ACC1-expressing pathogenic T helper 2 cell populations facilitate lung and skin inflammation in mice

Takahiro Nakajima1, Toshio Kanno1, Satoru Yokoyama1, Shigemi Sasamoto1, Hikari K. Asou1, Damon J. Tumes2, Osamu Ohara3, Toshinori Nakayama4,5, and Yusuke Endo4,6

T cells possess distinguishing effector functions and drive inflammatory disorders. We have previously identified IL-5–producing Th2 cells as the pathogenic population predominantly involved in the pathology of allergic inflammation. However, the cell-intrinsic signaling pathways that control the pathogenic Th2 cell function are still unclear. We herein report the high expression of acetyl-CoA carboxylase 1 (ACC1) in the pathogenic CD4+ T cell population in the lung and skin. The genetic deletion of CD4+ T cell–intrinsic ACC1 dampened eosinophilic and basophilic inflammation in the lung and skin by constraining IL-5 or IL-3 production. Mechanistically, ACC1-dependent fatty acid biosynthesis induces the pathogenic cytokine production of CD4+ T cells via metabolic reprogramming and the availability of acetyl-CoA for epigenetic regulation. We thus identified a distinct phenotype of the pathogenic T cell population in the lung and skin, and ACC1 was shown to be an essential regulator controlling the pathogenic function of these populations to promote type 2 inflammation.

Introduction

Allergic diseases including atopic dermatitis (AD) and asthma have been increasing worldwide and are a significant public problem in most developed countries. These disorders are driven by type 2 cytokines, including IL-4, IL-5, and IL-13 derived from Th2 helper 2 (Th2) cells and type 2 innate lymphoid cells (ILC2s; Fahy, 2015; Guttman-Yassky et al., 2011; Nakayama et al., 2017). Recently, tissue-derived checkpoint cytokines including IL-25, IL-33, and thymic stromal lymphopoietin secreted from injured epithelial cells and keratinocytes have also been implicated in Th2-associated diseases through their amplification of Th2 cytokine-mediated responses (Holgate, 2012) and driving IL-5 and IL-13 production by ILC2s.

We previously identified IL-33–dependent IL-5–producing allergen-specific pathogenic Th2 (Tpath2) cells in allergic eosinophilic airway inflammation (Endo et al., 2014; Nakayama et al., 2017). IL-5 is critical for generation, recruitment to inflammatory sites, and activation of eosinophils (Dougan et al., 2019) that play a key role for severe asthma and chronic AD (Aleman et al., 2016; Islam et al., 2011). IL-13 induces IgE production by B cells, tissue fibrosis, and mucus production in the airways (Brandt and Sivaprasad, 2011; Gour and Wills-Karp, 2015), decreases expression of skin structural proteins, and induces itchiness in AD. T cell–derived IL-3 is also involved in skin inflammation via basophil activation and migration (Leyva-Castillo et al., 2013).

Mammalian target of rapamycin (mTOR) integrates the signaling pathways associated with nutrient levels, energy status, cell stress, and growth factor–mediated signaling (Saxton and Sabatini, 2017). Emerging evidence has demonstrated that metabolic reprogramming toward an anabolic phenotype during naive-to-effector T cell differentiation is coordinated by mTOR signaling. The absence of mTORC1 signaling inhibits naive CD4+ T cell differentiation into effector cells (Delgoiffe et al., 2009), mTOR complex 2 (mTORC2) induces Th2 cell differentiation by inhibiting suppressor of cytokine signaling 5 (SOCS5), which represses IL-4–dependent STAT6 signaling (Stark et al., 2019). In contrast, the suppression of both mTORC1 and mTORC2 promotes differentiation of induced regulatory T cells (Chapman and Chi, 2014). Thus, mTOR plays a pivotal role in orchestrating metabolic programs during T cell differentiation, with
mTORC1 and mTORC2 exerting distinct effects on downstream molecular signaling. The abrogation of mTOR signaling with rapamycin also reportedly suppresses the function of ILC2s in vitro and alleviates IL-33–induced airway inflammation in vivo (Salmond et al., 2012).

Altered lipid metabolism is required for the early activation of CD4+ T cells via mTOR–mediated alterations to fatty acid synthesis (Angela et al., 2016). De novo fatty acid synthesis is initiated by the activation of sterol regulatory element–binding protein 1 (SREBP-1; Stark et al., 2019), which mediates the proliferation and controls the function of CD8+ T cells through the transcriptional regulation of acetyl-CoA (Ac-CoA) carboxylase α (ACCI, the gene product of Acaca; Lee et al., 2014). ACCI, a rate-limiting enzyme of fatty acid biosynthesis, contributes to Th17 cell differentiation in mouse and human obesity (Endo et al., 2015a), directs cell fate decisions during memory CD4+ T cell generation, and positively regulates follicular helper cell numbers in vivo (Endo et al., 2019). The fatty acid uptake program is also crucial for activation of T cells and maintenance of tissue-resident memory T cells (Angela et al., 2016; Pan et al., 2017). However, while mounting evidence has highlighted the importance of lipid metabolism for T cell differentiation and function, the role of T cell lipid metabolism in allergic diseases remains unclear.

We herein show that ACCI drives two distinct pathogenic T cell populations to facilitate allergic inflammation in vivo. We found that the numbers of ST2hi IL-5–producing Tpath2 cells in the lung and eosinophilic airway inflammation were dependent on ACCI. Similarly, genetic deletion of ACCI abrogated MC903–induced skin inflammation by inhibiting the production of IL-3 by skin CD4+ T cells. Mechanistically, ACCI–mediated de novo fatty acid biosynthesis controlled the maximal activation of glycolysis, which is required for IL-5 production by Tpath2 cells. Our study therefore indicates that ACCI is an essential metabolic regulator that controls the inflammatory function of pathogenic CD4+ T cells in the lung and skin.

Results
IL-5–producing Tpath2 cells express high levels of ACCI

In previous studies, we discovered a population of IL-5hi–expressing memory Th2 cells in an OVA antigen–specific model of memory Th2 cell formation. These cells were ST2hiCXCR3hiCD62Llo, and we named them pathogenic memory Th2 (Tpath2) cells (Endo et al., 2015b; Endo et al., 2014; Endo et al., 2011) owing to their heightened ability to induce eosinophilic airway inflammation. We further found that IL-33 signaling via ST2 in memory Th2 cells greatly enhances the Tpath2 phenotype (high IL-5 production) and confers pathogenicity to antigen–specific memory Th2 cells in eosinophilic airway inflammation in both mice and humans (Endo et al., 2015b). We therefore use the term Tpath2 to refer to memory CD4+ T cells that produce the cytokine IL-5, have cell surface expression of ST2, respond to IL-33, and are pathogenic in terms of the induction of eosinophilic airway inflammation. Although IL-33–ST2 signaling is known to contribute to phosphoinositide 3-kinase–mediated mTOR activation, resulting in the regulation of metabolic homeostasis (Weichhart et al., 2015), its role in the pathogenicity of Th2 cells and allergic inflammation remains poorly understood.

To investigate the role of mTOR signaling in the induction of Tpath2 cells, we first analyzed memory Th2 cells prepared as demonstrated in Fig. S1 A (Endo et al., 2011). We use the name “antigen–specific Tpath2 cells” for in vivo–generated OVA antigen–specific ST2hi memory Th2 cells exposed to IL-33. We use the term “polyclonal Tpath2 cells” for cells that were not antigen specific but resembled memory (CD44hiCD62Llo) CD4+ T cells and consisted of the polyclonal population of cells with the Tpath2 phenotype. The phosphorylation of 4EBP1 and S6 ribosomal protein, which are major downstream molecules of mTOR signaling, was significantly up-regulated in response to IL-33 stimulation (Fig. 1 A). As reported previously, we confirmed that IL-33 induced the production of IL-5 and IL-13 by antigen–specific Tpath2 cells and polyclonal Tpath2 cells (Fig. S1 B; Endo et al., 2015b; Yamamoto et al., 2018). Rapamycin, a pharmacologic inhibitor of mTOR signaling, clearly suppressed the IL-33–mediated augmentation of IL-5 and IL-13 in antigen–specific Tpath2 and polyclonal Tpath2 cells (Fig. 1, B and C). In contrast, IL-4 production was slightly decreased by rapamycin (Fig. S1, C and D). Similarly, the mRNA expression of Il5 and Il13 was significantly decreased by rapamycin treatment (Fig. 1, D and E). These results suggested that IL-33–mediated mTOR activation was crucial for the induction of IL-5–producing Tpath2 cells.

