Prevention of food allergy development and suppression of established food allergy by neutralization of thymic stromal lymphopoietin, IL-25, and IL-33

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Background: Food allergy (FA) is an increasing problem that has no approved treatment. The pro-T\(\text{H}2\) cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) are associated with FA, and mAbs to these cytokines are reported to suppress murine FA development.

Objective: We sought to determine whether anti–pro-T\(\text{H}2\) cytokine mAbs can block both FA maintenance and induction.

Methods: IgE-mediated FA was induced in BALB/c mice by oral gavage with medium-chain triglycerides (MCTs) plus egg white (EW) and was characterized by increased numbers of lamina propria T\(\text{H}2\) cells, mast cells, and eosinophils, shock (hypothermia), mast cell degranulation (increased serum mouse mast cell protease 1), increased serum IgG1 anti-EW and IgE levels, and increased IL-4 and IL-13 secretion after MCT/EW challenge. Mice were injected with anti–IL-25, IL-33 receptor, and/or TSLP mAbs before initial oral gavage with MCT/EW to suppress FA development; treatment with the same mAbs was initiated after FA development to suppress established FA. Results: Injection of an mAb to IL-25, IL-33 receptor, or TSLP strongly inhibited FA development. No single mAb to a pro-T\(\text{H}2\) cytokine could suppress established FA, and optimal FA suppression required treatment with a cocktail of all 3 anti–pro-T\(\text{H}2\) mAbs. Treatment with the 3-mAb cocktail during initial MCT/EW immunization induced EW tolerance.

Conclusion: All of the pro-T\(\text{H}2\) cytokines are required to induce our model of FA, whereas any pro-T\(\text{H}2\) cytokine can maintain established FA. Pro-T\(\text{H}2\) cytokines prevent oral tolerance. Combined treatment with antagonists to all 3 pro-T\(\text{H}2\) cytokines or with an inhibitor of pro-T\(\text{H}2\) cytokine production might be able to suppress established human FA. (J Allergy Clin Immunol 2018;141:171-9.)

Key words: Anaphylaxis, antibodies, cytokines, food allergy, mice, treatment, triglycerides

Food allergy (FA) affects approximately 8% of children and approximately 4% of adults in the United States, where it is responsible for 50,000 emergency department visits and approximately 150 deaths per year. 1-3 Furthermore, the incidence of FA has been rapidly increasing in the United States and other developed countries. 4,5 There is no approved therapy for this disorder other than avoidance of foods that cause allergic symptoms and injection of epinephrine once symptoms have developed.

In common with other allergic disorders, FA is primarily a type 2 cytokine disorder, with IL-4, IL-5, IL-9, and IL-13 having pathogenic roles in mouse models of this disease. 4,6-8 These cytokines induce FA by promoting IgE production, mastocytosis, eosinophilia, increased smooth muscle contractility, intestinal mastocytosis, and intestinal epithelial permeability.

Recently, 3 cytokines, thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, which can be produced by epithelial cells, 9,15,16 have been shown to act through multiple mechanisms on multiple cell types to promote a type 2 cytokine response 16-18; for this reason, we refer to them collectively as “pro-T\(\text{H}2\) cytokines.” The abilities of the pro-T\(\text{H}2\) cytokines to induce production of the T\(\text{H}2\) cytokines that are directly responsible for FA led to the hypothesis that the pro-T\(\text{H}2\) cytokines might be involved in FA induction and even maintenance. The hypothesis that pro-T\(\text{H}2\) cytokines are involved in FA induction is supported by results that have been published during the past few years by several teams of investigators 17-22; however, no published studies have
evaluated whether pro-Th2 cytokines are also important for FA maintenance.

Most of these observations that implicate pro-Th2 cytokines in patients with FA have been made in mouse models of this disease. One important variable in murine FA modeling has been the protocols used to induce disease. In general, these have either primed mice by inoculating them with food allergens through a nonenteric route (eg, the skin, lungs, or peritoneum [the latter with alum]) before challenging them orally23-25 or using a toxin (eg, chola toxin or staphylococcal enterotoxin B) as an oral adjuvant to sensitize mice to a coadministered food.26,27 Recently, we participated in a study that demonstrated that inoculation of mice with food (peanuts or ovalbumin) along with a common food constituent and additive, medium-chain triglycerides (MCTs), induces IgE-dependent peanut or ovalbumin FA, respectively, without requiring priming through a nonenteric route or the use of a conventional adjuvant.28 Studies of the mechanisms involved in FA induction with this protocol demonstrated that MCT ingestion increases intestinal epithelial permeability, as well as intestinal epithelial expression of each of the pro-Th2 cytokine genes.29 However, this study did not test whether any or all of the pro-Th2 cytokines were required for disease induction or maintenance in this system. We have now used the FA model to test the roles of each pro-Th2 cytokine in disease pathogenesis. Our results indicate that disease induction in this model can be blocked by inhibiting any of the pro-Th2 cytokines, whereas optimal suppression of established disease requires neutralization of all of these cytokines.

