

Accepted Manuscript

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PII: S0091-6749(16)30427-4

DOI: [10.1016/j.jaci.2016.04.035](https://doi.org/10.1016/j.jaci.2016.04.035)

Reference: YMAI 12154

To appear in: *Journal of Allergy and Clinical Immunology*

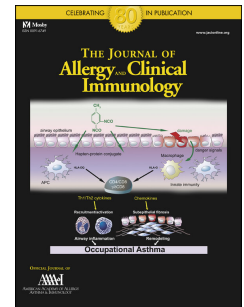
Received Date: 2 December 2015

Revised Date: 3 April 2016

Accepted Date: 12 April 2016

Please cite this article as: Yang G, Feng Y, Luo X-Q, Liu Z-Q, Geng X-R, Wang S, Zheng P-Y, Feng B-S, Liu Z-G, Yang P-C, Li H-B, Wu S-D, Alternation of circadian clock modulates forkhead box protein-3 gene transcription in CD4⁺ T cells in the intestine, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.04.035.

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**Alternation of circadian clock modulates forkhead box protein-3 gene transcription
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Gui Yang, MD^{a,b,d}, Ying Feng, PhD^a, Xiang-Qian Luo, MD, PhD^{b,c}, Zhi-Qiang Liu, MD,
PhD^{a,b,d}, Xiao-Rui Geng, MD^{b,d}, Shuai Wang, PhD^{b,d}, Peng-Yuan Zheng, MD, PhD^e,
Bai-Sui Feng^f, MD, PhD, Zhi-Gang Liu, MD, PhD^b, Ping-Chang Yang, MD, PhD^b, Hua-Bin
Li, MD, PhD^c, Shan-Dong Wu, PhD^a

From a, Zheda Dixun Bio-Gene Engineering Co., LTD; Hangzhou, 310052, China. b,
The center of Allergy & Immunology, Shenzhen University School of Medicine,
Shenzhen, 518060, China. c, Department of Otolaryngology, Head and Neck Surgery,
Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. d,
Longgang ENT Hospital, Shenzhen, 518116, China. e, Department of
Gastroenterology, the Fifth Hospital, Zhengzhou University, Zhengzhou 450000, China.
f, Department of Gastroenterology, the Second Hospital, Zhengzhou University,
Zhengzhou 450014, China.

Yang G, Feng Y and Luo XQ equally contributed to this work.

Corresponding authors: Dr. Shan-Dong Wu and Dr. Hua-Bin Li

1, Dr. Shan-Dong Wu, Zheda Dixun Bio-Gene Engineering Co., LTD; Hangzhou,
310052, China. Email: shandongrwu@sina.com

2, Dr. Hua-Bin Li, Department of Otolaryngology, Head and Neck Surgery, Xinhua
Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. Email:
allergyli@163.com

Capsule Summary
Alternation of circadian clock may contribute to the initiation of food allergy, which
may be prevented by suppressing the expression of NFIL3.

Keywords: Intestine; Allergy; Circadian clock; Regulatory T cell.

35 To the editor:

36 The alteration of the circadian clock is associated with the pathogenesis of a
37 large number of disorders in the body¹. Prompted by the fact that some patients
38 complained that their food allergy occurred after engaging their career with regular
39 day/night-shift-rotation for several years, we carried out a survey on identifying a
40 possible connection between the day/night-shift-rotation and food allergy. Within
41 668 nurses with regular day/night-shift-rotation, the incidence of food allergy was
42 86/668 (12.9%), while in those without engaging the regular day/night-shift-rotation
43 subjects, the incidence of food allergy was 26/668 (3.9%) (Table 1). The serum levels
44 of specific IgE and IL-4 in food allergy patients were significantly higher than in
45 healthy subjects. The frequency of CD4⁺ CD25⁺ Foxp3⁺ regulatory T cells (Treg) in food
46 allergy patients was significantly lower than in healthy subjects (Table 1, section 2). A
47 correlation analysis with the data showed a positive correlation ($r = 0.3664$, $p < 0.01$)
48 between the day/night-shift-rotation and the incidence of food allergy. Most food
49 allergies (81.4%) of this group occurred 3-4 years after they engaged in the
50 day/night-shift-rotation career (Table 1, section 3). The data imply that there is a
51 possible connection between the day/night-shift-rotation life style and the initiation
52 of food allergy.

