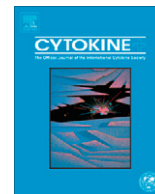




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## Eosinophil-derived interferon-lambda contributes to initiation of allergen-related inflammation in the intestine

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### ABSTRACT

**Background and aims:** Epithelial barrier dysfunction plays a critical role in the initiation of a number of immune diseases; the causative factors are not fully understood. The present study aimed to elucidate the mechanism by which the eosinophil-derived interferon (IFN)-lambda induced the gut epithelial barrier dysfunction.

**Methods:** The duodenal biopsies were obtained from patients with or without food allergies. The eosinophils and IFN $\lambda$  expression were observed by immune staining. Intestinal epithelial cell line, T84 cells, and a mouse model were employed to observe the effect of IFN $\lambda$  on the epithelial barrier function and the initiation of skewed T helper (Th)2 polarization in the mouse intestine.

**Results:** IFN $\lambda$  expression was observed in over 80% human eosinophils of the subjects with or without food allergies. Exposure to microbial products, lipopolysaccharide or peptidoglycan, could induce eosinophils to release IFN $\lambda$ . Exposure to IFN $\lambda$  could induce intestinal epithelial barrier dysfunction via inducing the epithelial cell apoptosis. Concurrent exposure to microbial products and food antigens could induce aberrant antigen specific Th2 polarization and Th2 pattern inflammation in the intestine.

**Conclusions:** Eosinophils express IFN $\lambda$  that can induce intestinal epithelial barrier dysfunction and promotes the initiation of the aberrant Th2 polarization in the intestine.

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### 1. Introduction

The antigen absorption in the intestinal mucosa is an essential step to initiate immune responses in the intestine. Under physiological conditions, the absorption of antigens is restricted by the integrated epithelial barrier on the intestinal mucosal surface. However, the integration of the intestinal epithelial barrier can be compromised by a number of factors, such as under psychological stress [1,2], or severe microbial infection [3,4], etc. These conditions may cause the tight junction open, increase in the intracellular permeability, or epithelial cell apoptosis resulting in the physical defect in the epithelial barrier; yet, the mechanisms underlying these conditions are not fully understood.

Food allergy indicates a condition that the immune system in the intestine aberrantly responds to normal food components.

The pathological feature of food allergy is a skewed T helper (Th)2 polarization, elevated levels of antigen specific IgE, mastocytosis and eosinophilia in the intestinal mucosa [5]. The initiation of the skewed Th2 polarization is not fully understood yet; multiple factors may be involved in the induction of food allergy.

Interferon (IFN) $\lambda$  is a newly described cytokine, including IFN $\lambda$ 1 [interleukin (IL)-29], IFN $\lambda$ 2 (IL-28A) and IFN $\lambda$ 3 (IL-28B) three subtypes; the IFN $\lambda$ 1 is only expressed in human. IFN $\lambda$  is actively involved in the immune regulation, such as inducing target cell apoptosis [6,7], suppressing IL-13 expression [8], facilitating regulatory T cell development [6,9] and fighting against viral infections. It has been found that IFN $\lambda$  can be produced by several cells types, including epithelial cells [10], dendritic cells [6] and macrophages [11]. It is still not fully understood how the production of IFN is initiated by those cells.

Eosinophils (Eos) are an inflammatory cell type. The eosinophilia is found in a number of diseases, such as allergic disorders, parasite infections and some special inflammations like eosinophilic esophagitis [12]. Apart from producing a number of chemical mediators, such as the major basic protein (MBP), eosinophil cationic protein, eosinophil peroxidase, etc., we also noted that Eos also produced IFN $\lambda$  in our previous study ([6] data not shown);