We previously reported that ACCI–mediated fatty acid biosynthesis is required for the early activation and proliferation of naive and memory CD4+ T cells (Angela et al., 2016). Furthermore, we and other groups have reported using CD4-CreAcacaα/α (hereafter Acaca+/−) mice that Th17 cells are highly dependent on the de novo fatty acid biosynthesis pathway (Berod et al., 2014; Endo et al., 2015a). These previous findings suggest that the fatty acid biosynthesis pathway may also be involved in the differentiation of Tpath2 cells. Thus, we next analyzed the expression of ACCI in lung lymphocytes from healthy mice. We found that ST2hi IL-5–producing cells were predominately detected in the ACCIhi population and rarely observed in the ACCIlo population (Fig. S1 E). IL-33 treatment strongly increased IL-5–producing ACCIhi lymphocytes in the lung (Fig. S1 F). Furthermore, in IL-33–treated inflammatory lungs, most of the ST2hi IL-5–producing cell population consisted of TCRβhiCD4+ polyclonal Tpath2 cells and ILC2s (TCRβ−CD4−), whose ACCI protein expression was higher than ST2hiIL-5− and ST2hiIL-5− cell populations (Fig. 1, F and G). We also found that IL-33 treatment resulted in the up–regulation of genes related to the fatty acid biosynthesis pathway, including Acaca, Elovl1, Elovl5, Fads2, Scd1, Scd2, Acs1β, and Fasn in Tpath2 cells (Fig. 1 H and Fig. S1 G). The inhibition of mTOR by rapamycin treatment suppressed the up-regulation of fatty acid biosynthesis genes in antigen–specific Tpath2 and polyclonal Tpath2 cells (Fig. 1 I and Fig. S1 G). Taken together, these results suggest that IL-33–mediated mTOR activation induces fatty acid biosynthesis, which enables Th2 cells to differentiate into IL-5–producing Tpath2 cells.
Figure 1. ST2\textsuperscript{hi} IL-5–producing Tpath2 cells express high levels of ACC1. (A) p4E-BP1 and pS6 in memory Th2 cells were analyzed by FACS. MFI, mean fluorescence intensity. (B and C) Intracellular staining profiles of IL-5 and IL-13 in antigen-specific Tpath2 cells (B) or polyclonal Tpath2 cells (C) treated with or without rapamycin. (D and E) Relative expression of IL-5 and IL-13 in antigen-specific Tpath2 cells (D) or polyclonal Tpath2 cells (E) treated with or without rapamycin. (F) Flow cytometry analysis of IL-5 and ST2 expression in lung lymphocytes and ST2\textsuperscript{hi}IL-5\textsuperscript{+} cells. (G) Normalized expression of ACC1 in lung lymphocytes. (H and I) Heatmaps of gene expression changes in antigen-specific Tpath2 cells treated with IL-2, IL-33, or rapamycin.
ACCI controls the development of ST2hi IL-5–producing type 2 pathogenic lymphocytes

We next analyzed the involvement of ACC1 in the development of Tpath2 cells using the inducible ERT2-Cre–driven Acaca-deficient mouse system. Cultivation of ERT2-Cre+Acaca<sup>n/n</sup> antigen-specific Tpath2 cells with 4-hydroxy tamoxifen (4-OHT) significantly reduced both protein and mRNA levels of IL-5 and IL-13 (Fig. 2, A and B). Similar to the findings in antigen-specific Tpath2 cells, IL-5 and IL-13 were also significantly reduced in ERT2-Cre+Acaca<sup>n/n</sup> polyclonal Tpath2 cells cultured with 4-OHT (Fig. S2, A and B). IL-4 production was unaffected by the genetic deletion of Acaca in antigen-specific and polyclonal Tpath2 cells (Fig. S2, C and D; Endo et al., 2019). Similarly, treatment of antigen-specific or polyclonal Tpath2 cells with 5–(tetradecyloxy)-2-furoic acid (TOFA), a pharmacologic inhibitor of ACC1, substantially suppressed the production of IL-5 and IL-13 to levels similar to those in rapamycin-treated cells (Fig. 2, A and B). Similar to the findings in antigen-specific Tpath2 cells, IL-5 and IL-13 were also significantly reduced in ERT2-Cre+Acaca<sup>n/n</sup> polyclonal Tpath2 cells cultured with 4-OHT (Fig. S2, A and B). IL-4 production was unaffected by the genetic deletion of Acaca in antigen-specific and polyclonal Tpath2 cells (Fig. S2, C and D; Endo et al., 2019). Similarly, treatment of antigen-specific or polyclonal Tpath2 cells with 5–(tetradecyloxy)-2-furoic acid (TOFA), a pharmacologic inhibitor of ACC1, substantially suppressed the production of IL-5 and IL-13 to levels similar to those in rapamycin-treated cells (Fig. 2, A and B).

To further investigate the effect of ACC1 on cytokine production in vivo, we examined a papain-induced lung inflammation model using mice in which the biotin carboxyl carrier protein domain in the Acaca gene had been conditionally deleted in CD4<sup>+</sup> T cells, as illustrated in Fig. 3 A (Yamamoto et al., 2018). As shown in Fig. 3 B, ST2<sup>hi</sup> IL-5–producing Tpath2 cells in the lungs were decreased in the Acaca<sup>−/−</sup> group. We also assessed the bronchoalveolar lavage (BAL) fluid and found that the number of infiltrated cells and eosinophils in the BAL fluid was significantly decreased by the genetic deletion of Acaca in CD4<sup>+</sup> T cells (Fig. 3 C). In contrast, IL-5 production of CD44<sup>hi</sup>CD4<sup>+</sup> cells, including ILC2s, was similar between the Acaca<sup>−/−</sup> and Acaca<sup>+/+</sup> groups (Fig. 3 D).

Papain is known to induce IL-33, IL-25, and thymic stromal lymphopoietin production by airway epithelial cells (Halim et al., 2014). Among them, IL-33 plays a critical role in the induction of IL-5–producing Tpath2 cells and ILC2s and the pathogenesis of eosinophilic airway inflammation (Endo et al., 2015b; Yamamoto et al., 2018). We therefore next directly administered IL-33 to the mice and assessed the role of ACC1 in the pathogenesis of asthma onset (Fig. 3 E). Similar to our findings in the papain-induced asthma model, IL-5–producing Tpath2 cells in the lung were reduced in the Acaca<sup>−/−</sup> group (Fig. 3 F). Furthermore, the number of eosinophils in the BAL fluids, infiltration of inflammatory cells in the lungs, mucus production, and mRNA levels of inflammatory markers for eosinophilic inflammation were also reduced in the Acaca<sup>−/−</sup> group (Fig. 3 G–J).

Next, Acaca<sup>−/−</sup> and Acaca<sup>+/−</sup> mice were intranasally challenged with OVA antigen after immunization with OVA/alum, and their ability to induce allergic airway inflammation was assessed (Fig. 4 A). Compared with the Acaca<sup>+/−</sup> group, the Acaca<sup>−/−</sup> group showed a significant decrease in the number of ST2<sup>hi</sup> IL-5–producing CD4<sup>+</sup> T cells in the lung and eosinophils in the BAL fluid, similar to results using the IL-33– or papain-induced lung inflammation models (Fig. 4, B and C). No obvious changes in numbers of the TCR<sup>B</sup>CD4<sup>+</sup>ST2<sup>hi</sup>IL-5<sup>+</sup> population that contains ILC2s were observed between the two groups (Fig. 4 D). Histologic analysis also revealed a similar reduction in mononuclear cell infiltration into the peribronchial regions of the lungs (Fig. 4 E). Periodic acid–Schiff (PAS) staining and the measurement of Gob5 or inflammatory markers in the lung tissues showed decreased production of mucus in the Acaca<sup>−/−</sup> group (Fig. 4, F and G). Notably, a significant reduction of total serum IgE and OVA–specific IgE in the Acaca<sup>−/−</sup> group was detected (Fig. 4, H and I). These results indicate that the genetic deletion of Acaca in CD4<sup>+</sup> T cells results in a reduction in ST2<sup>hi</sup> IL-5–producing Tpath2 cells and a decrease in eosinophilic inflammation in the lungs.