53 Published data indicate that the altered circadian clock (AC) compromises the
54 intestinal epithelial barrier integrity². Epithelial barrier dysfunction facilitates the
55 initiation of food allergy³. Therefore, we inferred that AC contributed to the

pathogenesis of food allergy. To test this, we treated mice with ovalbumin (OVA; a specific antigen) and AC or RC (regular circadian clock). As shown by Fig. 1, mice of AC group showed intestinal allergy like signs, including high serum levels of IL-4, IL-5, IL-13 (Fig. 1A) and OVA-specific IgE (Fig. 1B), profound infiltration of mast cell and eosinophil in the intestinal mucosa (Fig. 1C), high frequency of OVA-specific CD4⁺ T cell proliferation after exposure to the specific antigen OVA in the culture (Fig. 1D; Fig. S1 in online repository), and intestinal epithelial barrier dysfunction (Fig. 1E, 1F). None of the RC group mice developed food allergy (Fig. 1A-F). The results suggest that AC facilitates the development of intestinal allergy.

Previous studies suggest that the abnormality of Treg is associated with the pathogenesis of food allergy⁴. Since AC facilitates the development of food allergy as shown by Fig. 1, we inferred that AC might interfere with Tregs in the intestine. To test this, we assessed Tregs in the mouse intestine after AC. As analyzed by flow cytometry, about 9.94% Tregs were detected in the CD4⁺ T cells in RC mice while less (5.92%) Tregs were detected in the CD4⁺ T cells in AC mice (Fig. S2 in online repository). The results suggest that AC does reduce the Treg population in the intestine, which may be one of the important factors in the development of food allergy occurred after AC. To test the inference, we adoptively transferred OVA-specific Tregs to the AC mice in the course of sensitization. Indeed, the AC-facilitated food allergy in the mice was abolished (Fig. S3 in online repository).

The aberrant expression in some of the circadian clock-related proteins is associated with the pathogenesis of immune disorders⁵. Based on the results of Fig. S1, we reasoned that one or some of the circadian clock-related proteins interfered with the expression of Foxp3 in CD4⁺ T cells. Thus, after treating with AC, we isolated CD4⁺ T cells from the mouse intestine; the cells were analyzed by chromatin immunoprecipitation (ChIP) assay. The results showed that, among the tested molecules, the nuclear factor interleukin-3 (NFIL3) was uniquely high at the Foxp3 promoter locus in the CD4⁺ T cells from AC-treated mice, which did not occur in the CD4⁺ T cells from RC-treated mice (Fig. S4A).

To elucidate if such a phenomenon also occurred in human, we analyzed the peripheral CD4⁺ T cells from subjects after night shifts (AC subjects). The results showed that before night shift, the mRNA levels of NFIL3 in CD4⁺ T cells of AC subjects were similar to the subjects with the RC-living style (RC subjects). After night shift, the levels of NFIL3 in the CD4⁺ T cells were increased in a night shift time-dependent manner, which returned to the normal levels within 2 days after the night shifts (Fig. S4B). The NFIL3 was also increased at the Foxp3 promoter locus (Fig. S4C). Collectively, the data suggest that AC increases the NFIL3 levels in peripheral CD4⁺ T cells; the NFIL3 can bind to the Foxp3 promoter in the CD4⁺ T cells.

The above data implicate that NFIL3 may modulate the expression of Foxp3 in CD4⁺ T cells. To corroborate the results, we overexpressed NFIL3 in CD4⁺ T cells (Fig. S5A); the CD4⁺ T cells were transfected with a Foxp3 promoter luciferase reporter

gene (Fig. S5B). The results showed that, after activation by anti-CD3/CD28 and TGF- β in the culture, the luciferase activity was significantly increased in the CD4⁺ T cells, which was abolished by the overexpression of NFIL3 (Fig. S5C). The results suggest that NFIL3 represses the expression of Foxp3 by CD4⁺ T cells.

