DISCUSSION

Multiple lines of evidence support the concept that dysregulation of the intestinal immune response to commensal microbes is a predisposing factor for inflammatory bowel disease[5,29–31]. Such chronic inflammatory responses compromise the homeostasis of the intestinal ecosystem and often result in dysbiosis[32,33]. Therefore, commensal microbes may have undergone adaptation to curtail host immune responses over the course of coevolution[1–3]. We have now demonstrated that bacterial colonization induced an early IL-2 response in the colonic mucosa that in turn led to the accumulation of T_{reg} cells, at least in part through vigorous proliferation, that overwhelmed the activation of T_{eff} cells. The local proliferative activity of colonic T_{reg} cells was maximal before weaning and gradually decreased with age. This suggested that vigorous proliferation of colonic T_{reg} cells was induced early after birth in parallel with the establishment of the commensal microflora. This model was congruent with the observation that the expression of *Uhrf1* was much higher in colonic T_{reg} cells from 2-week-old infant mice than in those from adult mice. Similarly, *Uhrf1* expression by colonic T_{reg} cells was upregulated after the inoculation of GF mice with intestinal microflora. In contrast, *Uhrf1* expression by splenic T_{reg} cells of the same mice remained unchanged before and after the inoculation, consistent with the minimal proliferative response in the spleen. Thus, the expression of *Uhrf1* was positively correlated with the proliferative activity of T_{reg} cells, and Uhrf1 deficiency had a substantial effect on the local population expansion of T_{reg} cells in response to bacterial colonization. The data as a whole supported our conclusion that local proliferation of T_{reg} cells was the main downstream consequence of Uhrf1 expression. Notably, the ablation of Uhrf1 had a substantial effect on the suppressive function of T_{reg} cells. We propose that this defect was due to the compromised proliferative response of Uhrf1-deficient T_{reg} cells, because proliferating T_{reg} cells had higher expression of functional molecules than did cells in the nonproliferative compartment. Therefore, colonic tissue acts as a privileged site in conferring functional maturity on T_{reg} cells. Given that Uhrf1-deficient mice spontaneously developed colitis, this immunoregulatory mechanism ensured by Uhrf1-dependent proliferation of T_{reg} cells was essential for the establishment of a symbiotic host-microbe relationship without inflammation.

Our data identified Uhrf1 as an IL-2-responsive molecule. In the intestine, both T cells and dendritic cells can produce IL-2 (refs. 34,35). We also confirmed that IL-2 was produced by both T cell populations and non-T cell populations, among which $CD4^+$ T cells mainly contributed to IL-2 production after colonization by commensals (data not shown). We found that colonization with the '17-mix' strains of Clostridia from human feces[23] drove T_{conv} cells to produce IL-2, which in turn upregulated *Uhrf1* in T_{reg} cells; this resulted in their active proliferation. In our *ex vivo* experiments, T_{conv} cells from mice colonized with 17-mix produced IL-2 only in the presence of autoclaved 17-mix (data not shown), which indicated that some of the T_{conv} cells in the mice colonized with 17-mix produced IL-2 in an antigen-specific manner. These observations raise the possibility that stimulation of T cells through the T cell antigen receptor with bacterial antigens may initiate activation of the IL-2–Uhrf1 pathway.

Like Uhrf1-deficient mice, mice lacking either IL-2 or one of its receptors (IL-2Rα or IL-2Rβ) spontaneously develop chronic colitis due to an excessive response to commensal bacteria[36,37]. Moreover, these mice develop lethal lymphoid hyperplasia and autoimmune disorders characterized by hemolytic anemia[38,39]. We did not observe such systemic autoimmune disorders in Uhrf1-deficient mice. Therefore, among the many biological functions of IL-2, the role of the IL-2–Uhrf1 pathway is itself confined to the maintenance of gut immunological homeostasis. Given that genetic polymorphisms in *IL2* and *IL2RA* are closely associated with the development of human inflammatory bowel disease[40], our findings may provide molecular insight into the pathogenesis of this disease.