ACCI controls the production of common β cytokines, including IL-3 and GM-CSF, in memory Th2 cells

To further investigate the effect of ACC1 on cytokine production of CD4<sup>+</sup> T cells, we performed RNA-sequencing (RNA-seq)
Figure 2. **ACC1 controls the development of ST2hi IL-5-producing pathogenic lymphocytes.** (A) Intracellular staining profiles of IL-5 and IL-13 in ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl Tpath2 cells treated with or without 4-OHT (100 nM). (B) Quantitative RT-PCR analysis of Il5 and Il13 in ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl Tpath2 cells cultured as in A. (C) Intracellular staining profiles of IL-5 and IL-13 in antigen-specific Tpath2 cells treated with or without TOFA. (D) Quantitative RT-PCR analysis of Il5 and Il13 in antigen-specific Tpath2 cells cultured as in C. (E) Expression profiles of ST2 on antigen-specific Tpath2

Nakajima et al. Journal of Experimental Medicine 5 of 20

ACC1 controls lung and skin allergic inflammation

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analyses using ERT2-Cre:\Acaca\B/\B Tpath2 cells. We found that the expression values (fragments per kilobase of transcript per million mapped reads [FPKM]) of Il3 and Csf2 were substantially decreased in ACC1-deficient Tpath2 cells, as well as those of Il5 and Il13 (Fig. 5 A and Fig. S3 A). Expression of Il4 was not affected by the genetic deletion of ACC1, as in Fig. S2 C. These data suggested that common β cytokines, including Il5, Il3, and Csf2, were controlled in the same way by ACC1 in Tpath2 cells. As reported previously, the FPKM of Il17a and Il17f was also decreased in the Acaca\B/\B group, although the magnitude was substantially lower (Fig. S3 B; Endo et al., 2015a). Levels of other cytokines, such as Ifng, Il6, Il24, Il1a, Ilb, Il6, Il21, and Il22, were equivalent between Acaca\B/\B and Acaca\B/\B Tpath2 cells (Fig. 5 A and Fig. S3 B). We next confirmed the mRNA expression of Il3 and Csf2 in Acaca\B/\B Tpath2 cells, and a similar trend to the RNA-seq analyses was detected (Fig. 5 B). Similarly, TOFA treatment significantly reduced the expression of Il3 and Csf2 in Tpath2 cells (Fig. 5 C). Furthermore, we also detected a reduction in IL-3 and GM-CSF production by Acaca\B/\B or TOFA-treated Tpath2 cells (Fig. 5 D and Fig. S3 C). In addition, we observed a reduction in IL-3 and GM-CSF production by CD44hiCD4+ T cells in inflamed lungs collected from papain-treated mice (Fig. 5 E). These results suggest that ACC1 controls common β cytokines, including IL-5, IL-3, and GM-CSF, in Tpath2 cells.

**CD4+ T cell-intrinsic ACC1 controls MC903-induced skin inflammation via IL-3-mediated basophil activation**

The pathogenesis of AD involves interaction between Th2 cells, ILC2s, and basophils (Ito et al., 2011; Leyva-Castillo et al., 2013; Siracusa et al., 2013). Although IL-3 derived from activated CD4+ T cells is essential for basophil activation in the pathology of AD, the cell-intrinsic factors regulating IL-3–producing T cells remain poorly understood (Leyva-Castillo et al., 2013). We therefore used a mouse model of calcipotriol (MC903)-induced AD to investigate whether CD4+ T cell–intrinsic ACC1 influences skin inflammation (Fig. 5 A). In addition to the high expression of ACC1 in IL-5–producing Th2 cells in the lung, we found that IL-3–producing CD4+ T cells express slightly higher levels of ACC1 than non–IL-3–producing cells in inflamed skin and draining lymph nodes (dLNs; Fig. 6 A and Fig. S4 B). Furthermore, a significant reduction in IL-3–producing CD4+ T cells in the skin and dLNs was detected in MC903-treated Acaca\B/\B mice (Fig. 6 B and Fig. S4 C). The proportion of CD44hiCD4+ T cells in the skin was also decreased in the Acaca\B/\B group (Fig. S4 D). In contrast, IL-5–producing CD4+ T cells in the skin and dLNs were rarely observed (Fig. S4, E and F). Consistent with these results, we found that the Acaca\B/\B group showed reduced swelling, reddening, and scaling of the ears compared with the Acaca\B/\B group (Fig. 6, C and D). In addition, a histological examination of the skin showed improved hyperkeratosis and epidermal hyperplasia following the genetic deletion of CD4+ T cell–intrinsic ACC1 (Fig. 6 E). The difference in the clinical scores between groups reflected a reduction in inflammation in the Acaca\B/\B group (Fig. 6 F).

To determine whether IL-3 produced from ACC1-expressing CD4+ T cells in the skin was functionally relevant to basophil activation, we next analyzed the phenotype of basophils that had infiltrated into inflamed skin. Although the number of basophils in the ear skin was similar between Acaca\B/\B and Acaca\B/\B mice (Fig. S4 G), the cell surface expression of CD63, an activation marker of basophils, was substantially reduced in Acaca\B/\B mice (Fig. 6 G). Thus, we postulated that ameliorated MC903-induced skin inflammation in Acaca\B/\B mice was caused by the suppression of IL-3–mediated basophil activation. We therefore next assessed whether IL-3 administration restored the phenotype of skin inflammation and basophil activation in MC903-treated Acaca\B/\B mice (Fig. S4 H). Since the biological half-life of IL-3 is very short, we used a mixture of IL-3 and anti-IL-3 to prolong the half-life and enhance the biological effects in vivo (Finkelman et al., 1993; Ohmori et al., 2009). As expected, IL-3 injection into Acaca\B/\B mice restored the phenotype, including the gross pathological findings and the histopathologic assessment of skin inflammation, to that in the Acaca\B/\B mice (Fig. 6, H and I). Similarly, the ear thickness and clinical score in the IL-3–treated Acaca\B/\B mice were also improved (Fig. 6, J and K), and CD63 expression on basophils in the Acaca\B/\B mice was increased to the same extent as in the Acaca\B/\B mice by IL-3 injection (Fig. 6 L). The expression of Cdc203c, a basophil-specific activation marker, was also augmented by the injection of IL-3 into Acaca\B/\B mice (Fig. 6 M). In contrast, the IL-3 production of CD44hiCD4+ T cells in dLNs was not affected by IL-3 injection (Fig. S4 I). These results suggest that CD4+ T cell–intrinsic ACC1 controls MC903-induced skin inflammation via IL-3–mediated basophil activation.