On the other hand, naïve CD4⁺ CD25⁻ T cells were overexpressed with NFIL3 by transfecting with NFIL3 plasmids (Fig. S5A). The cells were treated with the Foxp3 polarization condition and analyzed by flow cytometry. The results showed that about 71.4% Foxp3⁺ CD4⁺ T cells were induced, while almost no Foxp3⁺ CD4⁺ T cells were detected in the NFIL3-overexpressing CD4⁺ T cells (Fig. S5D-E). In addition, a complex of Foxp3 and NFIL3 was detected in the CD4⁺ T cells with NFIL3 overexpression after the treatment of the Foxp3 polarization (Fig. S5F). The results indicate that NFIL3 physically contacts Foxp3 to repress the expression of Foxp3 in CD4⁺ T cells.

Since Foxp3 binds GATA3 to prevent the expression of IL-4⁶, after the AC treatment, we assessed the expression of IL-4 in CD4⁺ T cells. The results showed that at the IL-4 promoter locus, the levels of p300, acetylated H3K4, Pol II and GATA3 were significantly increased (Fig. S6A-D). The levels of IL-4 were also increased in the CD4⁺ T cells (Fig. S6E-F). The results suggest that AC-induced NFIL3 may be the most important factor to increase IL-4 expression in CD4⁺ T cells in AC-treated mice. To test the inference, we treated NFIL3-deficient mice with AC. Indeed, the expression of

117 IL-4 was not altered in CD4⁺ T cells (Fig. S6). the AC-treated NFIL3-deficient mice did
118 not show food allergy signs (Fig. S7).

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144 **Conflict of interest:** The authors declare there is no conflict of interest in this paper.

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146 **Acknowledgement:** This study was supported by grants from the innovation of
147 science and Technology Commission of Shenzhen Municipality
148 (JCYJ20150402090413008, JCYJ20140418095735611 and ZDSYS201506050935272),
149 the Natural Science Foundation of China (81373176, 31570932, 31400856, 81571790
150 and 81501573).

Table 1

Section 1: Food allergy survey

	Subjects with Day/night-shift	Subjects with non-Day/night-shift	Healthy subjects
Participants, n	668	668	40
Male/female, n	334/334	334/334	20/20
Age, mean in years (SD)	38.5(16)	37.8(15)	38.8(18)
Allergens	n (%)	n (%)	
Total food allergy patients	86(12.9)	26(3.9)*	0
Allergen distribution			
Fish	18 (20.9)	5 (19.2)	0
Shell fish	12 (13.9)	3 (11.5)	0
Milk	11 (12.8)	3 (11.5)	0
Tree nuts	10 (11.6)	3 (11.5)	0
Soy	10 (11.6)	1 (3.8)	0
Fruit	8 (9.3)	6 (23.1)	0
Sesame seed	6 (6.9)	0	0
Wheat	6 (6.9)	0	0
Egg	6 (6.9)	5 (19.2)	0
Peanut	5 (5.8)	5 (19.2)	0
With other allergies			
Asthma	1 (0.15%)	1 (0.15%)	
Allergic rhinitis	2 (0.3%)	0	
Allergic dermatitis	1 (0.15%)	2 (0.3%)	
Body mass index	20.3 ± 2.5	19.9 ± 2.7	
Serum vitamin D (ng/ml)	30.8 ± 1.9	30.5 ± 1.3	
Serum cortisol (ng/ml)	19.8 ± 2.4	20.2 ± 2.3	
Hight (m)	1.59 ± 0.06	1.58 ± 0.06	
Weight (kg)	61.5 ± 3.5	60.6 ± 3.2	
BMI (kg/m ²)	24.2 ± 2.8	23.9 ± 2.1	