We identified *Cdkn1a* (which encodes p21) as a target of Uhrf1 and showed the importance of the Uhrf1-p21 axis in the proliferation of T_{reg} cells. p21 has a vital role in controlling the proliferation, differentiation and tumorigenesis of many cell types[41]. The mechanisms for the regulation of *Cdkn1a* transcription are not yet fully elucidated, although it seems to be regulated via multiple pathways that may be different in various cell types. A possible link between Uhrf1 and p21 has been reported in embryonic stem cells and HeLa human cervical cancer cells[42]. The authors of that study[42] speculate that Uhrf1 recruits the histone lysine methyltransferase G9a to the *Cdkn1a* promoter to achieve accumulation of the repressive histone modification H3K9me2. *Cdkn1a* has a proximal promoter and a distal promoter in which CpG islands and a CpG cluster, respectively, are present. In intestinal epithelial cells, the proximal promoter is almost completely unmethylated; however, the distal promoter is partially methylated, which is negatively correlated with *Cdkn1a* expression[43]. In agreement with that observation, deficiency in Uhrf1 led to aberrant expression of *Cdkn1a* due to hypomethylation of its distal promoter region in T_{reg} cells. It is well documented that signaling via transforming growth factor-β (TGF-β) transactivates *Cdkn1a* expression as a canonical pathway[44,45]. Given that TGF-β, which is abundant in the intestinal tissue, is essential for the induction and maintenance of T_{reg} cells, it is conceivable that intestinal T_{reg} cells may be under continuous pressure to upregulate *Cdkn1a*. In this context, Uhrf1-dependent methylation of CpG sites may function to prevent the unwanted *Cdkn1a* expression that leads to a disadvantage in the progression of T_{reg} cells through the cell cycle.

Taking all of the observations noted above into account, we propose a model for establishment of gut immunological homeostasis based on reciprocal interaction between T_{reg} cells and T_{eff} cells. First, colonizing bacteria should be initially recognized by antigen-presenting cells such as dendritic cells. Second, the antigen-loaded antigen-presenting cells elicit an early IL-2 response by stimulating T_{eff} cells through antigen presentation. Third, the early IL-2 reponse provides a cue for T_{reg} cells to proliferate and simultaneously upregulate *Uhrf1* expression. Fourth, Uhrf1 represses the cell cycle–dependent kinase inhibitor p21 via methylation of *Cdkn1a* (which encodes p21) to safeguard the continuing proliferation of T_{reg} cells. Fifth, the actively proliferating T_{reg} cells become functionally mature and in turn prevent excessive immune responses to the colonizing microbiota. In conclusion, our study has provided a new mechanistic link between proliferation-dependent maturation of T_{reg} cells and containment of the inflammatory response to commensal microbiota.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. GEO: microarray and MeDP-Sequencing analysis data, GSE56544.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.O. and Y.F. did a large part of the experiments together with D.T., K.A., Y.F., M.T., T.I., T.O., Y.I.K. and K. Ha.; Y.O., Y.F., T.A.E. and J.S. analyzed the data; M.N., S.T. and S.H. provided materials; S.O. prepared GF mice; T.D., H.M., O.O., K. Ho., H.O. and H.K. provided experimental protocols and intellectual input into the study; T.D. and H.O. edited the manuscript; K. Ha. and H.K. conceived of the study; and K. Ha. designed the experiments, analyzed the data and wrote the manuscript (together with Y.O. and Y.F.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animal experiments. *Uhrf1*$^{fl/fl}$ mice (generated as in **Supplementary Fig. 2**) were backcrossed onto a C57BL/6 background. For the generation of mice with T cell-specific Uhrf1 deficiency, *Uhrf1*$^{fl/fl}$ mice were crossed with *Cd4*-Cre mice (The Jackson Laboratory) and then *Foxp3*^{hCD2} mice[14]. *Uhrf1*$^{fl/fl}$*Cd4*-Cre*Foxp3*^{hCD2} mice were housed under SPF conditions unless otherwise specified. IQI mice (CLEA Japan) were maintained in GF conditions in vinyl isolators in the animal facilities of the RIKEN Center for Integrative Medical Sciences and Graduate School of Medical Life Science, Yokohama City University. Feces from SPF C57BL/6 mice were suspended in PBS or were treated with 3% (vol/vol) chloroform in PBS to generate chloroform-resistant bacteria, and GF IQI and *Uhrf1*$^{fl/fl}$*Cd4*-Cre *Foxp3*^{hCD2} mice were inoculated with aliquots of those suspensions by intragastric intubation[1]. Mice treated with chloroform-resistant bacteria were maintained in the gnotobiotic vinyl isolator for 3–4 weeks. Gnotobiotic mice associated with the 17-strain mixture of Clostridia (17-mix) were generated as described[23].