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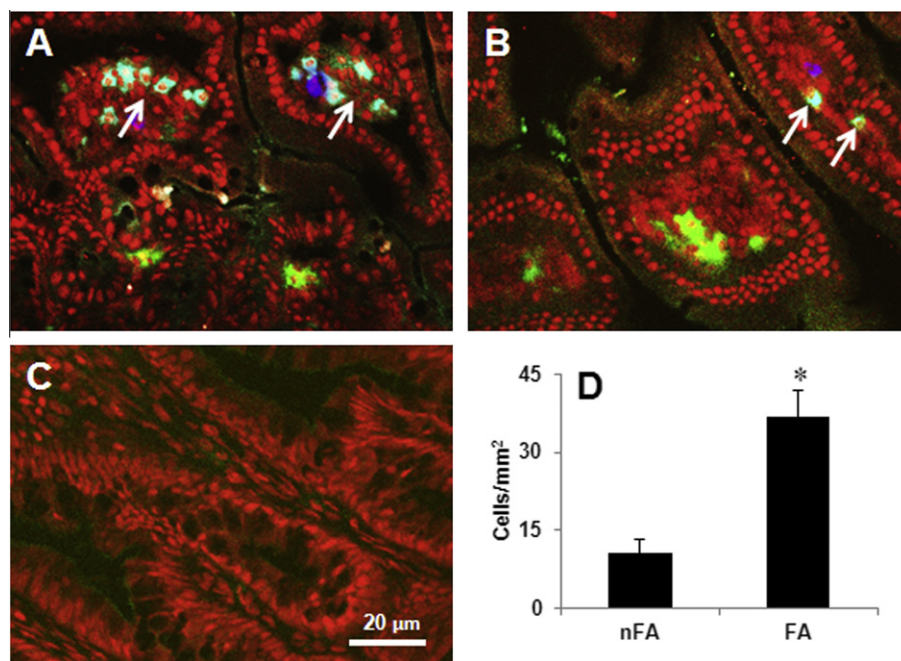


**Fig. 1.** Eos express IFN $\lambda$  in the intestine. Duodenal biopsies were processed for immunohistochemistry. The representative confocal images show that the Eo marker, MBP, was stained in green; the IFN $\lambda$  was stained in blue. The light blue color (pointed by arrows) was merged by the colors of green and blue. The nuclei were stained in red. (A) Samples were obtained from 15 patients with food allergy. (B) Samples were obtained from 15 non-food allergy patients. (C) An isotype control. (D) The bars indicate the double positive cell counts (mean  $\pm$  SD). \* $p < 0.01$ , compared with food allergy (FA) group. nFA, non-FA. Original magnification: 200 $\times$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the phenomenon has not been reported yet as we checked the literature throughout the PubMed database. Since Eos are one of the effector cell types in allergic diseases, Eos express Toll like receptor (TLR)2 and TLR4 [13], we postulated that the Eo-derived IFN $\lambda$  might be involved in the initiation of the skewed Th2 polarization in the intestine. The present data show that upon exposure to the ligands of TLR2 or TLR4, Eos release IFN $\lambda$ ; the latter affects the integrity of intestinal epithelial barrier function that contributes to the initiation of aberrant Th2 polarization and Th2 pattern inflammation in the intestine.

## 2. Materials and methods

### 2.1. Patients

Thirty patients with peptic ulcer with or without food allergies were included in the present study. The diagnosis of food allergy is described in [Supplementary materials](#). The patients did not use any anti-allergy drugs at least 1 month before the study. The demographic data of patients are presented in [Supplementary Table 1](#).

### 2.2. Duodenal biopsy

Duodenal biopsies were taken under endoscopy for a purpose of ruling out malignant disorders in the duodenum. One piece of biopsies was taken from the edge of the ulcer; another piece was taken from non-ulcer mucosa. The procedures using human tissue in the study were approved by the Research Ethic Committee at Nanjing Medical University. Informed written consent was obtained from each subject.

### 2.3. Enzyme-linked immunoassay (ELISA)

Levels of serum specific IgE, intestinal IL-4 and IFN $\lambda$  in tissue extracts and culture supernatant were measured by ELISA with commercial reagent kits following the manufacturer's instruction.