**Pharmacologic deletion or inhibition of ACC1 by topical treatment attenuates MC903-induced skin inflammation**

Next, to evaluate the effect of ACC1 on AD more accurately, we topicaly treated ERT2-Cre:\Acaca\B/\B mice with 4-OHT and selectively deleted the Acaca gene from skin lesions (Fig. 7 A). Topical 4-OHT treatment resulted in a significant reduction in IL-3–producing CD4+ T cells in the skin though partial genetic deletion of Acaca in ear CD45+ cells (Fig. 7 B and Fig. S4 J). Consistent with the findings in Acaca\B/\B mice, the ear thickness, swelling, and reddening were ameliorated in the ERT2-Cre:\Acaca\B/\B group compared with the ERT2-Cre:\Acaca\B/\B group (Fig. 7, C and D). Similarly, histological analysis and clinical scoring showed a reduction in hyperkeratosis and
Figure 3. Genetic deletion of CD4+ T cell–intrinsic ACC1 restrains Tpath2 cell responses and reduces airway inflammation in vivo. (A) Experimental protocol for papain-induced lung inflammation model. (B) Intracellular staining profiles of ST2 and IL-5 in lung TCRβ+CD4+CD44hi cells. The percentage of ST2hiIL-5+ cells is shown. (C) The absolute cell number of eosinophils and total cells in the BAL fluid collected from Acaca+/+ or Acaca−/− mice intranasally administered with papain. (D) Intracellular staining profiles of ST2 and IL-5 in lung CD44hiCD4+ cells isolated from Acaca+/+ or Acaca−/− mice. (E) Experimental protocol for IL-33–induced lung inflammation model. (F) Intracellular staining profiles of ST2 and IL-5 in stimulated lung TCRβ+CD4+CD44hi cells. The percentage of ST2hiIL-5+ cells is shown. (G) The absolute cell number of eosinophils and total cells in the BAL fluid collected from Acaca+/+ or Acaca−/− mice intranasally administered with IL-33. (H) Lung tissue sections of mice administered with IL-33 were fixed and stained with H&E (HE). A representative staining pattern is shown. Scale bars in bottom right corner represent 100 µm. Number of infiltrated inflammatory cells in lung per high power field (HPF) is shown. (I) Lung tissue sections of mice administered with IL-33 were fixed and stained with PAS. A representative staining pattern is shown. Scale bars in bottom right corner represent 100 µm. (J) Quantitative RT-PCR analysis of Gob5, Il5, Ccl11, Ccl17, and Ccl21 in the lung tissues of Acaca+/+ or Acaca−/− mice intranasally administered with IL-33. For each group, n = 6–7 (B–D and F–H) or n = 9–10 (J) biologically independent samples are shown. More than three independent experiments were performed with similar results (B–D and F–H). Mean values with SD are shown for B–D, F–H, and J. An unpaired two-tailed Student’s t test was applied for B–D, F–H, and J. Statistical significance (P values) is indicated as *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Figure 4. Genetic deletion of CD4+ T cell–intrinsic ACC1 reduces lung allergic inflammation in vivo. (A) Experimental protocol for OVA-induced lung inflammation model. (B) Intracellular staining profiles of ST2 and IL-5 lung TCRβ+CD4+CD44hi cells. The percentage of ST2hiIL-5+ cells is shown. (C) The absolute cell number of eosinophils and total cells in the BAL fluid collected from Acaca+/- or Acaca−/− mice intranasally administered with OVA as in A. (D) Intracellular staining profiles of ST2 and IL-5 in lung CD44hiCD4− cells isolated from Acaca+/- or Acaca−/− mice. (E) Lung tissue sections of mice administered with OVA were fixed and stained with H&E (HE). A representative staining pattern is shown. Scale bars in bottom right corner represent 100 µm. Number of infiltrated inflammatory cells in lung per high power field (HPF) is shown. (F) Lung tissue sections of mice administered with OVA were fixed and stained with PAS. A representative staining pattern is shown. Scale bars in bottom right corner represent 100 µm. (G) Quantitative RT-PCR analysis of Gob5, Il5, Ccl11, and Ccl17 in the lung tissues of Acaca+/- or Acaca−/− mice intranasally administered with OVA. (H and I) Total serum IgE (H) or OVA-specific IgE (I) was tested using ELISA. For each group, n = 6 (B–D, H, and I) or n = 5 (E–G) biologically independent samples are shown. More than three independent experiments were performed with similar results for B–I. Mean values with SD are shown for B–E and G–I. An unpaired two-tailed Student’s t test was applied for B–E and G–I. Statistical significance (P values) is indicated as *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Epidermal hyperplasia in the ERT2-Cre+Acacafl/fl group (Fig. 7, E and F).

Furthermore, topical treatment with rapamycin or the ACC1 inhibitor CP-640186 hydrochloride in the MC903-induced skin inflammation model strongly reduced MC903-induced swelling, reddening, and scaling of the ears and clinical scoring (Fig. 7, G–J). These inhibitors also suppressed IL-3 production by CD44hiCD4+ T cells in ear skin to a degree similar to that detected in Acaca−/− mice (Fig. 7 K). These results suggest that topical treatment of ACC1 inhibitor may have potential as a new therapeutic target for AD.

ACC1-dependent de novo fatty acid biosynthesis together with maximal glycolytic capacity controls IL-5–producing Tpath2 cells

The metabolic transition toward increased aerobic glycolysis and anabolic pathways in activated T cells represents general metabolic reprogramming during rapid T cell activation and proliferation (Slack et al., 2015). We previously showed that anabolic fatty acid metabolism plays an essential role in antigen stimulation–dependent metabolic reprogramming of memory CD4+ T cells (Angela et al., 2016). To investigate the effect of ACC1 deficiency on the metabolic state of IL-5–producing Tpath2 cells, we analyzed the mitochondrial function and rate of acid efflux in Acaca-deficient or TOFA-treated Tpath2 cells by monitoring the oxygen consumption rate (OCR; indicator of oxidative phosphorylation) and extracellular acidification rate (ECAR; indicator of glycolysis) with an extracellular efflux analyzer. Sequential treatment with the ATPase inhibitor oligomycin, the uncoupling agent carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, and the electron transport chain inhibitors rotenone and antimycin A revealed the basal oxygen consumption, ATP turnover, maximum respiratory capacity, and spare respiratory capacity (van der Windt et al., 2016). We found that the
Figure 6. ACC1 controls MC903-induced skin inflammation via IL-3–mediated basophil activation. (A) Intracellular staining profiles of IL-3 and ACC1 in stimulated ear-dLN CD44hiCD4+ T cells. Histogram shows ACC1 expression in IL-3+ or IL-3− cells. (B) Intracellular staining profiles of IL-3 in stimulated ear-dLN CD44hiCD4+ T cells. (C) Ear thickness of Acaca+/+ and Acaca−/− mice after daily MC903 treatment (2 nmol per ear). Values are differences in thickness from day 0. (D and E) Representative images of ears collected from Acaca+/+ or Acaca−/− mice subcutaneously administered with mIL-3/anti-IL-3 or PBS to shoulders (H). Histological analysis of ear sections of mice fixed and stained with H&E (I).
OCR and ECAR were lower in ACC1-deficient or TOFA-treated Tpath2 cells than in control cells (Fig. 8, A–D). These results indicate that ACC1-induced de novo fatty acid biosynthesis is required for the induction of metabolic reprogramming in Tpath2 cells.

We previously reported that extrinsic fatty acid supplementation restored the function of Acaca−/− Th17 cells and improved the proliferation, survival, and metabolic reprogramming of TOFA-treated activated CD4+ T cells (Angela et al., 2016; Endo et al., 2015a). We therefore next analyzed whether extrinsic fatty acid supplementation restored the function and cell proliferation of TOFA-treated Tpath2 cells. We found that extrinsic supplementation of TOFA-treated Tpath2 cells with oleic acid (OA) partially restored cell numbers (Fig. 8 E). Furthermore, extrinsic supplementation of TOFA-treated Tpath2 cells with OA restored OCR to levels nearly equivalent to those in control cells (Fig. 8 F). In contrast, intermediate restoration was observed for ECAR by the supplementation of TOFA-treated Tpath2 cells with OA (Fig. 8 G). Unexpectedly, IL-5 production by TOFA-treated polyclonal Tpath2 cells was not restored at all by OA supplementation alone (Fig. 8 H). We speculated that maximal activation of the glycolysis pathway is required for IL-5 production by Tpath2 cells. To address this, we added extra glucose to OA-treated Tpath2 cell cultures and assessed IL-5 production. As shown in Fig. 8 I, glucose supplementation together with OA dramatically restored IL-5 production by TOFA-treated Tpath2 cells. A similar trend was detected in the mRNA expression of Il5 (Fig. 8 J). These results suggest that de novo fatty acid biosynthesis together with maximal glycolytic capacity is essential for IL-5 production by Tpath2 cells.

**Acetate promotes chromatin accessibility at the Il3 locus in Acaca−/− Th2 cells**

We also evaluated the effect of extra glucose supplementation on IL-3 production by Th2 cells, but no significant changes were observed (Fig. S5 A). The short-chain fatty acid acetate is known to be an alternative carbon source for cancer cells or T cells, supporting their survival, proliferation, and function under nutrient-limited conditions (Comerford et al., 2014; Qiu et al., 2019). Furthermore, supplementation of acetate has been shown to enhance Ifng gene transcription in CD8+ T cells via the promotion of histone acetylation and chromatin accessibility (Qiu et al., 2019; Vodnala et al., 2019). To investigate whether the restriction of fatty acid biosynthesis in CD4+ T cells decreases the availability of cellular Ac-CoA for histone acetylation, we assessed global histone acetylation in TOFA-treated Th2 cells by Western blotting. We found that the acetylation of histone protein H3 was decreased in TOFA-treated Th2 cells compared with control cells (Fig. 9 A). We therefore further assessed the effects of supplemental acetate on IL-3 production by TOFA-treated or Acaca−/− Th2 cells. As expected, supplementation of acetate to TOFA-treated or Acaca−/− Th2 cell cultures restored the generation of IL-3–producing cells (Fig. 9, B and C). The gene expression of Il3 was also up-regulated by the supplementation of TOFA-treated or Acaca−/− Th2 cells with acetate (Fig. 9, D and E). To determine whether acetate enhances the production of IL-3 by TOFA-treated Th2 cells by promoting histone acetylation and supporting gene transcription, we assessed histone acetylation by performing a chromatin immunoprecipitation (ChIP) assay. We found that acetate supplementation increased H3K9 acetylation at the Il3 promoter region, where clusters are defined by similarity of DNase I– hypersensitivity site profiles (Duncliffe et al., 1997; Fig. 9 F and Fig. S5 B). To evaluate the importance of histone acetylation on IL-3 production more detail, we next analyzed whether administration of a histone acetyl transferase (HAT) inhibitor suppresses IL-3 production of Th2 cells. As shown in Fig. S5 C, the potent HAT inhibitor Curcumin suppressed IL-3 production by Th2 cells. We also assessed whether histone deacetylase (HDAC) inhibitors could enhance IL-3 production. Treatment of Th2 cells with trichostatin A (TSA), which is an organic compound that selectively inhibits the class I and II HDAC families of enzymes, enhanced IL-3 production (Fig. S5 D). These results suggest that histone acetylation is essential for the expression of Il3 by Th2 cells.