Section 2: Immune tests

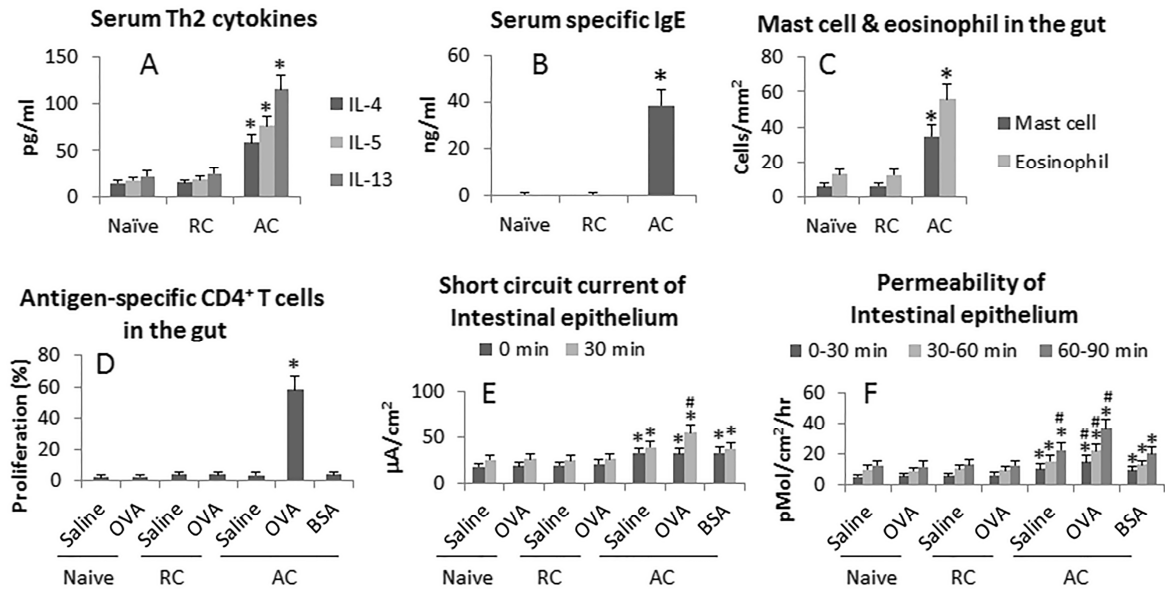
Parameters	Day/night-shift		Regular shift		Healthy
	FA	Non-FA	FA	Non-FA	
Specific IgE (kU/L)	15.3±21.6	<0.35	12.5±18.2	<0.35	<0.35
Treg (%)	2.14±2.06	5.95±5.14	2.28±2.35	6.28±7.29	6.68±6.26
Serum IL-4 (pg/ml)	45.8±25.9	12.6±18.6	39.5±31.3	9.8±15.4	10.5±8.6
SPT diameter (mm)	6.58±4.87	2.11±1.14	5.74±3.85	1.86±1.47	1.17±1.08

Section 3: occurrence of food allergy after the career of day/night-shift-rotation

Time (year)	Patients with food allergy	%
1-2	5	5.8
3-4	70	81.4

5-6	9	10.5
>7	1	1.2

Some patients were allergic to more than one allergen. Data are presented as mean $\pm$ SD from all subjects. *, $p < 0.01$, compared to the day-night-shift group (χ^2 test). #, $p < 0.01$, compared to the healthy subjects. Treg: Regulatory T cells. Day/night-shift = Day/night-shift rotation. SPT diameter: The diameter than the negative control site.



Online repository

Alternation of circadian clock modulates forkhead box protein-3 gene transcription in CD4⁺ T cells

Running title: Circadian clock modulates Foxp3 expression

Gui Yang, MD^{a,b,d}, Ying Feng, PhD^a, Xiang-Qian Luo, MD, PhD^{b,c}, Zhi-Qiang Liu, MD, PhD^{a,b,d}, Xiao-Rui Geng, MD^{b,d}, Shuai Wang, PhD^{b,d}, Peng-Yuan Zheng, MD, PhD^e, Bai-Sui Feng^f, MD, PhD, Zhi-Gang Liu, MD, PhD^b, Ping-Chang Yang, MD, PhD^b, Hua-Bin Li, MD, PhD^c, Shan-Dong Wu, PhD^a

From a, Zheda Dixun Bio-Gene Engineering Co., LTD; Hangzhou, 310052, China. b, The center of Allergy & Immunology, Shenzhen University School of Medicine, Shenzhen, 518060, China. c, Department of Otolaryngology, Head and Neck Surgery, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. d, Longgang ENT Hospital, Shenzhen, 518116, China. e, Department of Gastroenterology, the Fifth Hospital, Zhengzhou University, Zhengzhou 450000, China. f, Department of Gastroenterology, the Second Hospital, Zhengzhou University, Zhengzhou 450014, China.