### 2.4. Immunohistochemistry and TUNEL staining

Duodenal biopsies were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until use. Cryosections were prepared and fixed with cold acetone for 20 min. Sections were blocked by 1% bovine serum albumin (BSA) for 30 min and incubated with goat anti-human IFN $\lambda$  antibody (1:200) and mouse anti-MBP antibody (1:300), or isotype IgG (using as negative control) overnight at  $4^{\circ}\text{C}$ . After washing, sections were incubated with Cy5-labeled anti-goat and FITC-labeled anti-mouse second antibodies (1:300) for 1 h at room temperature. After washing, sections were mounted with cover slips and observed under a confocal microscope. The positive cells were counted in 20 fields (200 $\times$ ) per samples. For T84 cell staining, the cell suspension was smeared onto glass slides, dried overnight. The rest procedures were the same as above. To avoid the observer bias, the sections were coded; the observer was not aware of the codes.

After the MBP labeling, some sections were incubated for 1 h in freshly prepared TUNEL mixture containing TUNEL plus fluorescein tag in a dark humidified chamber at  $37^{\circ}\text{C}$ . After washing, sections were mounted with coverslips and viewed under a confocal microscope.

### 2.5. Detecting intestinal epithelial barrier function in vivo

BALB/c mice with eosinophilia were gavage-fed with ovalbumin (OVA) 5 mg/mouse in 0.3 ml saline. Mice were also i.p. injected with lipopolysaccharide (LPS; see [Fig. 3C](#) for dosage) or peptidoglycan (PGN; see [Fig. 3D](#) for dosage). Mice were sacrificed 6 h later. The jejunum segments were excised to extract the total proteins. The proteins were analyzed by Western blotting for the contents of the OVA.

### 2.6. Eosinophil depletion

Rats were i.p. injection with anti-CCR3 antibody (0.5 mg/rat) on day 3, 8 and 11, respectively. As shown by peripheral blood smear

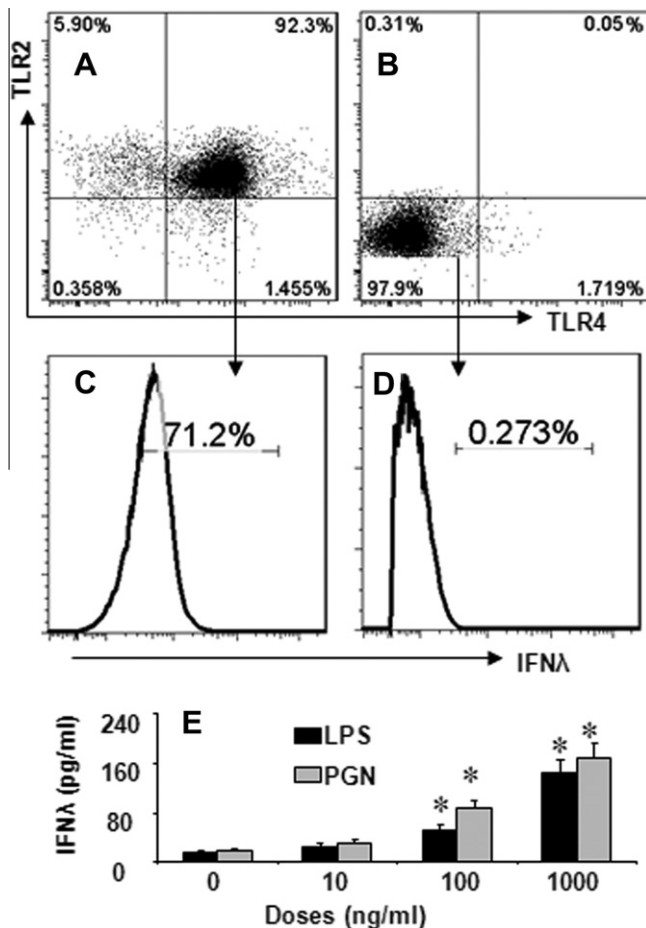


**Fig. 2.** EoL1 cells release IFN $\lambda$  upon TLR ligand stimulation. (A and B) The dot plots show TLR2 and TLR4 expressing EoL1 cells. (B) An isotype control. (C and D) The histograms indicate the expression of IFN $\lambda$  by the EoL1 cells in (A) and (B) (pointed by arrows). (E) The bars indicate the levels of IFN $\lambda$  in culture supernatants of EoL1 cells stimulated by LPS or PGN. The data were expressed as mean  $\pm$  SD from three experiments. \* $p$  < 0.01, compared with the dose "0" group.

and jejunal sections, eosinophils were observed in naïve control rats; no eosinophils were observed in rats treated with anti-CCR3 antibody.