For inhibition of the homing of extraintestinal T_{reg} cells to the gut[11], exGF mice were treated with a mixture (100 μg each per mouse) of mAb to integrin α4 (PS/2; Millipore) plus mAb to integrin β_7 (FIB504; Biolegend) or with control IgG (400533; Biolegend) on day 3 after bacterial colonization. The exGF mice were then subjected to an *in vivo* EdU-incorporation assay as described below.

Systemic population expansion of T_{reg} cells was induced as described[25]. SPF *Uhrf1*$^{fl/fl}$*Cd4*-Cre*Foxp3*^{hCD2} mice and their *Uhrf1*$^{+/+}$*Cd4*-Cre*Foxp3*^{hCD2} littermates were given intraperitoneal injection of complexes of IL-2 and mAb to IL-2 (JES6-1A12; R&D Systems) three times on days 0, 1 and 2, and proliferation of splenic T_{reg} cells was analyzed on day 5.

Protocols approved by Animal Studies Committees of RIKEN Yokohama Institute, The Institute of Medical Science, The University of Tokyo and Graduate School of Medical Life Science, Yokohama City University, were used for all animal experiments.

Preparation of lymphocytes. Lymphocytes from the cLP were prepared as described[46]. Colonic tissues were treated at 37 °C for 20 min with Hanks' balanced-salt solution (Wako Pure Chemical Industries) containing 1 mM dithiothreitol and 20 mM EDTA for removal of epithelial cells. The tissues were then minced and were dissociated for 30 min at 37 °C with collagenase solution containing 0.5 mg/ml collagenase (Wako Pure Chemical Industries) and 0.5 mg/ml DNase I (Roche Diagnostics), 2% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 12.5 mM HEPES, pH 7.2, in RPMI-1640 medium (Sigma-Aldrich) to obtain single-cell suspensions. After filtration, the single-cell suspensions were washed with 2% FCS in RPMI-1640 medium and were subjected to Percoll gradient separation. The spleen and mesenteric lymph nodes were mechanically disrupted into single-cell suspensions.

For quantitative PCR analysis, colonic mononuclear cells was subjected to cell sorting using FACSAriaII to isolate CD3ε$^+$CD4$^+$CD25$^+$FR4$^+$ or CD3ε$^+$CD4$^+$hCD2$^+$ T_{reg} cells, in IQI or Foxp3^{hCD2} reporter mice, respectively. Our preliminary experiments demonstrated that the CD3ε$^+$CD4$^+$CD25$^+$FR4$^+$ population almost exclusively consists of Foxp3$^+$ cells, consistent with a previous report[47].

Flow cytometry. The following mAbs were conjugated to biotin, fluorescein isothiocyanate, Alexa Fluor 488, phycoerythrin, peridinin chlorophyll protein–cyanine 5.5, phycoerythrin-indotricarbocyanine, allophycocyanin, Alexa Fluor 647, Alexa Fluor 700, allophycocyanin-Hilite7, eFluor 450, Pacific blue, Brilliant violet 421 or V500: anti–human CD2 (RPA-2.10), mAb to mouse CD25 (PC61), mAb to mouse CD44 (IM7), mAb to mouse CD45R/B220 (RA3-6B2), mAb to mouse CD62L (MEL-14), mAb to mouse Gr1 (RB6-8C5), mAb to mouse IL-2 (JES6-5H4), mAb to mouse interferon-γ (XMG1.2), mAb to mouse tumor-necrosis factor (MP6-XT22) and mAb to mouse Ter119 (TER-119; all from Biolegend); mAb to mouse CD3ε (145-2C11), mAb to mouse folate receptor 4 (eBio12A5), mAb to mouse Foxp3 (FJK-16s), mAb to mouse CTLA-4 (UC10-4B9) and mAb to mouse IL-10 (JES5-16E3; all from eBioscience); and mAb to mouse CD4 (GK1.5), mAb to mouse IL-17A (TC11-18H10.1) and mAb to mouse Ki67 (B56; all from BD Bioscience). Biotinylated polyclonal antibody to mouse Nrp1 (BAF566) was from R&D Systems.