METHODS

Mice

Seven- to 8-week-old BALB/c female mice were purchased from the National Cancer Institute. Animal work was approved by the Cincinnati Children’s Hospital Research Foundation Institutional Animal Care and Use Committee.

Reagents

MCTs (Nestle Health Science, Epalinges, Switzerland) were purchased at a local pharmacy. Anti–IL-33 receptor (IL-33R) mAb (clone 28B) was obtained from Sijen Pharmaceuticals (Raritan, NJ), 28F12, a hybridoma that produces anti-TSLP mAb, was a gift of Dr Andrew Farr, University of Washington. Egg white (EW) removed under sterile conditions from organic hen’s eggs was dialyzed against double-distilled water and centrifuged for 20 minutes at 3900 relative centrifugal force. The supernatant was concentrated with a stirred ultrafiltration cell unit (Millipore, Temecula, Calif) with a 10-kDa Diallo membrane. Protein concentrations were evaluated with a BCA protein assay kit (Pierce, Rockford, Ill), according to the manufacturer’s protocol.

Immunofluorescence and flow cytometry

To identify cell types among lamina propria (LP) and mesenteric lymph node (MLN) cells, single-cell suspensions prepared from these tissues were first stained with phycoerythrin (PE)–conjugated anti–c–c-Kit (clone 2B8, BioLegend, San Diego, Calif), PE-Cy7–conjugated anti-FcRγI (clone MAR-1; BioLegend), allopredychycin (APC)–conjugated anti–IL-17RB, fluorescein isothiocyanate–conjugated anti–β integrin (clone M293; BD Biosciences, San Jose, Calif), V500-conjugated anti-CD4 (clone RM4-5; BD Biosciences), and APC-Cy7–conjugated anti-CD3 (clone 145-2C11; BioLegend). Subsequently, cells were counterstained with PerCP-Cy5.5–conjugated mAbs against the lineage markers CD80 (clone 53-6.7; BioLegend), B220 (clone RA3-6B2; BioLegend), CD11c (clone HL3; BD Biosciences), and Gran-1 (clone RB6-8C5; BD Biosciences). For identifying dendritic cells, LP or MLN cells were first stained with PE-conjugated anti–MHC class II (clone NIMR-4; eBioscience, San Diego, Calif), APC-Cy7–conjugated anti-CD11c (clone N418; eBioscience), fluorescein isothiocyanate–conjugated anti-CD103 (clone M290; BD Biosciences), Pacific Blue–conjugated anti-CD11b (clone M170; BD Biosciences), V500-conjugated anti–Gr–1 (clone RB6-8C5; BioLegend), PE-Cy7–conjugated anti-CD3 (clone 145-2C11; BD Biosciences), APC-conjugated anti–CXCR1 (R&D Systems, Minneapolis, Minn), and biotinylated antibodies against the lineage markers Ter119 and CD19 (clones TER-119 and 1D3, respectively; BD Biosciences). Subsequently, cells were counterstained with PE-Cy7–labeled streptavidin (BD Biosciences). After staining, cells were analyzed with a FACSCanto II (BD Biosciences). The following cell types were identified by using the following markers: Th2 cells, type 2 innate lymphoid cells (ILC2s), mast cells (MCs), basophils, eosinophils, and dendritic cells.

FA induction

Mice were inoculated by means of oral gavage through an 18-gauge needle with a spherical tip with 0.1 mL of MCTs on days 0 and 3 and then inoculated by means of oral gavage with an emulsion (produced by thorough mixing, followed by brief sonication) of 100 μL of MCTs and 100 mg of EW (total volume, 400 μL), as specified in the protocols diagrammed in Figs 1-5. Mice were fasted for 4 hours before each oral treatment.

