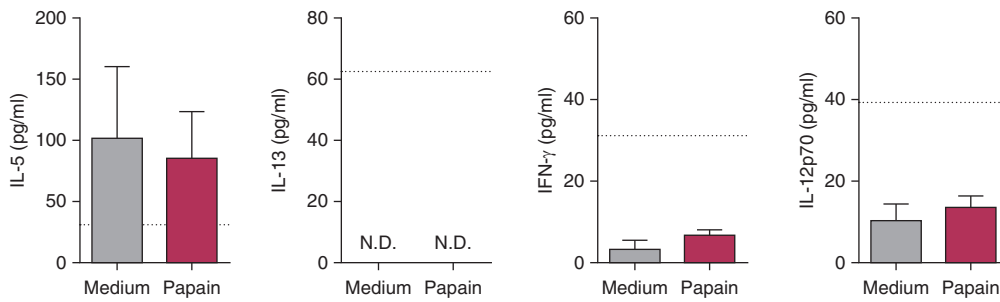
Nomenclature by Heilig and Tonegawa ([Heilig and Tonegawa, 1986](#)) is used to describe the murine Vγ gene throughout this study.

Numbers of replicates

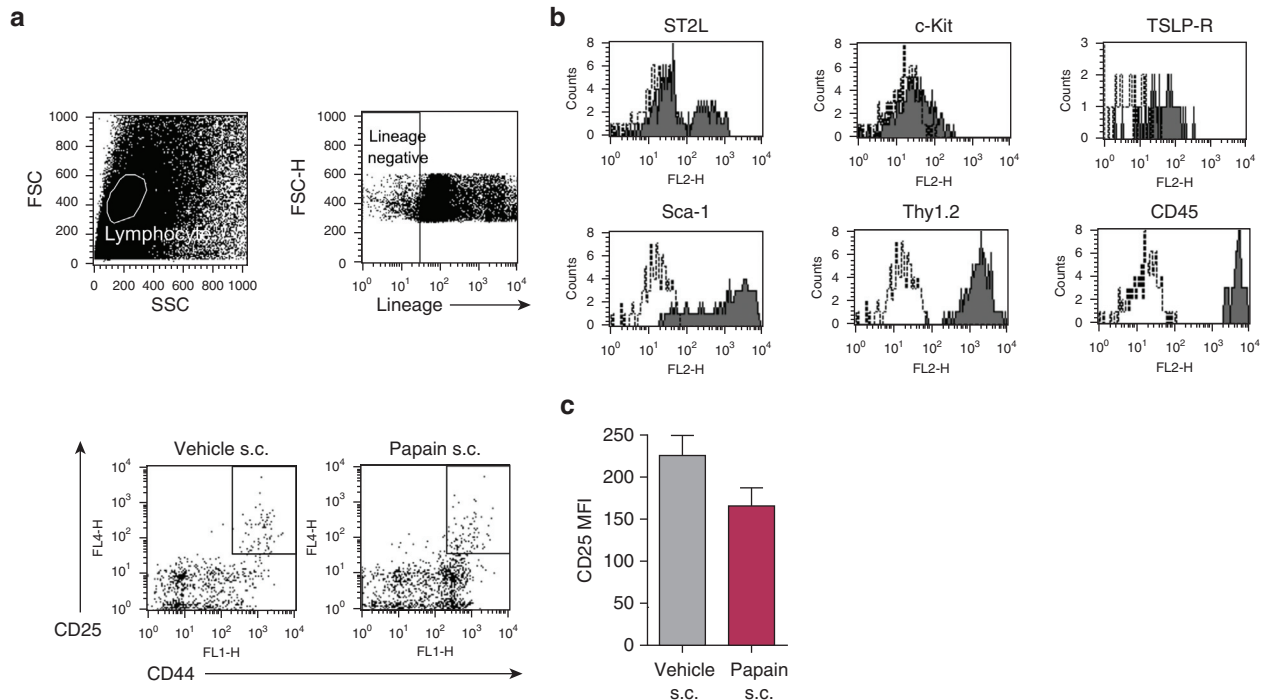
The numbers of replicates used in the experimental groups were indicated in Figure legends except for [Figures 3 and 6](#). For [Figure 3](#), data indicate the means ± SD of six ([Figure 3a and d](#), papain-specific IgE and IgG1 papain), five ([Figure 3d](#), total IgE wild-type vehicle and IL-17A^{-/-}), four ([Figure 3d](#), total IgE wild-type papain, and e), or seven ([Figure 3d](#), papain-specific IgE and IgG1 wild-type vehicle) mice and ten ([Figure 3b](#), wild-type vehicle and IL-17A^{-/-}) or eleven ([Figure 3b](#), wild-type papain) microscopic fields of Giemsa-stained ear skin specimens from five mice per group. For [Figure 6](#), data indicate the means of nine ([Figure 6a](#)) or six ([Figure 6c](#)) mice or the means ± SD of three ([Figure 6b](#)) mice and 28 microscopic fields from four ear lobes per group ([Figure 6d](#)) and are representative of three ([Figure 6a, b, and d](#)) or two ([Figure 6c](#)) independent experiments with similar results.