Yang G, Feng Y and Luo XQ equally contributed to this work.

Corresponding authors: Dr. Shan-Dong Wu and Dr. Hua-Bin Li.

1, Dr. Shan-Dong Wu, Zheda Dixun Bio-Gene Engineering Co., LTD; Hangzhou, 310052, China.

Email: shandongrwu@sina.com

2, Dr. Hua-Bin Li, Department of Otolaryngology, Head and Neck Surgery, Xinhua Hospital,
Shanghai Jiaotong University School of Medicine, Shanghai, China. Email: allergyli@163.com

Materials and methods

Mice

Male BALB/c mice and OVA-TCR transgenic DO11.10 mice (6-8 week old) were purchased from the Guangzhou Experimental Animal Center. The NFIL3-deficient mice were provided by the Shanghai Research Center for Model Organisms (Shanghai, China). The mice were maintained in a pathogen free environment with free access to food and water.

Ethic statement

The animal experimental procedures were approved by the Animal Ethic Committee at Shenzhen University. All the animal experiments were performed in accordance with the approved guidelines. The participation of human subjects in the present study was approved by the Human Ethic Committee at Zhengzhou University. An informed and written consent was obtained from each subject.

A survey of food allergy in human subjects with day/night-shift-rotation

The survey was carried out in the First Hospital, Second Hospital and Fifth Hospital of Zhengzhou University between 2013 and 2014. The staff nurses with or without the regular day/night-shift rotation (day shift and night shift were swapped at least once a week) were randomly (even numbers were selected; odd numbers were gave up) selected into this study. Subjects without the day/night-shift rotation in their daily life were randomly selected from the outpatients of the three hospitals. The diagnosis of food allergy was performed by physicians of

the department of gastroenterology based on the history of food allergy, positive results of skin test, and the serum levels of food allergen specific IgE were greater than 0.35 IU/ml. Exclusion criteria: (1) Subjects with allergic disorders before their nursing career; (2) Subjects with autoimmune diseases; (3) Subjects with cancer; (4) Subjects using immunosuppressants; (5) Subjects with severe organ diseases.

Skin prick test (SPT)

SPT of fish, shell fish, milk, tree nuts, soy, fruit, sesame seed, wheat, egg, peanut was performed for each subject controlled with both histamine phosphate (0.1%; positive control) and saline (negative control). The SPT was performed by applying a drop of the allergen extract on the skin of the volar aspect of the forearm. Skin reaction was evaluated 20 min after. The size of flare or wheal was measured. A flare or wheal of 3 mm greater than the negative control site were regarded as positive results.

Allergen-specific IgE (sIgE) measurements

Peripheral blood samples were collected from each human subjects (20 ml/person) via ulnar vein puncture. Serum aliquots were sent to the laboratory without any patient identification. We performed allergen sIgE detection using ImmunoCAP (Phadia, Uppsala, Sweden) according to the manufacturers' instructions. The sIgE detection range of ImmunoCAP® FEIA was 0.1-100 kU/L.

Serum levels of cortisol and vitamin D measurement

Serum levels of cortisol and vitamin D were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagent kits (Gudou Biotech, Shanghai, China) following the manufacturer's instructions.

Body mass measurement

Height and weight were measured for each participant. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Altered circadian clock and sensitization to ovalbumin (OVA)

Mice were treated with a 18-day schedule of altered circadian clock (AC) as reported by Yu et al (Yu X, Rollins D, Ruhn KA, Stubblefield JJ, Green CB, Kashiwada M, Rothman PB, Takahashi JS, Hooper LV. TH17 cell differentiation is regulated by the circadian clock. *Science*. 2013; 342 (6159): 727-30) or treated with regular circadian clock (RC). The mice were provided a specific mouse food, in which OVA was added to the regular mouse food at a ratio of 1/100. Each mouse took about 0.5-1.0 mg OVA daily from day 0 to day 18. The mice of both groups ingested comparable amounts of food and therefore OVA. Naïve controls mice were fed with regular mouse food (free of OVA).