Pro-Th2 cytokine blockade

IL-25, IL-33, and TSLP were blocked systemically by means of intraperitoneal injection of mice with the corresponding mAbs or 12 hours before each MCT or MCT/EW treatment. The quantities of blocking mAbs per week per mouse were based on preliminary studies that identified the doses required to block in vivo function: anti-TSLP, 0.5 mg; anti–IL-33R, 0.1 mg; and anti–IL-25, 0.5 mg.

Measurement of IL-4, IL-13, IFN-γ, antigen-specific IgG, IgE, and mouse mast cell protease 1 levels

In vivo IL-4 and IFN-γ cytokine secretion was measured by using an in vivo cytokine capture assay, as previously described.30,31 In vivo secretion of IL-13 was measured by using a similar procedure, except that mice were injected with 2 μg of biotin-labeled anti–IL-13 mAb (clone 54D1), and ELISA wells were coated with anti–IL-13 mAb 53F5 (both mAbs were obtained from AbVie, North Chicago, Ill). EW-specific IgG1 levels were measured by using an ELISA in which ELISA plates (Costar, Cambridge, Mass) were coated with EW (10 μg/mL) overnight and then washed and loaded with serial dilutions of mouse sera. After washing, wells were loaded sequentially with 1 μg/mL biotin–anti-mouse IgG1 (eBioscience), followed by 100 ng/mL horseradish peroxidase–streptavidin and SuperSignal ELISA

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<tr>
<td>APC: Allophycocyanin</td>
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<tr>
<td>EW: Egg white</td>
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<tr>
<td>FA: Food allergy</td>
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<tr>
<td>ILC2: Type 2 innate lymphoid cell</td>
</tr>
<tr>
<td>IL-33R: IL-33 receptor</td>
</tr>
<tr>
<td>LP: Lamina propria</td>
</tr>
<tr>
<td>MC: Mast cell</td>
</tr>
<tr>
<td>MCT: Medium-chain triglyceride</td>
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<tr>
<td>MLN: Mesenteric lymph node</td>
</tr>
<tr>
<td>MMCPI: Mouse mast cell protease 1</td>
</tr>
<tr>
<td>PE: Phycoerythrin</td>
</tr>
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<td>TSLP: Thymic stromal lymphopoietin</td>
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substrate, peroxide, and enhancer solution diluted 20-fold in 20 mmol/L Tris-Saline (pH 7.2; Pierce Biotechnology, Rockford, Ill). Serum levels of mouse mast cell protease 1 (MMCP1) and IgE were measured with the corresponding ELISA kits (eBioscience), according to the manufacturer’s protocols.

Anaphylaxis
The severity of anaphylactic shock was assessed by a change in rectal temperature measured by means of digital thermometry. 32,33

Statistics
Differences in temperature and concentrations of MMCP1, IL-4, IL-13, IFN-γ, IgE, and IgG1 anti-EW antibody were compared by using the Student t test (GraphPad Prism 4.0; GraphPad Software, La Jolla, Calif). A 1-tailed test was used to test hypotheses that MCT/EW immunization would increase the parameters studied, that an anti–pro-T H2 cytokine mAb or mAbs would decrease these parameters, and that increasing the number of anti–pro-T H2 cytokine mAbs used would further decrease these parameters. A 2-tailed t test was used to compare cell numbers (Fig 5). A P value of less than .05 was considered significant.

RESULTS
Pro-TH2 cytokine antagonists have a lasting effect on FA development
To determine whether our MCT/ovalbumin model of FA could be inhibited by systemic treatment with a combination of