SUPPLEMENTARY REFERENCES

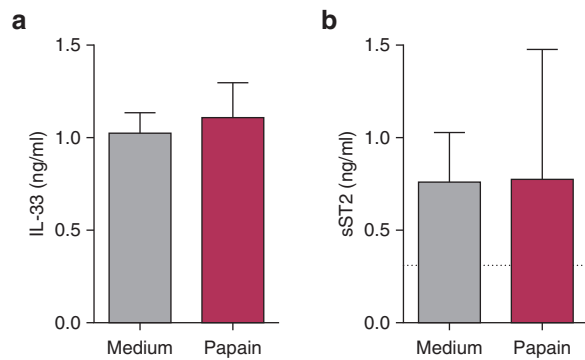
- Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012;36:451–63.
- Heilig JS, Tonegawa S. Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature* 1986;322:836–40.
- Kamijo S, Suzuki M, Hara M, Shimura S, Ochi H, Maruyama N, et al. Subcutaneous allergic sensitization to protease allergen is dependent on mast cells but not IL-33: distinct mechanisms between subcutaneous and intranasal routes. *J Immunol* 2016;196:3559–69.
- Kamijo S, Takeda H, Tokura T, Suzuki M, Inui K, Hara M, et al. IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation. *J Immunol* 2013;190:4489–99.
- Kim MH, Jin SP, Jang S, Choi JY, Chung DH, Lee DH, et al. IL-17A-producing innate lymphoid cells promote skin inflammation by inducing IL-33-driven type 2 immune responses. *J Invest Dermatol* 2020;140:827–37.e9.
- Koenecke C, Chennupati V, Schmitz S, Malissen B, Förster R, Prinz I. In vivo application of mAb directed against the gammadelta TCR does not deplete but generates “invisible” gammadelta T cells. *Eur J Immunol* 2009;39:372–9.
- Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Nakamizo S, Moniaga CS, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. *J Invest Dermatol* 2014;134:2122–30.
- Varughese KI, Ahmed FR, Carey PR, Hasnain S, Huber CP, Storer AC. Crystal structure of a papain-E-64 complex. *Biochemistry* 1989;28:1330–2.



Supplementary Figure S1. In vitro cytokine production from papain-stimulated skin tissue. Supplementary Figure S1 is supplementary for Figure 1. Data indicate the means $\pm$ SD of nine (IL-5, IL-13, and IFN- γ) or six (IL-12p70) ears. Detection limits are indicated by dotted lines. Data are representative of four (IL-5, IL-13, and IFN- γ) or two (IL-12p70) independent experiments with similar results.