For intracellular staining of cytokines, lymphocytes from the LP were cultured for 6 h in complete medium (RPMI-1640 medium containing 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 55 μM mercaptoethanol and 20 mM HEPES, pH 7.2) supplemented with 50 ng/ml PMA, 500 ng/ml ionomycin and GolgiPlug (BD Bioscience). The lymphocytes were then stained with mAb to CD3ε, mAb to CD4 and mAb to human CD2 (all identified above), followed by intracellular staining of interferon-γ, IL-17A, tumor-necrosis factor and IL-10 (antibodies identified above) with a Cytofix/Cytoperm kit (BD Bioscience). The stained samples were analyzed with a FACSCanto II or FACSAria II and with DIVA software (BD Biosciences) and FlowJo software, version 9.3.2 (Tomy Digital Biology).

***In vivo* EdU-incorporation assay.** For the detection of proliferating cells *in vivo*, GF and exGF mice received intraperitoneal injection of 3 mg EdU (5-ethynyl-2′-deoxyuridine) in 200 μl PBS, followed by administration of drinking water containing 0.8 mg/ml EdU for 2 d before the analysis. cLP cells that had incorporated EdU were visualized with a Click-it EdU Flow cytometry kit according to the manufacturer's instructions (Invitrogen).

Gene-expression profiling. Total RNA was extracted with TRIzol reagent (Life Technologies) according to a standard protocol and was subjected to microarray analysis with a GeneChip Mouse Genome 430 2.0 Array (Affymetrix). The data sets obtained were analyzed with GeneSpring GX 11 software (Agilent) and the Ingenuity pathway-analysis program (Ingenuity Systems).

Cell culture. CD3$^+$CD4$^+$CD44^{lo}CD62L^{hi} naive T cells were prepared from the spleen and lymph nodes by cell sorting as described above. Isolated naive CD4$^+$ T cells (5×10^5 cells per ml) were cultured for 3 d in complete RPMI-1640 medium supplemented with 5 ng/ml TGF-β and 10 ng/ml IL-2 (R&D Systems) and Dynabeads coated with mAb to CD3 and mAb to CD28 (Life Technologies) to induce differentiation into Foxp3$^+$ cells, then populations of differentiated cells were expanded up to an additional 4 d in the presence of 0.5 ng/ml TGF-β and 10 ng/ml IL-2. For cell-cycle analysis, induced T_{reg} cells were pulsed for 2 h with 10 μM EdU (Invitrogen). The cells were stained for EdU and 7-amino-actinomycin D with a Click-iT EdU flow cytometry kit before cell-cycle analysis with a FACSCanto II (BD) and FlowJo software, version 9.3.2 (Tomy Digital Biology).

***In vitro* suppression assays.** naive populations of CD3ε$^+$CD4$^+$hCD2$^+$ cells and CD3ε$^+$CD4$^+$CD62L^{hi}CD44^{lo} cells were purified as T_{reg} cells and responder cells, respectively, with the IMag Cell Separation System followed by cell sorting. For the preparation of antigen-presenting cells, splenocyte samples from C57BL/6J mice were depleted of Thy-1.2$^+$ cells and were irradiated with γ-irradiation (20 Gy). Responder cells labeled with carboxyfluorescein diacetate succinimidyl ester were cultured for 3 d together with T_{reg} cells at a ratio of 1:1 in the presence of antigen-presenting cells and mAb to CD3 (10 μg/ml; 145-2C11; eBioscience).