FIG 1. Pro-TH2 cytokine antagonists have a lasting effect on FA development. A, BALB/c female mice, 4 to 6 per group, were inoculated by oral gavage with 100 μL of MCTs on days 0 and 3 and then inoculated by oral gavage with MCT/EW emulsion every other day for 3 weeks. One group was injected intraperitoneally with a cocktail of anti-TSLP/anti–IL-33R/anti–IL-25 mAbs 12 hours before each MCT/EW dose, whereas the other group was injected intraperitoneally with isotype control mAbs. B, Rectal temperatures were determined for the hour after the last oral gavage inoculation (upper left panel), and mice were bled 4 hours after this inoculation. Treatment with anti–pro-TH2 cytokine mAbs and isotype control mAbs was then discontinued, but all mice were inoculated by oral gavage every other day for an additional 5 weeks with MCT/EW. Mice were again followed for decreases in rectal temperature for 1 hour after the last oral gavage inoculation (upper right panel). C, Mice were again bled 4 hours after this oral gavage inoculation, and total IgE, EW-specific IgG1, and MMCP1 levels were evaluated by means of ELISA. *P < .05 compared with isotype control–treated mice.
neutralizing mAbs to all of the pro-Th2 cytokines, we inoculated BALB/c female mice by oral gavage with MCTs on days 0 and 3 and then used oral gavage every other day with an MCT/EW emulsion. Mice in one group also received intraperitoneal injections of a combination of anti–IL-25, anti–IL-33R, and anti-TSLP mAbs 12 hours before each oral gavage inoculation with MCTs or MCT/EW, whereas mice in the other group were injected intraperitoneally with isotyope-matched control mAbs. After 3 weeks, mice that had received isotype control mAbs experienced an approximately 4°C decrease in rectal temperature by 30 minutes after oral gavage with MCT/EW (which was shown in a separate experiment to be IgE dependent, see Fig E1 in this article’s Online Repository at www.jacionline.org), whereas the temperature decrease after oral challenge was approximately 1.2°C in mice that had been treated with the anti–pro-Th2 mAb cocktail (Fig 1). This suppressive effect reflected a greater than 10-fold decrease in serum MMCP1 levels, which reflects mucosal MC degranulation, and IgG1 anti-EW antibody levels, as well as an approximately 3-fold decrease in total serum IgE levels. This suppressive effect was persistent; when these mice were inoculated by oral gavage with EW/MCT for an additional 5 weeks in the absence of mAb injections, the mice that had initially been treated with anti–pro-Th2 mAbs continued to show considerable suppression of development of shock and IgG1, IgE, and MMCP1 responses (Fig 1).

**FIG 2.** IL-25, IL-33, and TSLP are all required for development of FA in EW plus MCT–inoculated mice. A, BALB/c mice, 4 to 6 per group, were fasted for 4 hours and left untreated or inoculated by oral gavage with 100 μL of MCT on days 0 and 3. MCT-treated mice were then inoculated by oral gavage with MCT/EW emulsion every other day for 3 weeks. B, Mice were also injected intraperitoneally 12 hours before each MCT/EW inoculation with anti-TSLP mAb, anti–IL-25 mAb, anti–IL-33R mAb, a cocktail of anti-TSLP/anti–IL-33/anti–IL-25 mAbs, or isotype control mAbs 12 hours before each MCT/EW dose. Rectal temperatures were determined for the hour after the last oral gavage inoculation. Mice were bled 4 hours after this inoculation. C, IL-4, IL-13, and IFN-γ secretion was evaluated by using an in vivo cytokine capture assay, whereas serum levels of MMCP1, IgE, and IgG1 anti-EW were determined by using an ELISA. In this and other figures, brackets with asterisks indicate a statistically significant \( P < .05 \) difference between the groups connected by the bracket.
IL-25, IL-33, and TSLP are all required for FA development in EW plus MCT–inoculated mice

To determine which of the pro-T\(\text{H}_2\) cytokines are required for FA development in our model, mice were not immunized or were inoculated by means of oral gavage with MCTs and then EW/MCT, as in our initial experiment, and were treated intraperitoneally with isotype control mAbs or anti–TSLP, anti–IL-25, or anti–IL-33R mAbs or a combination of all 3 (Fig 2, A). After 3 weeks of this treatment, shock (>1°C of hypothermia) in response to EW/MCT challenge developed in mice treated with the control mAbs but not in mice treated with any of the anti–pro-T\(\text{H}_2\) cytokine mAbs (Fig 2, B). Suppression of development of shock (hypothermia) was complete in mice treated with anti-TSLP mAb, anti–IL-25 mAb, or the mAb cocktail, whereas a small temperature decrease was seen in anti–IL-33R mAb–treated mice. Anti-TSLP mAb suppressed IL-4 and IL-13 responses to basal levels and was more effective than either anti–IL-25 or anti–IL-33R mAb at suppressing the IL-4 and MMCP1 responses (Fig 2, C). Anti-TSLP and anti–IL-33R mAbs were more effective than anti–IL-25 mAb at suppressing IL-13 production. The mAb cocktail was slightly more effective than any of the single mAbs at suppressing the MMCP1 response but otherwise resembled anti-TSLP mAb in its effects; there was a nonsignificant trend toward decreased MMCP1 levels in anti–IL-25 and anti–IL-33 mAb–treated mice. Importantly, the effects of the anti–pro-T\(\text{H}_2\) cytokines resulted from suppression of the T\(\text{H}_2\) response without a corresponding shift to a T\(\text{H}_1\) response, as judged from the lack of a significant increase in IFN-\(\gamma\) secretion in anti–pro-T\(\text{H}_2\) cytokine mAb–treated mice (Fig 2, C).