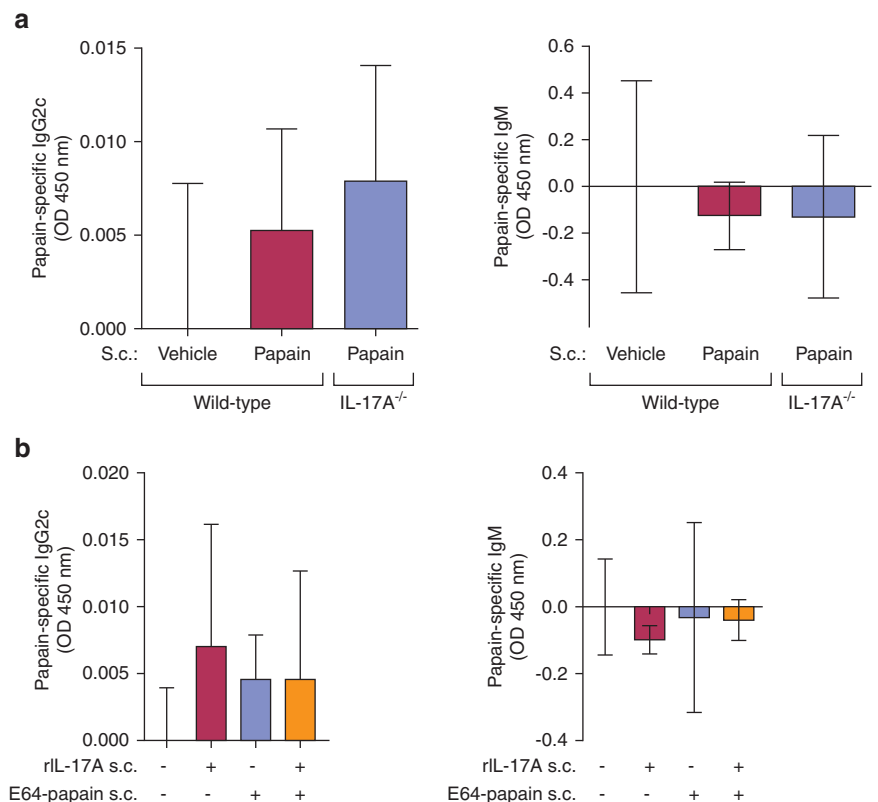


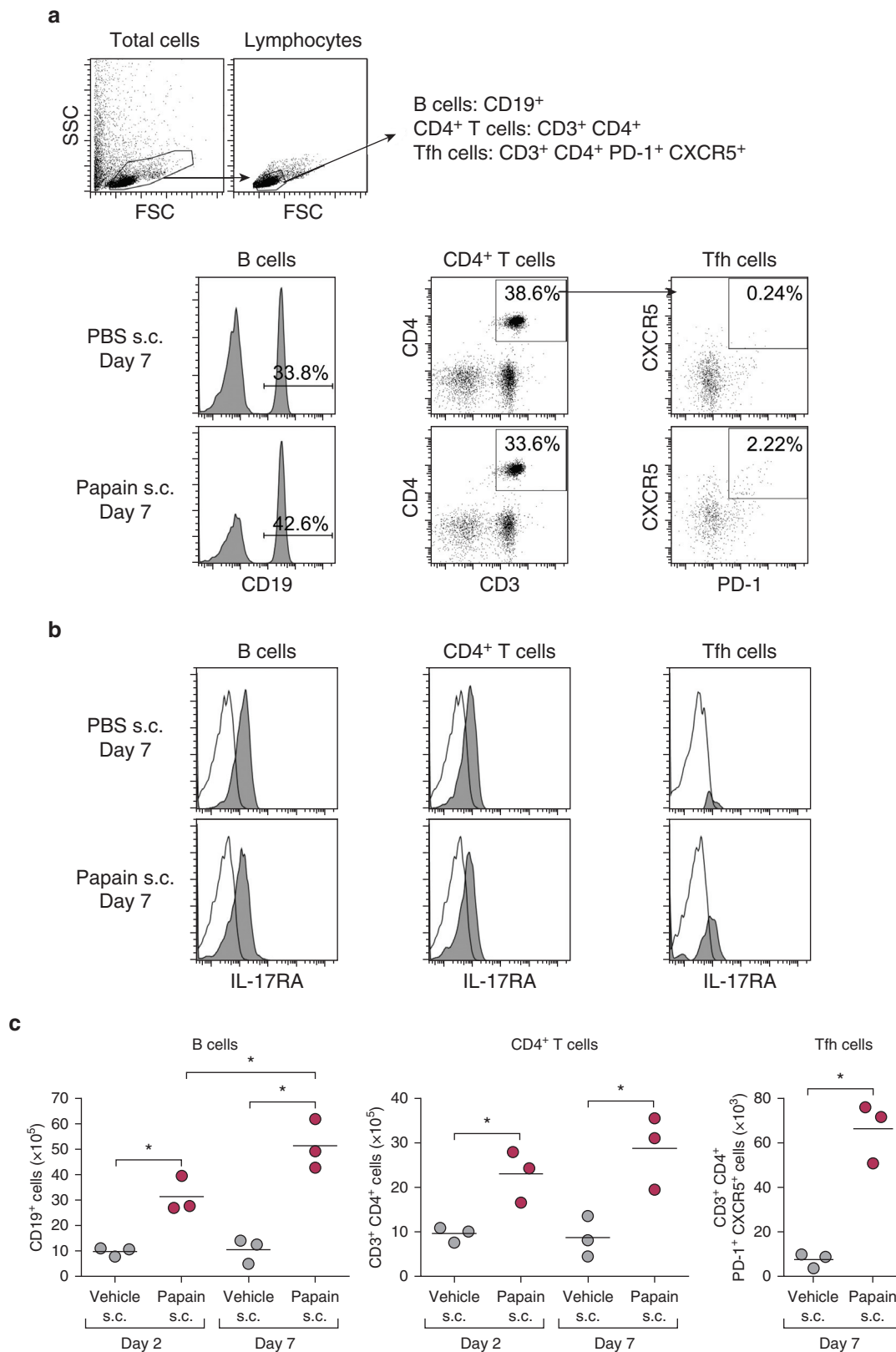
Supplementary Figure S2. The s.c. administration of papain failed to activate skin ILC2s. Papain (10 μ g per ear) was subcutaneously administered to both ears of wild-type mice. (a–c) After 3 hours, a single-cell suspension was prepared from the ear skin. (a) Detection and (b) surface marker expression profile of lineage⁻CD25⁺CD44⁺ lymphoid cells in the ear skin tissue. Shaded histograms show staining with target-specific mAbs, whereas open histograms show staining with isotype control mAbs. (c) MFI of CD25 in lineage⁻CD25⁺CD44⁺ lymphoid cells. Data indicate means $\pm$ SD of (c) three ears. Data are representative of (a, b) three or (c) two independent experiments with similar results. FSC, forward scatter; ILC2, group 2 innate lymphoid cell; MFI, mean fluorescence intensity; s.c., subcutaneous; SSC, side scatter; TSLP, thymic stromal lymphopoietin.



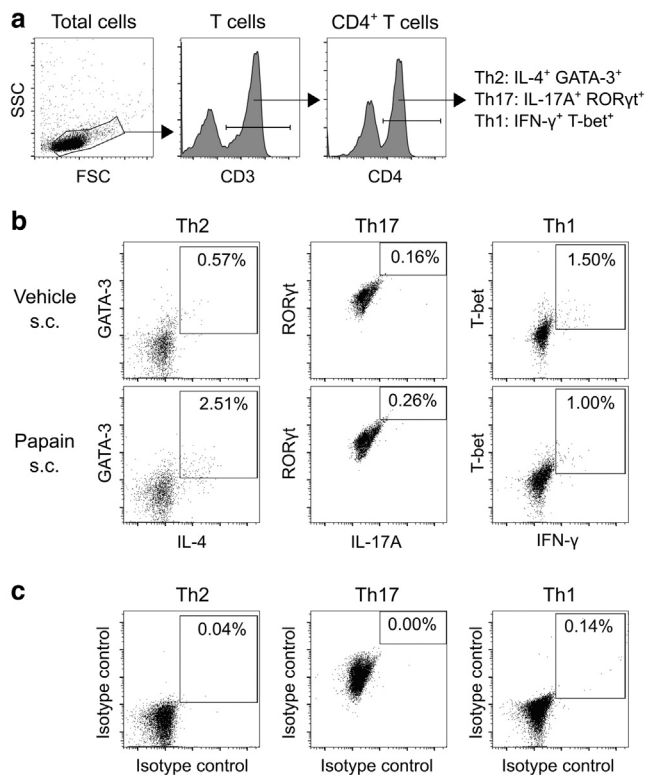
Supplementary Figure S3. Production of IL-33 and sST2 by a lung explant culture. Lung explants were cultured in the presence or absence of papain (100 µg/ml) for 24 hours. (a) IL-33 and (b) sST2 concentrations in culture supernatants were measured by ELISA. Data indicate the means $\pm$ SD of six lung explant cultures. Data are representative of two independent experiments with similar results. * $P < 0.05$ by t -test. sST2, soluble ST2.

Supplementary Figure S4. Serum levels of papain-specific IgG2c and IgM. Supplementary Figure S4a and b are supplementary for Figures 3d and 5a, respectively. (a) Papain (10 µg per ear) was subcutaneously administered to both ears of wild-type or *IL-17A*^{-/-} mice on day 0. (b) rIL-17A (1 µg per ear) and E64-papain (5 µg per ear) were administered to wild-type mice on day 0. Sera were collected on day (a) 11 or (b) 14, and papain-specific IgG2c and IgM were measured by ELISA. Data indicate the means $\pm$ SD of (a) ten (wild-type vehicle), eight (wild-type papain), seven (*IL-17A*^{-/-}), or (b) five mice. Data are representative of three independent experiments with similar results. * $P < 0.05$ by one-way ANOVA with Tukey's post hoc test. OD, optical density; rIL, recombinant IL; s.c., subcutaneous.