DNA-methylation analysis. Genomic DNA from CD3ε$^+$CD4$^+$hCD2$^+$ cells and CD3ε$^+$CD4$^+$hCD2$^-$ cells derived from mesenteric lymph nodes of male mice were extracted with an AllPrep DNA/RNA extraction kit (Qiagen), then were fragmented to approximately 200 base pairs by high-intensity focused ultrasound (Covaris) and were precipitated with histidine-tagged recombinant MBD1 ('methyl-CpG-binding-domain protein 1')[48]. After amplification by PCR, DNA fragments of the proper size were subjected to cluster generation and sequencing analysis with a HiSeq 1000 system (Illumina). Sequenced 'reads' were mapped to the mm9 assembly of the mouse genome (National Center for Biotechnology Information) with Bowtie software for the alignment of short DNA sequences. Peaks for each population were 'called' by model-based analysis of ChIP-seq data with a *P*-value threshold of less than 10^{-5}. The difference in methylation for a gene in one condition relative to its methylation in another condition was calculated with the normalized 'reads' mapped from 4 kilobases upstream to 4 kilobases downstream of its transcription start site. Transcription start sites were defined according to annotation on the Entrez database (National Center for Biotechnology Information).

Genomic bisulfite sequencing of the *Cdkn1a* promoter was done as described[17] with an EpiTect kit (Qiagen). The amplified fragments were cloned

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with a TOPO TA cloning kit (Invitrogen) and were subsequently sequenced with the BigDye Terminator Cycle Sequencing system (Applied Biosystems) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The PCR primers were designed with MethPrimer software (Li laboratory, Department of Urology, University of California, San Francisco). The sequences of the primer sets were as follows: 5′-ATATGTTGGTTTTTGAAGAGGG-3′ and 5′-ATCCCAAAAAATCCCACTATATC-3′.

Quantitative PCR. Total RNA was isolated from colonic tissues with an RNeasy mini kit (Qiagen) and was subjected to reverse transcription with a ReverTra Ace kit according to the manufacturer's instructions (Toyobo). The cDNA samples were amplified with a Thermal Cycler Dice Real Time System (TAKARA BIO), SYBR premix Ex Taq (TAKARA BIO) and the primer sets specific for mouse genes (sequences in **Supplementary Table 1**).

ChIP–quantitative PCR analysis. The MAGnify ChIP system (Life Technologies) was used as described[7], with a few modifications, for ChIP assays. Splenic $CD4^+CD25^+$ T cells were cultured for 3 d with Dynabeads Mouse T-Activator CD3-CD28 (Life Technologies) in the presence of 10 ng/ml IL-2 and 5 ng/ml TGF-β. The cells were allowed to 'rest' for 6 h in RPMI-1640 medium (Sigma-Aldrich) containing 0.1% FBS and then were stimulated for 1.5 h with or without 100 ng/ml IL-2. The cells were fixed for 10 min at 37 °C (in a water bath) with 1% formaldehyde, and the reaction was quenched by the addition of 125 mM glycine. Crude nuclei were isolated in SDS lysis buffer and were sonicated with a Microson (Misonix) and then a focused ultrasonicator (Covaris S220; Covaris) for the generation of chromatin fragments approximately 100–700 base pairs in length. The acoustic parameters were optimized as follows: duty cycle, 5%; intensity, 140 W; cycle and burst: 200 and 5 min. After evaluation of sample quality with an Agilent 2100 Bioanalyzer (Agilent), the sheared chromatin samples were immunoprecipitated overnight at 4 °C under gentle rotation with magnetic Protein A/G beads immobilized with anti-STAT5 (9363; Cell Signaling) or rabbit IgG (MAGnify ChIP kit; Life Technologies). After extensive washing of samples, immunocomplexes were eluted for 30 min at 55 °C, then were treated for 1 h at 65 °C with proteinase K for reversal of crosslinking. After extraction of DNA, quantitative PCR analysis was done with the following primer set specific for the promoter region of *Uhrf1*: 5′-TCCCTTTCTCCTCTCCCAGG–3′ and 5′-CTGCCGGCTATGCTCACTTT-3′.