Established FA is effectively suppressed by an anti–pro-T\(\text{H}_2\) mAb cocktail

Because induction of our model of FA was most effectively suppressed by either anti-TSLP mAb or a cocktail of all 3 anti–pro-T\(\text{H}_2\) cytokine mAbs, we evaluated the ability of each of these mAb treatments to suppress FA that had been established by means of oral gavage inoculation of mice with MCTs and then

FIG 3. Established FA is suppressed by an anti–pro-T\(\text{H}_2\) mAb cocktail. A, BALB/c mice were fasted for 4 hours and sensitized with 2 oral doses of MCTs on days 0 and 3. Then mice were treated with MCT/EW emulsion every other day for 4 weeks. Mice that had a 4°C maximum temperature decrease were divided into 3 groups of 5 mice per group. All groups were inoculated by oral gavage with MCT/EW emulsion twice a week for 4 more weeks. The different groups were also injected intraperitoneally with anti-TSLP mAb, with the cocktail of anti-TSLP/anti–IL-33R/anti–IL-25 mAbs, or with isotype control mAbs 12 hours before each MCT/EW inoculation. B, Decreases in rectal temperature were determined for the hour after the last MCT/EW inoculation. C, Mice were bled 4 hours after the last oral gavage inoculation for determination of serum MMCP1 levels (Fig 3, C) and serum IgE levels and IgG1 anti-EW titers (Fig 3, D). *\(P < .05\).
FIG 4. Combined pro-T2 cytokine blockade is required for effective suppression of established FA. A, BALB/c mice were fasted for 4 hours and then inoculated by oral gavage with 100 μL of MCTs on days 0 and 3. Mice were then kept unimmunized or were inoculated by oral gavage with MCT/EW emulsion twice a week for 4 weeks. Mice that had significant shock (more than 4°C maximum temperature decrease) were divided into 5 groups of 5 mice per group. All groups were then inoculated by oral gavage with MCT/EW emulsion twice a week for an additional 3 weeks. Different groups of MCT/EW-immune mice were injected intraperitoneally with the following mAb combinations 12 hours before each oral gavage inoculation with MCT plus EW: anti-TSLP plus anti–IL-33R mAb, anti-TSLP plus anti–IL-25 mAb, anti–IL-25 plus anti–IL-33R mAb, anti-TSLP plus anti–IL-33R plus anti–IL-25 mAb, or isotype control mAbs. B, Maximal decreases in rectal temperature were determined for the hour after the oral gavage inoculation just before initiation of mAb treatment (day 0) and for the hour after the oral gavage inoculations after 14 and 24 days of mAb treatment. C, Mice were bled 4 hours after the day 24 oral gavage inoculation to determine IL-4 and IL-13 secretion, MMCP1 response, and serum IgE and IgG1 anti-EW levels. *P < .05.
EW/MCT for a total of 4 weeks before initiation of mAb treatment (Fig 3, A). Mice were then inoculated by means of oral gavage with MCT/EW for an additional 4 weeks but also received one of the intraperitoneal mAb treatments. At the end of this 4-week treatment period, the hypothermia response to EW/MCT oral challenge was not affected by anti-TSLP mAb by itself but was considerably suppressed by the mAb cocktail (Fig 3, B and D). In the same experiment the MMCP1 response to MCT/EW challenge was not affected by anti-TSLP mAb alone but was suppressed by approximately 80% by the mAb cocktail (Fig 3, C); the cocktail was also more effective than anti-TSLP mAb alone at suppressing serum IgE and IgG1 anti-EW antibody levels (Fig 3, B and D).

In an additional experiment with mice that were induced to have FA before initiation of mAb treatment (Fig 4), 24 days of treatment with the mAb cocktail totally suppressed the development of shock and decreased the MMCP1 response to oral challenge by greater than 90%. The same treatment decreased IL-4 and IL-13 responses to oral challenge by 80% to 90% and total serum IgE and IgG1 anti-EW antibody levels by approximately 50%. A combination of anti-TSLP and anti-IL-33R mAbs showed less complete ability to suppress FA in this time frame, whereas combinations of anti-TSLP and anti-IL-25 or anti-IL-25 and anti-IL-33R mAbs were even less effective (Fig 4).