Given the above findings, we propose that selective ACC1 expression and active fatty acid biosynthesis can be new hallmarks of cellular metabolism characterizing Tpath2 cells that facilitate granulocyte activation–dependent allergic inflammation (Fig. 9 G and Fig. S5 E).

**Discussion**

Pathogenic CD4+ T cell populations drive chronic inflammatory disorders in response to cell-extrinsic environmental factors, such as alarmins and cytokines (Saravia et al., 2019; Walker and McKenzie, 2018). However, the cell-intrinsic metabolic pathways that control pathogenic CD4+ T cell function are unclear. We found that ACC1-dependent fatty acid biosynthesis plays a crucial role in Tpath2 cell differentiation and the pathogenesis of type 2 inflammation in the lung and skin. Furthermore, CD4+ T cell–intrinsic ACC1 deletion attenuated allergic responses during eosinophilic and basophilic inflammation. Mechanistically, mTORC1-ACC1 orchestrated cell metabolism, fatty acid biosynthesis, and maximal activation of glycolysis to facilitate IL-5 production by Tpath2 cells. In addition, we found that IL-3 production was controlled by an epigenetic mechanism, as...
Figure 7. ACC1 deletion or inhibition to whole cells in ear region by topical treatment of 4-OHT or inhibitors reduces MC903-induced skin inflammation. (A) Experimental protocol for MC903-induced skin inflammation model with ERT2-Cre Acacafl/fl or ERT-Cre Acacafl/fl mice. (B) Intracellular staining profiles of IL-3 in ear CD45CD44hiCD4 T cells in 4-OHT-treated ERT-Cre Acacafl/fl or ERT-Cre mice. (C) Ear thickness of ERT-Cre Acacafl/fl or ERT-Cre mice given 4-OHT or EtOH (vehicle) topically. Values are differences in thickness from day 0. Statistical significance indicates difference between ERT-Cre Acacafl/fl with 4-OHT group and the others. Cont., control. (D and E) Representative images of ears of ERT-Cre Acacafl/fl or ERT-Cre mice given 4-OHT topically (D). Ear tissue sections of mice were fixed and stained with H&E (E). Scale bars represent 100 µm. (F) Clinical score of ear redness and scaling. (G) Experimental protocol for MC903-induced skin inflammation model treated with rapamycin or CP-640186. (H) Ear thickness of mice treated topically with rapamycin or CP-640186 versus control mice. Values are differences in thickness from day 0. Statistical significance indicates difference between rapamycin or CP-640186 group and control. (I) Representative images of rapamycin- or CP-640186–treated mice with control. (J) Clinical score of rapamycin- or CP-640186–treated mice with control, assessed by ear redness and scaling. (K) Intracellular staining profiles of IL-3 in ear CD45CD44hiCD4 T cells in rapamycin- or CP-640186–treated mice. For each group, n = 4 (B–F) or n = 6 (H–K) biologically independent samples are shown. More than three independent experiments were performed with similar results for B–E and H–K. Mean values with SD are shown for B, C, F, H, J, and K. Two-way ANOVA was applied for C and H. An unpaired two-tailed Student’s t test was applied for B, F, J, and K. Statistical significance (P values) is indicated as *, P < 0.05; **, P < 0.01; ****, P < 0.0001.
Figure 8. **ACC1-dependent de novo fatty acid biosynthesis together with maximal glycolytic capacity controls IL-5-producing Tpath2 cells.** (A) OCR of ERT2-Cre\(^{+}\)Acaca\(^{-}\)\(^{-}\) Tpath2 cells treated with or without 4-OHT. FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone. (B) ECAR of Tpath2 cells cultured as in A. (C) OCR of Tpath2 cells after 5-d cultivation with IL-33 plus TOFA or without TOFA. (D) ECAR of Tpath2 cells after 5-d cultivation with IL-33 plus TOFA or without TOFA. (E) The graph shows the number of Tpath2 cells after 5-d culture with TOFA in the presence or absence of OA (50 µM). (F) OCR of...
evidenced by the fact that supplementation of acetate to Acaca−/− Th2 cell cultures restored histone acetylation at the Il3 promoter region (Fig. S5 E). Taken together, our findings indicate that the ACC1-dependent fatty acid biosynthesis pathway serves as an essential metabolic checkpoint controlling Th2 cell pathogenicity and the development of allergic inflammation.

Raptor-mTORC1 signaling has been found to coordinate glycolysis and lipid biosynthesis to mediate TCR stimulation-induced exit from quiescence in Th2 cells (Yang et al., 2013). Another study reported that antigen-driven mTORC1/REBP-mediated lipid metabolism plays a critical role in the activation and proliferation of CD8+ T cells (Kidani et al., 2013). We now show that IL-33-induced mTORC1 activation is critical for the ACC1-mediated fatty acid biosynthesis and pathogenic function of Tpath2 cells. Consistent with our findings, IL-33 induces mTORC1 activation and phosphorylation of downstream effector proteins through ST2 signaling and the activation of phosphoinositide 3-kinase in ILC2s (Salmond et al., 2012). Of note, ACC1 expression in ST2hi IL-5–producing Tpath2 cells was selectively higher than in non-ST2hi IL-5–producing cell populations and ST2lo populations. This result indicates that mTORC1 activation differs between ST2hi IL-5–producing Tpath2 cells and non-ST2hi IL-5–producing cells. A possible explanation for this difference is that different glucose or amino acid availability controls the activation status of mTORC1 signaling in these populations (Jewell and Guan, 2013; Saxton and Sabatini, 2017). Indeed, glycolysis activity characterized by ECAR was lower in TOFA-treated and ACC1-deficient Tpath2 cells than in control cells. Furthermore, extrinsic glucose and OA supplementation to TOFA-treated Tpath2 cell cultures significantly restored IL-5 production. Previous findings suggest that cell-intrinsic Arginase 1 regulates proliferation and effector function of ILC2s via the control of maximal glycolytic capacity (Monticelli et al., 2016). Thus, the effector function, especially IL-5 production, in Tpath2 cells or ILC2s appears to be strictly regulated by an active metabolic circuit coordinated by mTORC1, ACC1-mediated fatty acid biosynthesis, and maximal glycolytic activity.

In contrast to IL-5, ACC1-dependent IL-3 production is controlled by epigenetic mechanisms. While transcriptional regulation of Il3 is not well characterized, T cell–selective DNase I hypersensitivity sites at the Il3 locus have been reported (Dundcliffe et al., 1997). Our data highlight the role of acetate as an alternative carbon source for histone acetylation at the Il3 promoter region in Th2 cells when synthesized fatty acids are limited. Acetate is known to enhance IFNγ production from CD8+ T cells during prolonged glucose restriction (Qiu et al., 2019; Vodnala et al., 2019). Furthermore, acetate and butyrate can promote Foxp3 expression to induce the differentiation of regulatory T cells in the gut (Furusawa et al., 2013). Thus, the gene group including Il3, Csf2, Ifng, and Foxp3 appears to be favorably regulated by feeding of cellular Ac-CoA into histone proteins. Although the detailed mechanisms underlying how ACC1 controls the availability of cellular Ac-CoA for histone acetylation remain unclear, our previous data have shown that cellular Ac-CoA likely feeds into the cellular pool of metabolites in the tricarboxylic acid cycle in Acaca−/− Th1 and Th2 cells (Endo et al., 2019).