Transfection of siRNA. Cells were transfect with siRNA through the use of an Amaxa Nucleofector kit according to the manufacturer's protocol (Ronza) with minor modifications. For this, 4 μg of negative control siRNA or pooled siRNA targeting *Cdkn1a* conjugated to the fluorescent dye Hilyte 488 (Nippon Gene) was added to Nucleofector solution containing 1×10^6 cells, followed by electroporation (Program: X-001). The cell cycle of cells containing Hilyte 488 was analyzed with Hoechst 33342, a cell-permeable DNA-binding dye, 24 h after electroporation. Transfection efficiency was approximately 10–15%. The sequence of the *Cdkn1a*-specific siRNA was as follows: 5′-GUUGCGCCGUGAUUGCGAU-3′, 5′-CCAGCCUGACAGAUUUCUA-3′ and 5′-GAACGGUGGAACUUUGACU-3′.

Immunoblot analysis. For immunoblot analysis, whole-cell extracts were prepared in RIPA lysis buffer containing a 'cocktail' of protease inhibitors (Nacalai Tesque). Equal amounts of cell lysate were separated by 5–20% gradient SDS-PAGE (Biorad). After transfer, proteins on Immobilon-P membranes (Millipore) were probed with the following primary antibodies: mAb to p21 (SX118; BD Pharmingen), mAb to GAPDH (6C5; Santa Cruz) and polyclonal antibody to Uhrf1 (M-132; Santa Cruz), together with horseradish peroxidase–conjugated antibody to mouse IgG (7076; Cell Signaling Technology) and antibody to rabbit IgG (7074; Cell Signaling Technology). The specific binding of the antibodies was visualized by an enhanced chemiluminescence detection system (Nacalai Tesque) and a LAS-3000 luminescent image analyzer (Fuji Film).

Histology. Prefixed colonic tissue sections were deparaffinized and rehydrated and were stained with either hematoxylin and eosin or Alcian blue–nuclear fast red. Specimens were histologically examined for the assignment of scores for the degree of colitis based on the following criteria: inflammatory infiltrates, mucosal hyperplasia and loss of goblet cells.

Adoptive-transfer experiments. Experimental colitis was induced in mice with deficient in recombination-activating gene 1 ($Rag1^{-/-}$) by adoptive transfer of $CD4^+CD25^-CD45RB^{hi}$ T cells as described[28]. Splenocyte samples fromC57BL/6 mice were enriched for $CD4^+$ T cells with the IMag Cell Separation System. The resultant $CD4^+$ T cells were labeled with fluorescein isothiocyanate–conjugated antibody to mouse CD3ε (145-2C11; BD Biosciences) and phycoerythrin-conjugated antibody to mouse CD45RB (16A; BD Biosciences), and $CD3\varepsilon^+CD4^+CD45RB^{hi}$ cells were isolated by sorting with a FACSAria II (BD Biosciences). The $Rag1^{-/-}$ recipients were given 1×10^5 $CD4^+CD25^-CD45RB^{hi}$ T cells via the tail vein and were analyzed at 6 weeks after transfer. For the experiment in **Supplementary Figure 6**, $CD4^+CD25^+$ T cells from $Uhrf1^{fl/fl}Cd4$-Cre or $CD4^{Cre}Uhrf1^{+/+}$ mice (8×10^4 cells per mouse) were transferred to $Rag1^{-/-}$ recipients together with $CD4^+CD25^-CD45RB^{hi}$ T cells from $CD45.1^+$ C57BL/6 mice (1×10^5 cells per mouse).

In the experiment in **Figure 7**, $CD4^+hCD2^+$ or $hCD2^-$ T cells from the spleen and peripheral lymph nodes of $Foxp3^{hCD2}$ mice (2×10^6 cells per mouse) were injected intravenously into 4- to 5-week-old $Uhrf1^{fl/fl}Cd4$-Cre$Foxp3^{hCD2}$ mice. The development of colitis in recipient mice was analyzed at 12 weeks of age.