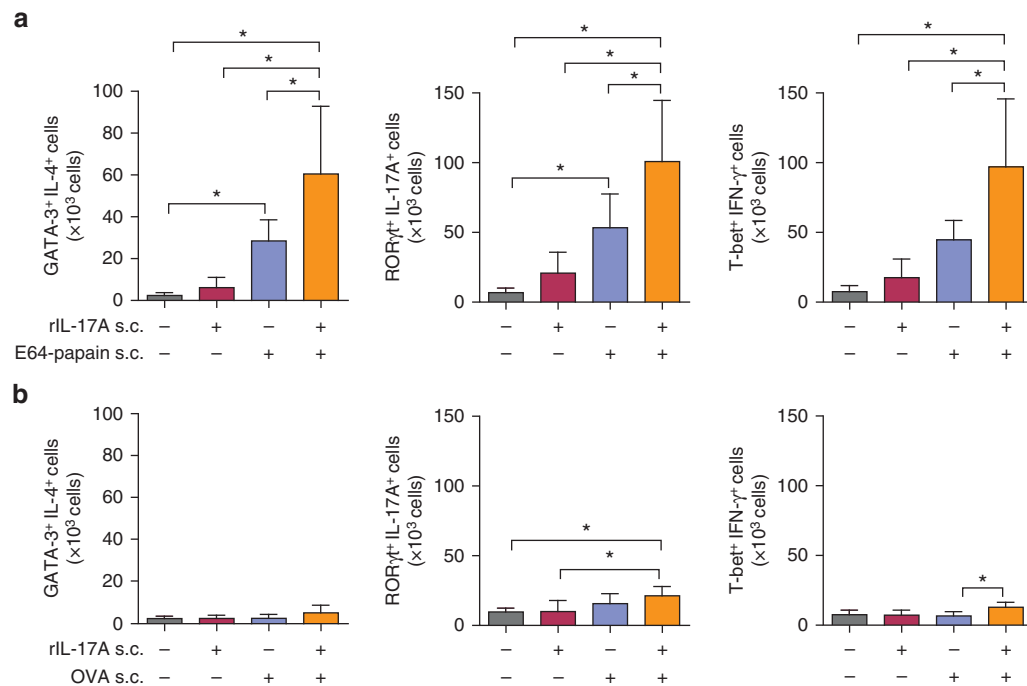




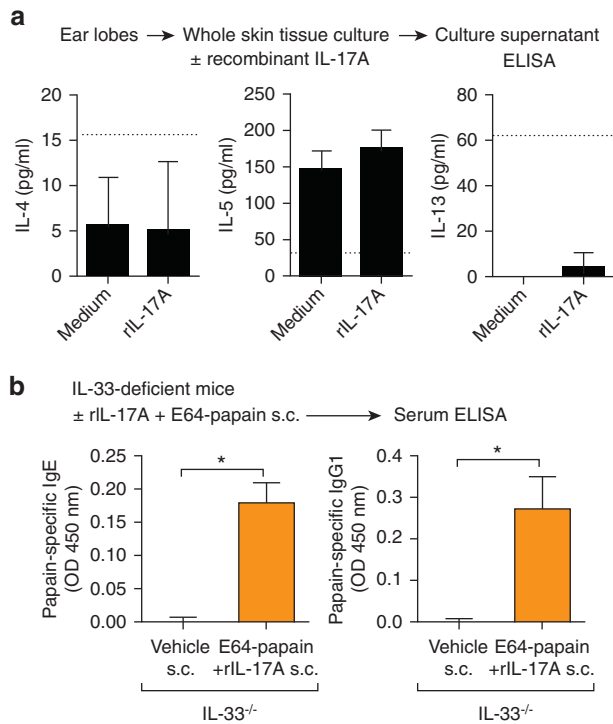
Supplementary Figure S5. B cells, CD4⁺ T cells, and Tfh cells in DLNs express IL-17RA and expands in response to s.c. papain. Papain (10 μ g per ear) was subcutaneously administered to both ears of wild-type mice. (a) Gating strategies, (b) IL-17RA expression, and (c) absolute numbers of B cells, CD4⁺ T cells, and Tfh cells in DLNs on day 2 or day 7 after s.c. papain injection. Shaded and open histograms show fluorescence intensity of target-specific mAbs and isotype control mAbs, respectively. Data indicate the means of three mice and are representatives of two independent experiments with similar results. * $P < 0.05$ by t -test. DLN, draining lymph node; FSC, forward scatter; PD-1, programmed cell death 1; s.c., subcutaneous; SSC, side scatter; Tfh, T follicular helper.



Supplementary Figure S6. Gating strategies for Th2, Th17, and Th1 cells in DLNs. Supplementary Figure S6 is supplementary for Figure 3e and Supplementary Figure S7. Papain (10 μg per ear) was subcutaneously administered to both ears of wild-type mice. (a) Gating strategy for CD3⁺ CD4⁺ Th cells in DLNs on day 10. (b) CD3⁺ CD4⁺ Th cells were further subdivided into Th2 (GATA-3⁺ IL-4⁺), Th17 (RORγt⁺ IL-17A⁺), and Th1 (T-bet⁺ IFN-γ⁺) cells. (c) Fluorescence levels of DLN cells stained with isotype-matched control Abs. Data are representatives of two independent experiments with similar results. Ab, antibody; DLN, draining lymph node; FSC, forward scatter; s.c., subcutaneous; SSC, side scatter; Th, T helper.