Assessment of food allergy in mice

The mice were sacrificed on day 19 and subjected to the assessment of the allergy status in the intestine following our established procedures (Yang et al. *J Allergy & Clin Immunol* 2014; 133: 1702-8).

Assessment of Th2 cytokines and allergen specific IgE: The blood samples were collected from each mouse. The sera were isolated from the blood samples by centrifugation. The serum levels of IL-4, IL-5, IL-13 and OVA-specific IgE were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagent kits (Kits of IL-4, IL-5 and IL-13 were purchased from R&D Systems. Kit of OVA-specific IgE was purchased from Shuoheng Biotech; Guangzhou, China) following the manufacturer's instructions.

Assessment of infiltration of mast cell and eosinophil in the intestinal mucosa: A jejunal segment was excised from each mouse after sacrifice and fixed with 4% formalin overnight. The tissue was processed for paraffin sections. The sections were stained with 0.5% toluidine blue (for mast cell staining) or hematoxylin and eosin (for eosinophil staining). Mast cells and eosinophils were counted on 20 fields ($\times 400$) of each sample under a light microscope. The slides were coded. The observers were not aware of the code to avoid the observer bias.

Assessment of OVA-specific intestinal CD4⁺ T cells: The small intestine was collected from each mouse at sacrifice. Lamina propria mononuclear cells (LPMC) were isolated from the intestinal tissue following our established procedures (Yang et al. J Allergy & Clin Immunol 2014; 133: 1702-8). CD4⁺ CD25⁻ T cells and CD11c⁺ DCs were isolated from the LPMCs by magnetic cell sorting (MACS) with commercial reagent kits (Miltenyi Biotech) following the manufacturer's instructions. The purity of the cells were greater than 95% as checked by flow cytometry. The CD4⁺ T cells (labeled with CFSE) and DCs were cultured (at 10^5 cells: 2×10^4 cells/well) in the presence of OVA (5 μ g/ml) or BSA (5 μ g/ml; control), PMA (20 ng/ml) and ionomycin (1 μ g/ml) for 3 days. The cells were analyzed with a flow cytometer.

Assessment of intestinal epithelial barrier function: The permeability to a macromolecular tracer (horseradish peroxidase or HRP, MW = 44 kDa) of the jejunal epithelial layer was determined by Ussing chamber technique following our established procedures (Zheng et al. Gut. 2009; 58: 1473-9) and used as an indicator of the epithelial barrier function. Briefly, 4 adjacent pieces of the jejunum (2 cm each) were cut from each mouse (cut at 10 cm from the stomach) and mounted on Ussing chambers. The electrical current crossing the tissue (a measure of net ion transport) was determined in the voltage-clamp mode (zero volts potential difference) and expressed as short-circuit current (I_{sc} , in $\mu A/cm^2$). To measure the permeability of the epithelial layers, HRP was added to the epithelial side chambers at 10 $\mu mol/L$. The serosal buffer was sampled at 30-minute intervals for 90 minutes. The concentration of intact HRP in the samples was determined by assaying enzyme activity using a modified Worthington method. Briefly, 150 μl of sample were added to 800 μl of phosphate buffer containing 0.003% H_2O_2 and 80 $\mu g/ml$ o-dianisidine. Enzyme activity was determined from the rate of increase in OD at 460 nm. Fluxes were calculated according to standard formulae and were expressed as $pmol/cm^2/hour$.

***In vitro* generation of Foxp3⁺ Tregs**

$CD4^+$ $CD25^-$ T cells were isolated from the naïve DO11.10 mouse spleen; the cells were cultured in RPMI1640 medium containing transforming growth factor (TGF)- β (20 ng/ml), 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2mM L-glutamine for 6 days. The medium was changed on day 3. The $CD4^+$ $CD25^+$ $CD127^-$ T cells were isolated by magnetic cell sorting (MACS) to be used as Tregs in the cell culture experiments as well as the adoptive transfer in the mouse

model. The Foxp3⁺ cells were greater than 98% as checked by flow cytometry. The viability of the cells was greater than 99% as checked by Trypan blue exclusion assay.