Generation of mixed-bone marrow chimeras. Bone marrow cells isolated from femora of wild-type ($CD45.1^+$; 1×10^6 cells per mouse) and $Uhrf1^{fl/fl}$ *Cd4*-Cre or $Uhrf1^{+/+}Cd4$-Cre mice ($CD45.2^+$; 1×10^7 cells per mouse) were injected intravenously into $Rag1^{-/-}$ mice treated with γ-irradiation (8 Gy) before the injection. Six weeks later, the cLP of the recipient mice was analyzed by flow cytometry.

Immunofluorescence staining. Immunofluorescence staining of cross-sections of colonic tissues was done as described[49].

Statistical analysis. Differences between two or more groups were analyzed by Student's *t*-test or one-way ANOVA followed by Tukey's test. When variances were not homogeneous, the data were analyzed by the nonparametrical Mann-Whitney *U*-test or the Kruskal-Wallis test followed by the Scheffé test.

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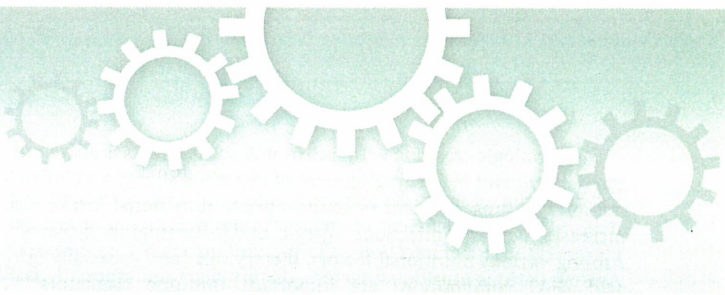

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Dietary ω3 fatty acid exerts anti-allergic effect through the conversion to 17,18-epoxyeicosatetraenoic acid in the gut

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ω3 polyunsaturated fatty acids (PUFAs) have anti-allergic and anti-inflammatory properties, but the immune-metabolic progression from dietary oil remains to be investigated. Here we identified 17,18-epoxyeicostetraenoic acid (17,18-EpETE) as an anti-allergy metabolite generated in the gut from dietary ω3 α-linolenic acid (ALA). Biochemical and imaging mass spectrometry analyses revealed increased ALA and its metabolites, especially eicosapentaenoic acid (EPA), in the intestines of mice receiving ALA-rich linseed oil (Lin-mice). In murine food allergy model, the decreased incidence of allergic diarrhea in Lin-mice was due to impairment of mast cell degranulation without affecting allergen-specific serum IgE. Liquid chromatography–tandem mass spectrometry-based mediator lipidomics identified 17,18-EpETE as a major ω3 EPA-derived metabolite generated from dietary ALA in the gut, and 17,18-EpETE exhibits anti-allergic function when administered *in vivo*. These findings suggest that metabolizing dietary ω3 PUFAs generates 17,18-EpETE, which is an endogenous anti-allergic metabolite and potentially is a therapeutic target to control intestinal allergies.

Food allergies affect the quality of life of patients and their families; they may even cause severe or fatal reactions. Although the prevalence of food allergy has increased recently, current standards of care remain focused on the elimination of dietary allergens because available means of prevention and treatment are inadequate[1]. The immunologic mechanisms in the development of food allergy involve the disruption of oral tolerance, induction of Th2-type responses, allergen-specific IgE production, and mast cell (MC) activation[2,3]. These immune responses have been studied in several murine models of food allergy (including ours)[4–8]. Using egg white ovalbumin (OVA) as a model food allergen, we induce allergic diarrhea in mice accompanied by aberrant Th2-type responses, increased OVA-specific serum IgE, and MC infiltration and degranulation in the large intestine[4]; this type I intestinal allergy is therefore similar to that of human patients with egg food allergy. Our subsequent study shows that the development of intestinal allergy is mediated by sphingosine 1-phosphate by controlling the trafficking of pathogenic cells, such as Th2 cells and MCs[9]. Therefore, various host-derived factors (e.g., cytokines, antibodies, and lipid mediators) are likely involved in the development of intestinal allergy.