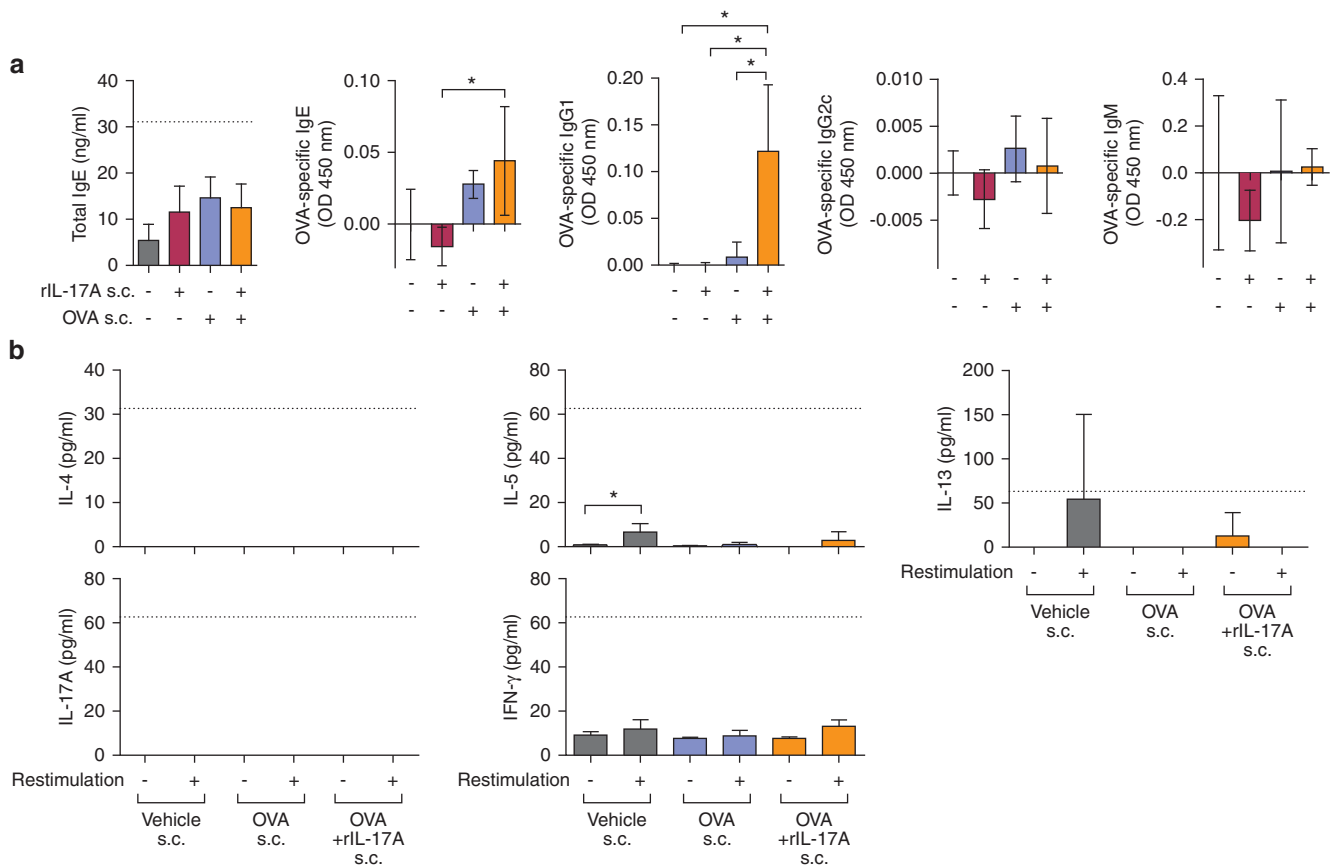


Supplementary Figure S7. Coadministration of rIL-17A and antigen enhanced DLN Th cell development. Supplementary Figures S7a and S7b are supplementary for Figure 5 and Supplementary Figure S9, respectively. rIL-17A (1 µg per ear) was subcutaneously coadministered with (a) E64-papain (10 µg per ear) or (b) OVA (5 µg per ear) to both ears on days 0 and 10. DLN cells were collected on day 11, and CD3⁺ CD4⁺ Th cell development was measured as in Supplementary Figure S6. Data indicate the means ± SD of (a) seven or (b) six mice from two independent experiments. **P* < 0.05 by one-way ANOVA with Tukey's post hoc test. DLN, draining lymph node; OVA, ovalbumin; rIL, recombinant IL; s.c., subcutaneous; Th, T helper.