An intriguing finding in the present study is that ACC1-dependent fatty acid biosynthesis appears to control specific cytokines, including IL-5, IL-3, and GM-CSF, in CD4+ T cells. In addition, we and other groups previously reported that IL-17A and IL-17F were impaired in Acaca−/− Th17 cells (Berod et al., 2014; Endo et al., 2015a). Among Th2 cytokines, IL-13 production is also partially decreased in Acaca−/− Tpath2 cells, whereas IL-4 is totally unaffected. RNA-seq analysis also revealed that other cytokines such as Ifng, Il6, Il9, and Il24 were unchanged by genetic deletion of Acaca in Tpath2 cells. IL-5 and IL-3 play a crucial role in the pathogenesis of type 2 inflammation, and IL-17A and GM-CSF have a pathogenic role in a variety of autoimmune diseases (Broughton et al., 2012; Lotfi et al., 2019; Maddur et al., 2012). Therefore, we speculate that a high expression of ACC1 is a key hallmark of pathogenic cytokine production by T cells. In contrast to the selective effect of ACC1 inhibition on cytokine production, rapamycin-treated or mTOR-deficient CD4+ T cells fail to differentiate into all Th1, Th2, or Th17 cells (Chi, 2012; Pollizzi and Powell, 2014). Furthermore, T cell–specific genetic deletion of Raptor, the scaffolding subunit of mTORC1, abrogates T cell priming, antigen-stimulated IL-2 production, and Th cell subset differentiation (Yang et al., 2013). These broad effects on T cell function by mTOR disruption make it reasonable to consider that mTOR is a critical regulator that integrates multiple inputs from environmental signals such as nutrients, growth factors, and cytokines.

In addition to our data suggesting the importance of fatty acid biosynthesis in Tpath2 cells and ILC2s, transient storage of fatty acids in lipid droplets was recently shown to regulate ILC2 responses in the lung (Karagiannis et al., 2020). That study showed esterification of fatty acids into triacylglycerols in ILC2s and that exogenous fatty acids are particularly important for mediating ILC2 proliferation (Karagiannis et al., 2020). Similarly, another report found that highly proliferating cells rely on external fatty acids to build complex lipids for cellular membranes (Yao et al., 2016). In conjunction with these reports, we previously reported that PPARγ-dependent fatty acid uptake programs are required for the rapid proliferation of activated CD4+ T cells (Angela et al., 2016). However, supplementation of fatty acids was able to rescue the proliferation of Tpath2 cells. Therefore, both de novo fatty acid biosynthesis and the uptake of
Figure 9. Acetate promotes chromatin accessibility at the Il3 locus in Acaca<sup>−/−</sup> Th2 cells. (A) Western blot analysis of acetylated histone H3K9 and total histone H3 in Th2 cells treated with or without TOFA (C control, T, TOFA). (B) Intracellular staining profiles of IL-3 in restimulated Th2 cells treated with TOFA in the presence or absence of acetate (5 mM). (C) Intracellular staining profiles of IL-3 in restimulated Acaca<sup>−/−</sup> Th2 cells treated with acetate (5 mM). Acaca<sup>−/−</sup> Th2 cells were differentiated from CD4-Cre<sup>−/−</sup>Acaca<sup>fl/fl</sup> mouse–derived naive CD4 T cells. (D) Quantitative RT-PCR analysis of Il3 in Th2 cells cultured as in B.
extrinsic fatty acids are required for Tpath2 cells and ILC2s to meet their lipid demands.

In summary, we demonstrated that ACC1 controls the production of IL-5 and IL-3, which consequently determines the pathology of Th2 cells in the lung and skin, respectively. Thus, ACC1 and the de novo fatty acid biosynthesis pathway may be potential therapeutic targets for the treatment of type 2 inflammatory diseases, including asthma and AD.

Materials and methods

Mice

Acaca−/− mice (Miao et al., 2006) were crossed with CD4-Cre and ERT2-Cre mice (Jackson Laboratory) 10 times and maintained on a C57BL/6 background. OVA-specific TCR-αβ (DO11.10) transgenic mice were provided by Dr. D. Loh (Washington University School of Medicine, St. Louis, MO), and backcrossed to BALB/c mice 10 times (Murphy et al., 1990). C57BL/6, BALB/c, and BALB/c nu/nu mice were purchased from Clea. All mice were used at 6–10 wk of age and were housed under specific pathogen-free conditions. The animal experiments were performed with protocols approved by the Institutional Animal Care and Use Committee of Kazusa DNA Research Institute (registration no. 30-1-002). Experiments and animal care were performed according to the guidelines of Kazusa DNA Research Institute.

Reagents

The reagents in this study are as follows: PE-conjugated anti-p4EBP1 (560285, clone M31-16), Alexa Fluor 647-conjugated anti-p56 (560465, clone N5-676), PE/Cy7- and BV421-conjugated anti-CD4 (PE/Cy7; 552775, BV650; 563747, clone RM4-5), PE- and BV421-conjugated anti-IL-33R (Loh15, 0.3 µM) plus antigen-presenting cells (irradiated splenocytes from BALB/c mice) in the presence of IL-2 (25 U ml−1), IL-4 (5 ng ml−1), and anti-IFNγ monoclonal antibody for 6 d in vitro. The effector Th2 cells (3 × 105) were transferred intravenously into BALB/c nu/nu recipient mice (Fig. S1 A). More than 5 wk after the cell transfer, KJ1+CD4+ T cells in the spleen were purified by auto-MACS (Miltenyi Biotec) and cell sorting (KJ1+CD4+CXCR3+CD62Lhigh T cells) (CD4+CD62Lhigh T cells were sorted from CD4+ cells using a FACS Melody cell sorter (BD Biosciences). Similarly, ILC2s (CD4+Lin−IL-7Rα−Thy1.2+) were sorted from Lineage− cells. CD4+CD62Lhigh T cells were plated onto 48-well tissue culture plates (Costar) that contained IL-2 (15 ng ml−1) and recombinant mouse IL-33 (10 ng ml−1) and then used as polyclonal Tpath2 cells after 5 d of culture. ILC2s were cultured with recombinant mouse IL-7 (10 U ml−1), recombinant mouse IL-25 (10 ng ml−1), and recombinant mouse IL-33 (10 ng ml−1) for 5 d.

The cell preparation protocol for antigen-specific Tpath2 cells was as follows. Splenic CD62L−KJ1−CD4+ T cells from DO11.10 OVA-specific TCR transgenic mice were stimulated with OVA peptides (LohI5, 0.3 µM) plus antigen-presenting cells (irradiated splenocytes from BALB/c mice) in the presence of IL-2 (25 U ml−1), IL-4 (5 ng ml−1), and anti-IFNγ monoclonal antibody for 6 d in vitro. The effector Th2 cells (3 × 105) were transferred intravenously into BALB/c nu/nu recipient mice (Fig. S1 A). More than 5 wk after the cell transfer, KJ1+CD4+ T cells in the spleen were purified by auto-MACS (Miltenyi Biotec) and cell sorting (KJ1+CD4+CXCR3+CD62Lhigh T cell population; BD FACS Melody or Aria III) and then used as antigen-specific memory Th2 cells. Antigen-specific memory Th2 cells were plated onto 48-well tissue culture plates (Costar) that contained IL-2 (15 ng ml−1) and recombinant mouse IL-33 (10 ng ml−1) and used as antigen-specific Tpath2 cells after 5 d of culture. For pharmacologic

Nakajima et al.

ACC1 controls lung and skin allergic inflammation

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Journal of Experimental Medicine

16 of 20
inhibition of ACC1, TOFA was dissolved in DMSO (10 mM) and administered at a final concentration of 10 µM. For gene deletion of Acaca, 4-OHT was added to ERT2-Cre\-Acaca\textsuperscript{A/A} or ERT2-Cre\-Acaca\textsuperscript{B/B} mouse–derived cells. 4-OHT was dissolved in EtOH (100 µM) and administered at a final concentration of 100 nM.