Collection of blood samples from nurses

Twenty nurses without food allergy were randomly recruited for this procedure. Before and immediately after the night shift, and one day after the shift, about 20 ml peripheral blood samples were drawn from the ulnar vein at each time point from each nurse. Mononuclear cells were isolated from the blood by the gradient density centrifugation. The cells were further isolated for CD4⁺ T cells by MACS with purchased reagent kits (Miltenyi Biotech) following the manufacturer's instructions.

Flow cytometry

In the surface staining, cells were stained with fluorochrome-labeled antibodies (or isotype IgG) for 30 min at 4 °C. In the intracellular staining, cells were fixed with 2% paraformaldehyde for 2 h at room temperature, and then treated with 0.5% saponin for 30 min. The cells were incubated with fluorochrome labeled antibodies (0.5 µg/ml, or isotype IgG) for 30 min at 4 °C. The cells were analyzed with a flow cytometer (FACSCanto II, BD Bioscience). The data were analyzed with the software Flow Jo. Data of isotype IgG were used as the gating reference. Antibodies for flow cytometry were purchased from BD Biosciences.

Real-time quantitative PCR (RT-qPCR)

The total RNA was extracted from the cells with TRIzol reagent, and the first-strand cDNA was synthesized with a reverse transcription kit. The real-time PCR was performed using SYBR Green Master Mix on a real time PCR device (MiniOpticon, Bio-Rad). The sequences of the primers used in this study are presented in Table S1. The data were calculated by the $2^{-\Delta\Delta Ct}$ method. (Reagents for RT-qPCR were purchased from Invitrogen).

Preparation of cytosolic and nuclear extracts

Cells were incubated with lysis buffer (10 mM HEPES, pH 7.4, 10 mM NaCl, 1.5 mM MgCl₂, 0.5 mM DTT, 0.2% Nonidet P-40, and 0.2 mM PMSF) at 4°C for 15 min, and centrifuged at 500 ×g for 10 min at 4°C. The supernatant was collected as the cytosolic extract. The pellet was added with nuclear extract buffer (20 mM HEPES-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF, and 1× protease inhibitor cocktail) and incubated for 15 min at 4°C, followed by centrifugation at 13,000 ×g for 10 min at 4°C. The supernatant was collected as the nuclear extract. The protein concentrations were determined by the Bradford method.

Immunoprecipitation

For the pre-clearing, the cellular extracts were incubated with protein-G beads (Sigma Aldrich) for 2 h at 4 °C, and centrifuged for 2 min at 13 000 rpm at 4 °C. The supernatant was incubated overnight with specific antibodies (or isotype IgG) and protein-G beads. The immunoprecipitates were collected and eluted with eluting buffer. The supernatant was then subjected to Western blotting.

Western blotting

The total proteins were extracted from cells; the extracts were fractioned by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred onto a PVDF membrane. After blocking with 5% skim milk for 30 min, the membrane was incubated with the primary antibodies (0.5 µg/ml, or isotype IgG) overnight at 4 °C and followed by incubation with the secondary antibodies (labeled by peroxidase) for 1 h at room temperature. The membrane was washed with TBST (Tris-buffered saline Tween 20) between incubations. The immune blots on the membranes were developed by ECL (enhanced chemiluminescence). The results were photographed with a KODAK Image Station 4000Pro (KODAK, Shanghai, China). The antibodies used in Western blotting were purchased from Santa Cruz Biotech.

Chromatin immunoprecipitation (ChIP)

ChIP assays were performed using a ChIP kit (Sigma Aldrich) following the manufacturer's instructions. Briefly, CD4⁺ T cell extracts were treated as indicated above and cross-linked with formaldehyde and sonication. Resulting cell lysates (input) were immunoprecipitated with respective antibodies (2.5 µg each, or isotype IgG). The precipitated protein-DNA complexes were subjected to proteinase (Sigma Aldrich) treatment. The primers used to confirm the binding of factors to the promoter region include: Foxp3 (gcctggttgtagaaggtct and gcttctcctttccagctcc) and IL-4 (aacgaggtcacaggagaagg and caggacagagaaagcatcgc). (The antibodies used in ChIP were purchased from Santa Cruz Biotech).