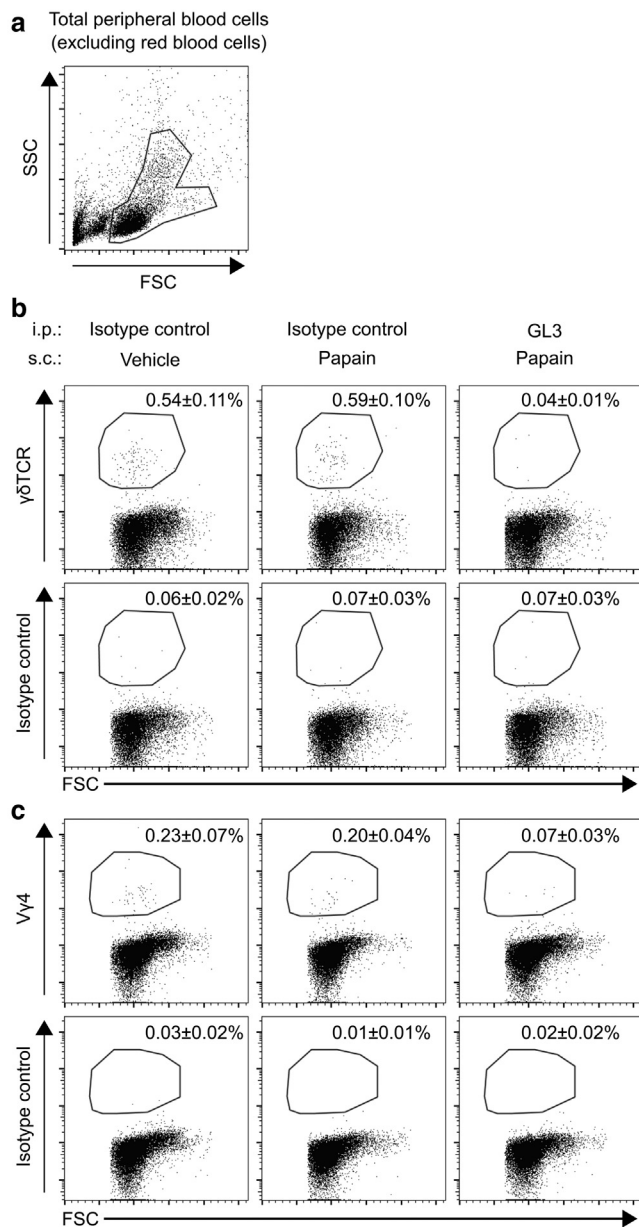


Supplementary Figure S8. rIL-17A failed to induce skin local production of Th2 cytokines in vitro but enhanced allergen-specific IgE and IgG1 production in the sera of IL-33-deficient mice.

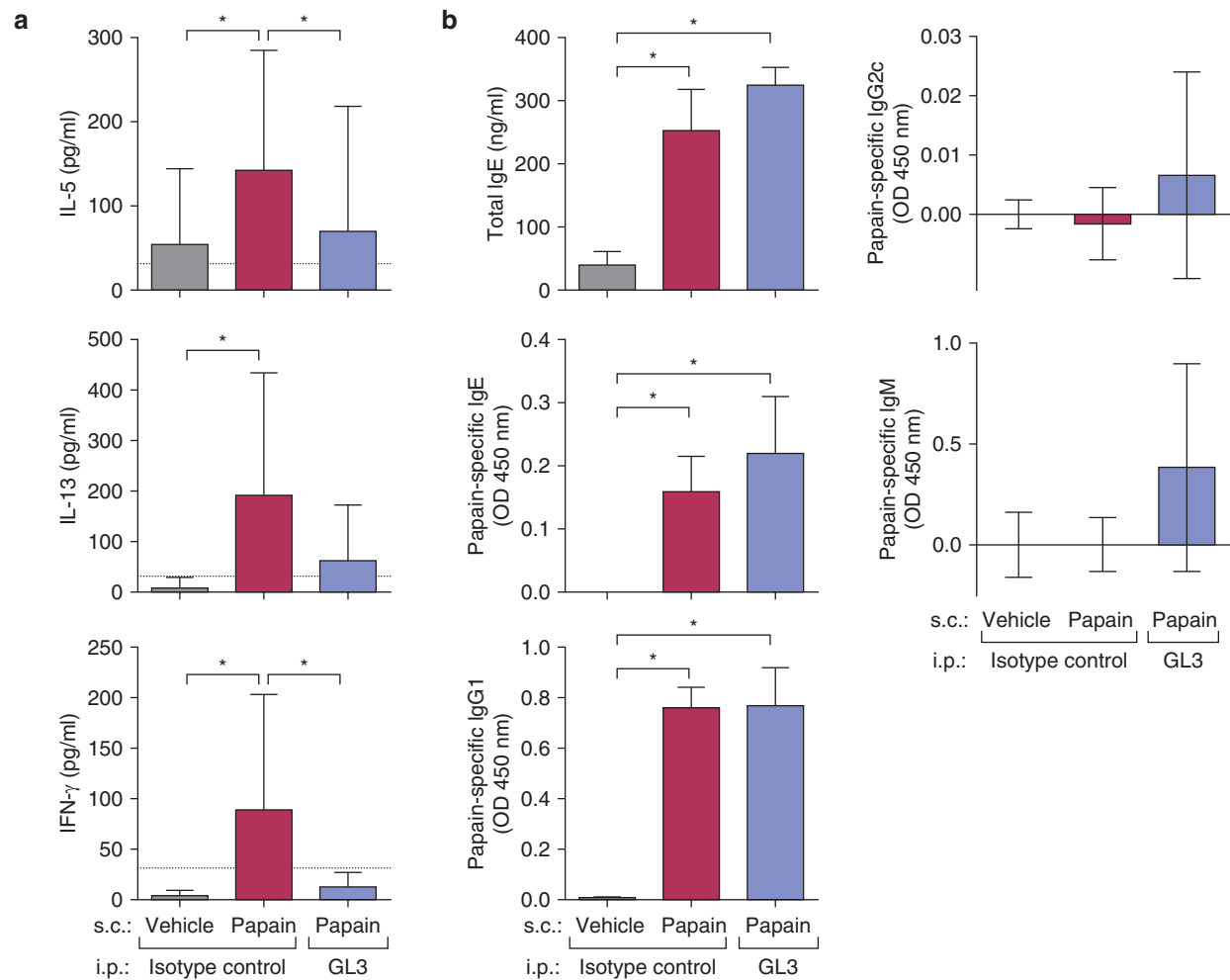
(a) The ear lobes of wild-type mice were cut, separated into ventral and dorsal sides, and incubated with or without rIL-17A (25 µg/ml) in vitro. After 24 hours, skin tissue culture supernatants were collected and cytokine concentrations were measured by ELISA. **(b)** rIL-17A (1 µg per ear) and E64-papain (5 µg per ear) were administered subcutaneously to the ear lobes of *IL-33*^{-/-} mice on day 0. Sera were collected on day 14 (papain-specific IgE) or 10 (papain-specific IgG1) and were measured by ELISA. Data indicated are the means ± SD of **(a)** three ears or **(b)** four mice and are representative of **(a)** two or **(b)** three independent experiments with similar results. Detection limits are indicated by dotted lines. **P* < 0.05 by one-way ANOVA with Tukey's post hoc test. OD, optical density; rIL, recombinant IL; s.c., subcutaneous; Th, T helper.