**Mouse T cell cultures**

For the preparation of effector Th2 cells, CD62L\textsuperscript{+}CD4\textsuperscript{+} T cells isolated from spleens of C57BL/6j mice were stimulated with plate-coated anti-TCRβ (5 µg ml\textsuperscript{-1}) plus anti-CD28 (1 µg ml\textsuperscript{-1}) antibodies in complete medium with IL-2 (25 U ml\textsuperscript{-1}), IL-4 (5 ng ml\textsuperscript{-1}), and anti-IFNγ (1 µg ml\textsuperscript{-1}) for 5 d in vitro. To assess IL-3 and GM-CSF production, CD62L\textsuperscript{+}CD4\textsuperscript{+} T cells isolated from C57BL/6j mice were cultured for 3 d under the Th2 skewing condition as shown above. For the OA supplementation assay, OA was dissolved in ethanol, with a concentration of 100 mM complexed with 5% BSA before use, and administered to cultured cells at a final concentration 50 µM. Acetate was prepared to 3 M and administered at a final concentration of 5 mM. Glucose was prepared to 0.28 M in complete medium and administered at a final concentration of 50 mM. Curcumin was dissolved in ethanol (10 mM) and administered at a final concentration of 2.5 µM. TSA was dissolved in DMSO (1 mM) and administered at a final concentration 3 nM.

**Flow cytometry analysis**

Cells were analyzed using flow cytometry (FACS Celesta; BD Biosciences). For intracellular cytokine staining, cells were restimulated with PMA (0.1 µg ml\textsuperscript{-1}; Sigma-Aldrich) and monensin (Sigma-Aldrich) for 6 h, followed by fixation and permeabilization. ILC2s were stimulated with IL-7 (10 U ml\textsuperscript{-1})/IL-33 (10 ng ml\textsuperscript{-1}) plus monensin (Sigma-Aldrich) for 6 h, followed by fixation and permeabilization. Data were analyzed with FlowJo software (v10.8.0).

**Lung inflammation model**

Mice were injected intranasally recombinant mouse IL-33 (100 ng in 20 µl sterile PBS) or papain (50 µg in 20 µl sterile PBS; Nacalai) for three consecutive days. BAL fluid was collected 24 h (papain) or 72 h (IL-33) after last administration. Lung histology and cells were assessed similarly. Mice were immunized with two intraperitoneal injections (days 1 and 8) of 100 µg OVA (dissolved in 100 µl sterile PBS; Sigma-Aldrich) emulsified in alum (100 µl; Thermo Fisher Scientific) and then challenged by intranasal administration of 10 µg OVA dissolved in 20 µl PBS on days 18–22. On day 23 (24 h after final challenge), BAL fluid, lung histology, and cells were analyzed.

**Histopathology**

Lung and skin tissues from euthanized mice were fixed in 10% formalin neutral buffer solution (Wako) and embedded in paraffin. H&E- or PAS-stained 5-µm sections were examined with a BZ-X800/X810 fluorescence microscope (Keyence; 20× or 40× objective and zoom of 1.0). Histological analysis was performed with ImageJ (v1.52n).

**ELISA**

Total IgE- and OVA-specific IgE ELISA kits were purchased from BioLegend (432401 and 439807) and used according to the manufacturer’s protocol.

**Metabolism analysis**

OCR and ECAR were measured with an XF96 analyzer (Agilent Technologies). Memory Th2 cells cultured with IL-33 were seeded at a density of 200,000 cells per well on a microplate. The cells were equilibrated for 1 h in unbuffered XF assay medium supplemented with 25 mM glucose and 1 mM sodium pyruvate before assay. Mixing, waiting, and measure times were 2, 2, and 4 min, respectively. The final concentrations of compounds were 0.2 µM oligomycin, 0.5 µM carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone, and 0.75 µM rotenone-antimycin-A. The details of cell preparation were previously described (Endo et al., 2019).

**Immunoblotting assay**

TH2 cells were washed with PBS and lysed with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific) according to the manufacturer’s protocol. The proteins in the lysates of the nuclear fraction were measured with protein assay dye reagent concentrate (Bio-Rad). The antibodies used for the immunoblot analysis were anti-Ac H3K9 (CST) and anti-total H3 (BioLegend). Immunoblotting was performed essentially as previously described (Yamamoto et al., 2018).

**RNA-seq and bioinformatic analysis**

Total cellular RNA was extracted with TRIzol reagent (Invitrogen). For cDNA library construction, we used TruSeq RNA Sample Prep Kit v2 (Illumina) according to the manufacturer’s protocol. Sequencing the library fragments was performed on the HiSeq 2500 system. For data analysis, read sequences (50 bp) were aligned to the mm10 mouse reference genome (University of California Santa Cruz, December 2011) using Bowtie (v0.12.8) and TopHat (v1.3.2). Fragments per kilobase of exon per million mapped reads (FPKM) for each gene were calculated using Cufflinks (v2.0.2). Genes with an absolute FPKM >1 (mean from duplicate samples) were defined as expressed genes. Gene set enrichment analysis was performed to determine the statistical significance of the enrichment of known transcriptional signatures in a ranked list of genes. We used 2,178 gene sets from the Molecular Signature Database C5 v3.0. Raw and processed sequencing data are available under GEO accession no. GSE185743.

**PCR genotyping for detection of ACC1 deletion**

PCR genotyping was performed by using the following primers: forward, 5′-AGTTCCCTAAGGAGCTGGACA-3′, and reverse, 5′-CCACTGCAATTCAGTCACCATC-3′. These primers were designed to sandwich loxp sites, as in a previous study (Mao et al., 2006).
MC903-induced skin inflammation model
MC903 (calcipotriol; MCE) was dissolved in ethanol (EtOH) and topically applied on mouse ears (2 nmol in 20 µl per ear). CP-640186 was dissolved in EtOH and topically applied on mouse ears (200 nmol in 20 µl per ear). Rapamycin was dissolved in EtOH and topically applied on mouse ears (1 nmol in 20 µl per ear). The same volume of EtOH was used as vehicle control. 4-OHT (Sigma-Aldrich) was dissolved in EtOH (50 µg in 20 µl). MC903, CP-640186, and rapamycin were administered daily, and 4-OHT was administered on days 1, 3, 6, and 8. 6 h after MC903 treatment, 4-OHT, CP-640186, or rapamycin was applied in the same volume as MC903 on ears. Ear thickness was measured daily with a caliper-type digital micrometer (Mitutoyo Corp.). A quarter of the ear was snap-frozen into liquid nitrogen for RNA isolation and extracted with TRIZOL reagent. Mouse recombinant IL-3 (Peprotech) was mixed with anti-IL-3 antibody (BioLegend) to prolong the biological half-life. To prepare IL-3 mixture for injection, IL-3 was adjusted to 20 µl with PBS (1 µg IL-3 and 5 µg anti-IL-3 per 20 µl), based on published methods (Ohmori et al., 2009).

Quantitative real-time PCR
Total RNA was isolated with the TRizol reagent (Invitrogen). cDNA was synthesized using oligo(dT) primers and Superscript II RT (Invitrogen). Quantitative real-time PCR was performed as described previously using a StepOnePlus real-time PCR system. The primers and Roche universal probes used were purchased from Thermo Fisher Scientific and Roche (Table S1). Gene expression was normalized using the Hprt mRNA signal or the 18S ribosomal RNA signal.

ChIP assay
ChIP assay was performed as described previously (Angela et al., 2016). In brief, 5 × 10⁶ Th2 cells (cultured for 3 d under Th2 skewing conditions) were fixed with 1% paraformaldehyde at 25°C for 10 min. The lysates were sonicated by a Covaris Focused-Ultrasonicator M220. Quantitative real-time PCR was performed as described previously (Angela et al., 2016). The primers and Roche universal probes used were purchased from Thermo Fisher Scientific and Roche (Table S1). Gene expression was normalized using the Hprt mRNA signal or the 18S ribosomal RNA signal.

Statistical analysis
Data are expressed as mean ± SD. The data were analyzed with Prism (v7.0e; GraphPad). Differences were assessed using two-tailed Student’s t tests or two-way ANOVA with Sidak’s multiple comparisons test. Differences with values of P < 0.05 were considered to be significant. Statistical details of experiments are indicated in the figure legends.