**DISCUSSION**

Our studies with MCT/EW-induced FA have resulted in 5 important findings that build on our previous observation that MCTs induce an intestinal epithelial IL-25, IL-33, and TSLP response:\n
1. treatment with a blocking mAb to any of these pro-Tg2 cytokines inhibits FA development; \n2. treatment with a cocktail of all 3 pro-Tg2 cytokine–blocking mAbs during oral exposure of immunologically naive mice to MCT/EW leads to EW tolerance instead of FA; \n3. treatment with all 3 mAbs is required to optimally suppress established FA; \n4. induction of FA in our system is accompanied by increases in LP Tg2 cells, MCs, eosinophils, and dendritic cells but not ILC2s; and \n5. increases in Tg2 cell, MC, and eosinophil numbers are

**Maintenance of cellular changes in mice with FA is pro-Tg2 cytokine dependent**

To evaluate the cellular changes that accompany the development of FA in our model, we inoculated mice twice a week by means of oral gavage for 5 weeks to induce FA (defined as a temperature decrease >2°C in response to oral gavage challenge) and then continued these oral gavage inoculations for an additional 5 weeks but injected mice intraperitoneally with all 3 anti–pro-Tg2 cytokine mAbs or isotype control mAbs 4 hours before each oral gavage inoculation (Fig 5, A). At the end of this 10-week period, control mAb–treated mice but not anti–pro-Tg2 cytokine mAb–treated continued to have hypothermia in response to oral gavage MCT/EW (data not shown). Studies of LP and MLN cells obtained at this time showed large significant increases in numbers of Tg2 cells and MCs and smaller significant increases in numbers of eosinophils and dendritic cells in the isotype control mAb–treated mice (Fig 5, B). No increases in ILC2 numbers were observed compared with those in untreated mice. Treatment with the cocktail of anti–pro-Tg2 cytokine mAbs suppressed increases in LP Tg2 cell, MC, and eosinophil numbers but not the increase in dendritic cell numbers. Induction of FA did not significantly increase any of these cell populations in MLNs (Fig 5, B).
suppressed by anti–pro-TH2 cytokine mAb treatment. Thus all 3 pro-TH2 cytokines are required to induce FA in our model, and once induced, any of these cytokines is sufficient to at least partially maintain this disorder.

Several previous studies have investigated the importance of the pro-TH2 cytokines for FA induction. Studies in which sensitization to peanut or ovalbumin was induced by means of epicutaneous administration of these antigens without additional adjuvants revealed requirements for IL-33 and TSLP in one case without investigating whether IL-25 was required \(^{18}\) or for TSLP and IL-25 in another case without investigating whether IL-33 was required. \(^{20}\) Additional studies that used a similar approach for sensitization reported a requirement for TSLP without investigating whether there was also a requirement for IL-25 or IL-33. \(^{17}\) These results are consistent with the results of our MCT/EW model in that, when taken together, they suggest that all 3 pro-TH2 cytokines are required to induce FA in the absence of additional adjuvants. In contrast, studies that used cholera toxin as an adjuvant for FA induction reported a requirement for IL-33 but not TSLP \(^{19}\); this suggests that at least some of the adjuvants that have commonly been used in murine FA models can bypass the requirement for the latter cytokine.

Although several studies have examined pro-TH2 cytokine requirements for FA induction, to the best of our knowledge, ours is the first to evaluate the clinically relevant question of whether pro-TH2 cytokines are required to maintain established FA. Because pro-TH2 cytokines are known to be important in the induction of type 2 cytokine production by TH2 cells \(^{16}\) and established TH2 cells can lose their ability to switch to production of other cytokines, \(^{34}\) it was possible that the pro-TH2 cytokines had little importance in maintaining type 2 cytokine–dependent FA. Indeed, had we only neutralized 1 pro-TH2 cytokine at a time, we might have reached that conclusion because neither anti-TSLP mAb alone (Fig 3) nor the combination of anti–IL-25 and anti–IL-33 mAbs (Fig 4) had a significant effect on established FA; even the combination of anti–IL-33 and TSLP mAbs had only a moderate suppressive effect. Only the combination of mAbs to all 3 pro-TH2 cytokines was able to strongly suppress established FA within 3 to 4 weeks (Fig 4). This suggests that once established, any of the pro-TH2 cytokines can maintain FA, at least to some extent. It is not yet known whether the pro-TH2 cytokine contribution to FA maintenance reflects a need for these cytokines to maintain type 2 cytokine production by TH2 cells. If so, this would suggest that TH2 commitment is not irreversible in vivo or that persistent cytokine production and/or survival of this population or its replacement with fresh TH2 cells requires continuing pro-TH2 cytokine production. Alternatively, continuing pro-TH2 cytokine stimulation of type 2 cytokine production by ILC2s, basophils, and/or MCs might be required to maintain a sufficient type 2 cytokine response to permit FA persistence. In either case, to the extent that our results reflect the pathophysiology of human FA, they suggest that successful therapy would require inhibition of the pro-TH2 cytokine triad or suppression of the production of all 3 of these cytokines, perhaps through an effect on intestinal epithelial cells. However, it is possible that studies with different animal models of FA would show different pro-TH2 cytokine dependence for FA maintenance.