Supplementary Figure S9. Coadministration of rIL-17A and OVA weakly induced OVA-specific IgE and IgG1 responses in sera. (a) Antibody responses. rIL-17A (1 μ g per ear) and OVA (5 μ g per ear) were administered to wild-type mice on day 0. Sera were collected on day 14, and antibody levels were measured by ELISA. Data indicated are the means $\pm$ SD of four mice and are representative of two independent experiments with similar results. (b) Th2 responses in DLNs. rIL-17A (1 μ g per ear) was subcutaneously coadministered with OVA (10 μ g per ear) to both ears of mice on days 0 and 10. DLN cells were collected on day 11 and restimulated in vitro with OVA. Culture supernatants were collected after 72 hours and cytokine concentrations were measured by ELISA. Detection limits are indicated by dotted lines. Data indicate the means $\pm$ SD of four mice. * P < 0.05 by one-way ANOVA with Tukey's post hoc test. DLN, draining lymph node; OD, optical density; OVA, ovalbumin; rIL, recombinant IL; s.c., subcutaneous; Th, T helper.



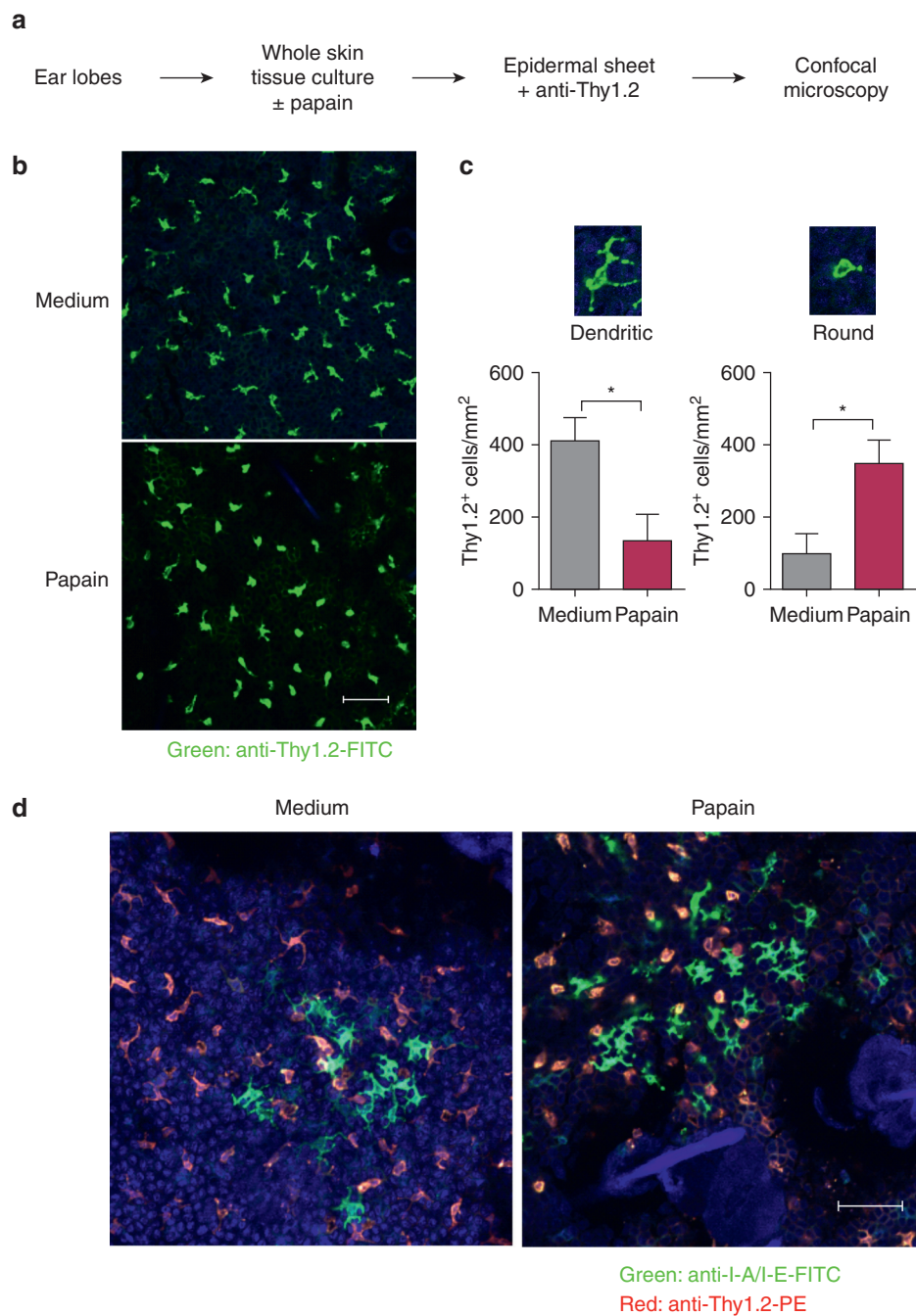
Supplementary Figure S10. Effect of a depleting anti- $\gamma\delta$ TCR antibody on $\gamma\delta$ TCR⁺ cells and V γ 4⁺ cells in peripheral blood. Wild-type mice were treated i.p. with 100 μ g per mouse of a depleting anti- $\gamma\delta$ TCR antibody (clone: GL3) or isotype control antibody on days -3, 0, 3, 7, and 13. Papain (10 μ g per ear) was subcutaneously administered to both ears on day 0. (a) FSC and SSC dot plot of peripheral blood cells after removal of red blood cells. (b) Depletion of $\gamma\delta$ TCR⁺ cells. The $\gamma\delta$ TCR⁺ cells were detected by the anti- $\gamma\delta$ TCR antibody (clone: UC7-13D5). (c) Depletion of V γ 4⁺ cells. Data indicate the means $\pm$ SD of five (Isotype control) or four (GL3) mice. Data are representative of two independent experiments with similar results. FSC, forward scatter; i.p., intraperitoneally; s.c., subcutaneous; SSC, side scatter.