NFIL3 overexpression

The NFIL3 DNA vector was constructed by GeneScript (Nanjing, China). 2×10^5 CD4⁺ T cells were seeded in each well of a 24-well tissue culture plate 1 day before transfection. The equimolar amount of the NFIL3 DNA vector was transfected into each well, according to the Lipofectamine 2000 Transfection Reagent manual from Invitrogen. The NFIL3 expression in the CD4⁺ T cells was assessed by Western blotting.

Statistics

The data are presented as mean $\pm$ SD. The difference between groups was determined by Student t test, or ANOVA if more than two groups. In the case of a distribution different from normal, the Mann-Whitney U test was used. A correlation assay with the day/night-shift-rotation as 1, non-day/night-shift-rotation as 0; subjects with food allergy as 1, non-food allergy as 0 was performed with Excel. $P < 0.05$ was set as a significant criterion.

Fig. S1. Assessment of OVA-specific CD4⁺ T cells. CD4⁺ T cells were isolated from the intestine, labeled with CFSE, and stimulated with OVA or BSA for 72 h in the presence of DCs. The gated flow cytometry histograms indicate the frequency of CD4⁺ T cell proliferation. Each group consists of 10 mice. Samples from individual mice were analyzed separately.

Fig. S2. AC suppresses Tregs in the intestine. A, the gated dot plots are CD4⁺ T cells of LPMCs. B-C, the gated dot plots are Tregs. D, the bars (mean $\pm$ SD; *, $p < 0.01$, compared with the RC group) are the summarized data of B-C. Each group consists of 10 mice. Samples from individual mice were processed separately.

Fig. S3. Adoptive transfer with Tregs suppresses AC-induced food allergy. The AC mice were treated with Tregs, or naive CD4⁺ T cells, at 10^6 cells/mouse via tail vein injection. The data of bars are presented as mean $\pm$ SD. *, $p < 0.01$, compared with the saline group. Samples from individual mice were processed separately. Each group consists of 10 mice.

Fig. S4. AC up regulates NFIL3 expression in CD4⁺ T cells. A, the bars indicate the NFIL3 promoter binding rate by AC molecules in mice treated with AC. B-C, the bars indicate the NFIL3 mRNA (B) and NFIL3 binding to Foxp3 promoter in peripheral B cells of subjects engaging day/night-shift rotation. *, $p < 0.01$, compared with the RC group (A), or the “Before” group (B and C). Samples from the individual subjects were analyzed separately.

Fig. S5. NFIL3 represses Foxp3 expression in CD4⁺ T cells. Medium: CD4⁺ T cells were cultured with medium alone. NFIL3 plasmid or control plasmid: CD4⁺ T cells were transfected with NFIL3-expression plasmids or control plasmids. The data of bars are presented as mean $\pm$ SD. *, $p < 0.001$, compared with the medium group. The data represent 3 independent experiments.

Fig. S6. AC-induced NFIL3 increases IL-4 expression in CD4⁺ T cells. #, NFIL3-deficient mice. The bars of A-D indicate the levels of p300, acetylated H3K4, Pol II and GATA3 at the IL-4 promoter locus. The data of bars are presented as mean $\pm$ SD. *, $p < 0.001$, compared with the medium group RC. The data represent 3 independent experiments.

Fig. S7. Deficiency of NFIL3 abolishes AC-induced food allergy in mice. NFIL3d: NFIL3-deficient mice. The data of bars are presented as mean $\pm$ SD. *, $p < 0.01$, compared to the RC group. Samples from individual mice were processed separately. Each group consists of 10 mice.

Table S1. Primers used in this study

Molecule	Forward	Reverse
HDAC1	gatgaggaggagagaaggtgg	aacttggggagaagatgggg
Foxp3	cctccactccacctaagca	ccttgtttgcgctgagagt
NFIL3	agcagaaccacgataaccca	atcttgctgagctgctggt
β -actin	gtgggaatgggtcagaagga	tcatcttttcacggttgcc