Our observations also demonstrate the cellular changes that accompany the development of our model of FA and the pro-TH2 cytokine requirement for maintenance of these changes. After 10 weeks of MCT/EW inoculation, we saw large increases in TH2 and MC numbers and smaller increases in eosinophil and dendritic cell numbers in the LP of treated mice without any increase in LP ILC2 or basophil numbers or an increase in any cell type in MLNs. These changes are similar to those observed in FA models that relied on intraperitoneal inoculation with alun or epicutaneous immunization to prime for FA development. \(^{12}\) More importantly, our data demonstrate that 5 weeks of treatment with mAbs that block all 3 pro-TH2 cytokines prevents or, more likely, reverses the increases in LP TH2 cell, MC, and eosinophil numbers but not dendritic cell numbers. The lack of association of FA models with an increase in LP ILC2 numbers is surprising in view of the potent pro-TH2 cytokine effects on ILC2 survival and proliferation \(^{37}\) and the considerable increase in ILC2 numbers in intestinal worm infection models. \(^{35-39}\) Our results suggest that 1 or more additional stimuli induced by worm infection but not the FA models is required for proliferation of these cells. They also suggest that cytokine production by TH2 cells and MCs might be the predominant source of TH2 cytokines in the FA models, although it is also possible that the pro-TH2 cytokines produced in these models increase ILC2 cytokine production without driving their proliferation.

The relevance of our observations to human FA is also uncertain; few studies of pro-TH2 cytokines have been performed in human subjects with FA, although TSLP and IL-33 have been associated with human eosinophilic esophagitis, \(^{40,41}\) and all 3 of the pro-TH2 cytokines have been associated with human atopic dermatitis, \(^{42-44}\) an FA-associated skin disorder. \(^{45,46}\) We are aware of only 2 clinical trials of antibodies to pro-TH2 cytokines in human allergic disorders: anti-TSLP mAb inhibited responses to allergen challenge in a small trial in patients with mild asthma, \(^{47}\) whereas an anti–IL-17RA mAb, which was designed to inhibit responses to IL-25 and other IL-17 family cytokines, had no significant effect in patients with moderate-to-severe asthma. \(^{48}\) No clinical trials with agents designed to suppress more than 1 pro-TH2 cytokine have been reported in PubMed or ClinicalTrials.gov; the results of our study suggest that such human trials might be required for clinically relevant suppression of established FA.

We thank Dr Andrew Farr for his gift of an anti-mouse TSLP mAb–secreting hybridoma, Janssen Pharmaceuticals for providing purified mAbs to mouse IL-25 and mouse IL-33, and AbbVie for providing purified antibodies to mouse IL-13.

Clinical implications: IL-25, IL-33, and TSLP are critical for murine FA induction and maintenance. Neutralization or blocking of these cytokines might provide a way to suppress established FA in human subjects.

REFERENCES


FIG E1. Development of hypothermia in response to ingested antigen is IgE dependent in mice with FA. BALB/c mice (4 per group) were inoculated by oral gavage with 100 μL MCTs for 3 days and then with MCT/EW until they had hypothermia in response to oral gavage inoculation. Mice were then injected intraperitoneally with 500 μg of anti-IgE mAb (EM-95), 500 μg of anti-FcγRIIB/RIII mAb (2.4G2), both mAbs, or isotype control mAbs. One day later, mice were challenged by means of oral gavage with MCT/EW, and rectal temperatures were followed for the next 60 minutes. *P < .05.