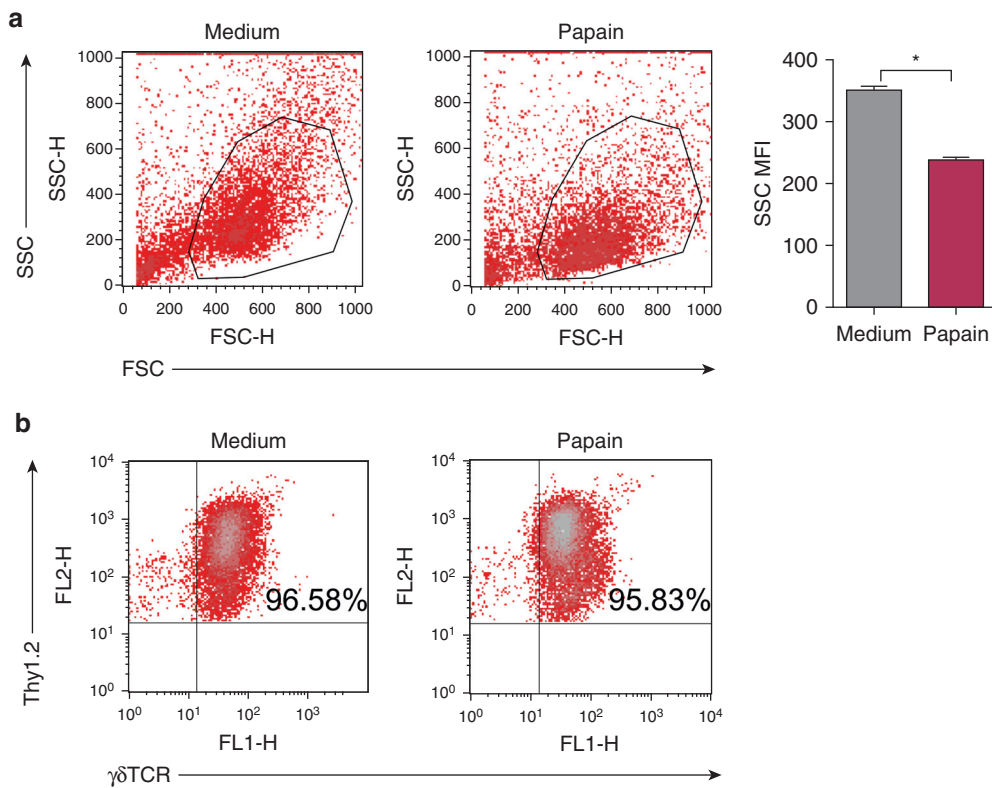


Supplementary Figure S11. Treatment with a depleting anti- $\gamma\delta$ TCR antibody reduced Th cytokine production by DLN cells but not serum antibody production in response to s.c. papain. Wild-type mice were treated i.p. with 100 μ g per mouse of a depleting anti- $\gamma\delta$ TCR antibody (clone: GL3) or isotype control antibody on days -3, 0, 3, 7, and 13. Papain (10 μ g per ear) was subcutaneously administered to both ears on day 0. (a) Th cytokine production by DLN cells isolated on day 14 and restimulated in vitro with E64-papain (30 μ g/ml) for 72 hours. Data indicate the means $\pm$ SD of nine (Isotype control) or eight (GL3) mice from two independent experiments. * P < 0.05 by Mann-Whitney U test. (b) Antibody responses in sera collected on day 12. Data indicate the means $\pm$ SD of five (Isotype control) or four (GL3) mice. * P < 0.05 by one-way ANOVA with Tukey's post hoc test. Data are representative of two independent experiments with similar results. DLN, draining lymph node; i.p., intraperitoneally; OD, optical density; s.c., subcutaneous; Th, T helper.

Supplementary Figure S12. Papain induced the activation of and morphological changes in Thy1⁺ DETCs in vitro.

(a) Experimental procedure. The ear lobes of wild-type mice were cut, separated into ventral and dorsal sides, and incubated with or without papain (100 µg/ml) for 1 hour in vitro. Ear epidermal sheets were prepared and stained with (b, c) FITC- or (d) PE-labeled anti-Thy1.2 mAb and (d) FITC-labeled anti-mouse I-A/I-E mAb. (b) Confocal microscopy of epidermal Thy1.2⁺ cells. Bar = 50 µm. (c) Epidermal Thy1.2⁺ cells with dendritic or round-shaped morphology were differentially counted. (d) Confocal microscopy of epidermal Thy1.2⁺ cells and MHC class II⁺ cells. Bar = 50 µm. Data indicate the means ± SD of (c) 28 (medium) or 21 (papain) microscopic fields from four or three ear lobes, respectively. **P* < 0.05 by *t*-test. Data are representative of three independent experiments with similar results. DETC, dendritic epidermal T cell; MHC, major histocompatibility complex.





Supplementary Figure S13. FSC and SSC profile and $\gamma\delta$ TCR expression in Thy1^+ DETCs. Ear lobes of wild-type mice were stimulated with papain in vitro as indicated in Figure 1a for 19 hours. Single-cell suspensions were prepared from ear epidermal sheets, and epidermal Thy1.2^+ cells were isolated using anti-mouse Thy1.2 microbeads and autoMACS. MACS-sorted Thy1.2^+ cells were stained with anti-mouse Thy1.2 mAb and anti-mouse $\gamma\delta$ TCR mAb for flow cytometry. **(a)** FSC and SSC dot plot of epidermal Thy1.2^+ cells from papain-stimulated skin tissue. **(b)** The expression of $\gamma\delta$ TCR in epidermal Thy1.2^+ cells. Data indicate the means $\pm$ SD of five ears. $*P < 0.05$ by t -test. Data are representative of **(a)** three or **(b)** two independent experiments with similar results. DETC, dendritic epidermal T cell; FSC, forward scatter; MFI, mean fluorescence intensity; SSC, side scatter.