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Figure S1. **Experimental protocol of memory Th2 cells and gating strategy of ST2hi IL-5–producing cells in vivo.** (A) Experimental protocol for the generation of memory Th2 cell. (B) Intracellular staining profiles of IL-5 and IL-13 in restimulated polyclonal Tpath2 cells. The percentages of IL-5+ and IL-13+ cells are shown with SD. Cells were cultured with IL-2 or IL-2 plus IL-33 in complete medium for 5 d. (C and D) Intracellular staining profiles of IL-5 and IL-4 in restimulated antigen-specific Tpath2 cells (C) or polyclonal Tpath2 cells (D) treated with or without rapamycin (100 nM). The percentages of IL-4+ cells are shown with SD. Cells were cultured with IL-33 (B) or IL-2 and IL-33 (C) in complete medium for 5 d. (E) Intracellular staining profiles and gating strategy for flow cytometric analysis of lung cells that express ACC1 low, intermediate, and high cells. Intracellular staining profiles of IL-5 and ST2 in each population. The percentages of ST2hiIL-5+ cells are shown with SD. FSC, forward scatter; SSC, side scatter. (F) Representative intracellular staining profiles of ACC1 and IL-5 in restimulated lung lymphocytes isolated from untreated C57BL/6 mice or C57BL/6 mice administered IL-33 intranasally. (G) Heatmap visualization of expression profiles of genes involved in fatty acid biosynthesis in IL-2–cultured and IL-2 plus IL-33–cultured polyclonal Tpath2 cells treated with or without rapamycin. The Z-score scales ranging from blue to red are shown in the bottom right corner. For each group, n = 3 (D) or 4 (B, C, and E–G) biologically independent samples are shown. More than three independent experiments were performed with similar results for B–G. Mean values with SD are shown for B–E. An unpaired two-tailed Student’s t test was applied for B–E. Statistical significance (P values) is indicated as *, P < 0.05; **, P < 0.01; ****, P < 0.0001.
Figure S2.  **ACC1 controls the development of ST2hi IL-5–producing pathogenic lymphocytes.** (A and D) Intracellular staining profiles of IL-5 and IL-13 (A) or IL-5 and IL-4 (D) in restimulated ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl polyclonal Tpath2 cells treated with or without 4-OHT. The percentages of IL-5+ and IL-13+ cells (A) or IL-4+ cells (D) are shown. (B) Quantitative RT-PCR analysis of Il5 and Il13 in ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl polyclonal Tpath2 cells treated with or without 4-OHT. (C) Intracellular staining profiles of IL-5 and IL-4 in restimulated ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl Tpath2 cells treated with or without 4-OHT. The percentages of IL-4+ cells are shown. (E) Intracellular staining profiles of IL-5 and IL-13 in restimulated polyclonal Tpath2 cells treated with TOFA or rapamycin. The percentages of IL-5+ and IL-13+ cells are shown. (F) Quantitative RT-PCR analysis of Il5 and Il13 in polyclonal Tpath2 cells treated with TOFA or rapamycin. (G) Expression profiles of ST2 on ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl polyclonal Tpath2 cells treated with 4-OHT or TOFA on day 5. The graph shows mean fluorescence intensity (MFI) ± SD values of ST2 in each group. (H) Quantitative RT-PCR analysis of Il1rl1 in ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl polyclonal Tpath2 cells treated with 4-OHT or TOFA. For each group, n = 4 biologically independent samples are shown (A–H). The cells isolated from ERT2-Cre−Acacafl/fl or ERT2-Cre+Acacafl/fl mice were used for A–D, G, and H. More than three independent experiments were performed with similar results for A–H. An unpaired two-tailed Student’s t test was applied for A–H. Statistical significance (P values) is indicated as **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.
ACC1 controls the production of common β cytokines, including IL-3 and GM-CSF, in memory Th2 cells. 

(A and B) Expression profile by RNA-seq of selected cytokine genes in Tpath2 cells. 

(C) Intracellular staining profiles of IL-3 and GM-CSF in restimulated polyclonal Tpath2 cells treated with or without TOFA. The percentages of IL-3+ cells or GM-CSF+ cells are shown with SD. For each group, n = 2 (A and B) or 4 (C) biologically independent samples are shown. More than three independent experiments were performed with similar results for C. Mean values with SD are shown for C. An unpaired two-tailed Student’s t test was applied for C. Statistical significance (P values) is indicated as ****, P < 0.0001.
Figure S4. **ACC1 controls MC903-induced skin inflammation via IL-3–mediated basophil activation.** (A) Experimental protocol for MC903-induced skin inflammation model. 1 nmol MC903 in 10 µl EtOH was applied topically on both sides of ear (total 2 nmol/20 µl per ear). (B) Intracellular staining profiles of IL-3 and ACC1 in ear CD45^+CD44^hiCD4^+ T cells. Histogram shows ACC1 expression of IL-3^+ or IL-3^- cells. Gray filled histogram indicates background, stained without primary antibody. (C) Intracellular staining profiles of IL-3 in ear CD45^+CD44^hiCD4^+ T cells. The percentage of IL-3^+ cells is shown. (D) Expression profiles of CD4 and CD44 on Acaca^-/- or Acaca^+/- skin CD45^+ cells. (E and F) Intracellular staining profiles of IL-5 in ear (E) or ear-dLN (F) CD45^+CD44^hiCD4^+ T cells. The percentage of IL-5^+ cells is shown. (G) The absolute number of skin basophils in Acaca^-/- or Acaca^-/- mice. (H) Experimental protocol for MC903-induced skin inflammation model with subcutaneous injection of mIL-3/anti-mIL-3 mixture or PBS. IL-3 mixture or PBS administration was performed on days 1, 3, 6, and 8 (details of reagent in Materials and methods). (I) Intracellular staining profiles of IL-3 in ear dLN CD44^hiCD4^+ T cells. The percentage of IL-3^+ cells is shown. (J) Confirmation of gene depletion in cells by tamoxifen-inducible and CD4-specific deletion of Acaca. PCR analysis indicates Acaca deletion in genomic DNA of ear CD45^+ cells (ERT-Cre^+ or ERT-Cre^-) and lymph-node CD4^+ cells (as positive control). Representative data are shown. (K) Intracellular staining profiles of IL-3 in stimulated ear CD45^+CD44^hiCD4^+ T cells. The percentage of IL-3^+ cells is shown. For each group, n = 3–4 (C and G); 4 (I and J); 5–6 (D–F); or 6 (B and K) biologically independent samples are shown. More than three independent experiments were performed with similar results for B–G and I–K. An unpaired two-tailed Student’s t test was applied for B–G, I, and K. Statistical significance (P values) is indicated as *, P < 0.05; **, P < 0.01; ***, P < 0.001; N.S., not significant.
Figure S5. **Acetate promotes chromatin accessibility at the Il3 locus in Acaca−/− Th2 cells.** (A) Intracellular staining profiles of IL-5 and IL-3 in restimulated Th2 cells treated with TOFA in the presence or absence of OA (50 µM) and glucose (50 mM). The percentage of IL-3+ cells is shown with SD. (B) Schematic representation of the murine Il3 locus. The locations of primers and exons are indicated. (C) Intracellular staining profiles of IL-3 in restimulated Th2 cells treated with or without Curcumin (2.5 µM). The percentage of IL-3+ cells is shown with SD. (D) Intracellular staining profiles of IL-3 in restimulated Th2 cells treated with or without TSA (3 nM). The percentage of IL-3+ cells is shown with SD. (E) Upper left: IL-33 stimulation induces fatty acid biosynthesis via the mTORC1-ACC1 axis in IL-5-producing Tpath2 cells. Fatty acid biosynthesis and maximal glycolytic capacity control a large amount of IL-5 production and eosinophilic inflammation in the lung. OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid. Lower left: Fatty acid biosynthesis in Tpath2 cells is inhibited by genetic deletion or pharmacologic inhibition of ACC1. Blockade of de novo fatty acid biosynthesis reduced glycolysis, which resulted in a dramatic decrease of IL-5 production in Tpath2 cells. Upper right: Antigenic TCR stimulation induces fatty acid (FA) biosynthesis via the mTORC1-ACC1 axis in IL-3–producing Th2 cells. Active fatty acid biosynthesis properly regulates the availability of cellular Ac-CoA into histone acetylation and induces IL-3 production in Th2 cells. Lower right: Fatty acid biosynthesis is suppressed by genetic deletion or pharmacologic inhibition of ACC1 in Th2 cells. As a result, cellular Ac-CoA likely feeds into the cellular pool of metabolites in the tricarboxylic acid cycle, which may decrease the usage of Ac-CoA for chromatin acetylation. The reduction of histone acetylation levels at the Il3 gene locus in Acaca−/− or TOFA-treated Th2 cells strongly decreases transcription and production of IL-3. For each group, n = 4 biologically independent samples are shown (A, C, and D). More than three independent experiments were performed with similar results for A, C, and D. Mean values with SD are shown for A, C, and D. An unpaired two-tailed Student’s t test was applied for A, C, and D. Statistical significance (P values) is indicated as ****, P < 0.0001.
Table S1 is provided online as an Excel file and shows specific primers and Roche Universal Probes used for quantitative RT-PCR of mouse genes.