## 2.7. Statistics

All values were expressed as the means  $\pm$  SD of at least three independent experiments. The values were analyzed using the two-tailed unpaired Student's  $t$ -test when the data consisted of two groups or by ANOVA when three or more groups were compared.  $p$  < 0.05 was accepted as statistically significant.

Other experimental procedures were listed in [Supplementary materials](#).

## 3. Results

### 3.1. Eos express IFN in the intestine

In the first attempt, we observed the expression of IFN $\lambda$  by Eos in the duodenal biopsies. As shown by immunohistochemistry, Eos were observed in the duodenal biopsy sections of the patients with or without food allergies. The number of Eos was significantly more in the patients with food allergies than that in non-food allergy ( $p$  < 0.01). Three types of positive cells, MBP $^{+}$ , IFN $\lambda^{+}$  and MBP $^{+}$  IFN $\lambda^{+}$ , were observed. The rate of MBP $^{+}$  IFN $\lambda^{+}$  cells was

85%  $\pm$  11.8% of the total MBP $^{+}$  cells in food allergy group and 82.1%  $\pm$  14.8% of the total MBP $^{+}$  cells in non-food allergy group ( $p$  > 0.05; [Fig. 1](#)). In addition, similar results were also observed in human tissues of the skin, tonsil, colon, lung, kidney and stomach (data not shown). To confirm the data that Eos express IFN $\lambda$ , we infected mice with parasites to induce the eosinophilia in the intestine. The nucleated cells were isolated from the jejunum and examined by flow cytometry. The results showed that the rate of MBP $^{+}$  IFN $\lambda^{+}$  cells was 92.5%  $\pm$  15.6% in the total MBP $^{+}$  cells from six mice with parasite infection and 91.1%  $\pm$  16.6% in naïve mice. The data indicate that the number of Eos is significantly more in the duodenal mucosa of patients with food allergy than those of non-food allergy. Over 80% Eos in human and over 90% Eos in mice express IFN $\lambda$ .

### 3.2. Eos release IFN $\lambda$ upon activation by TLRs

Previous reports indicate that Eos express TLR2 and TLR4 [\[13\]](#). We next examined the expression of TLRs on the Eo cell line, EoL1 cells. As shown by flow cytometry, over 90% EoL1 cells express both TLR2 and TLR4 ([Fig. 2A and B](#)). The EoL1 cells also expressed IFN $\lambda$  ([Fig. 2C and D](#)). We then added the ligands of TLR2 (peptidoglycan; PGN; 100 ng/ml) [\[14\]](#) and TLR4 (lipopolysaccharide; LPS; 100 ng/ml) to activate the EoL1 cells in culture for 3 days. As assessed by ELISA, both TLR2 and TLR4 ligands could respectively induce the release of IFN $\lambda$  from EoL1 cells ([Fig. 2E](#)).

### 3.3. Intestinal epithelial cells express the IFN $\lambda$ receptors (IFN $\lambda$ R)

Eos are dispersed immediately under the subepithelial region as well as in the epithelial layer. Such an anatomical relation between Eos and the epithelium implies that the Eo-derived IFN $\lambda$  may affect the epithelial cells. By immunohistochemistry, the expression of IFN $\lambda$ R was observed on the epithelial cells of human duodenal biopsies as well as on the human intestinal cell line, T84 cells ([Fig. 3](#)).

### 3.4. Eo-derived IFN $\lambda$ induces gut epithelial barrier dysfunction

The data in [Figs. 2 and 3](#) imply that Eo-derived IFN $\lambda$  may affect the intestinal epithelial barrier function. To test the hypothesis, we cultured the human intestinal epithelial cell line, T84 cell monolayers, in transwells to confluence. EoL1 cells were added to the basal chambers of transwells in the presence of LPS or PGN. The results showed that upon the exposure to LPS or PGN, the transepithelial electric resistance (TER) was markedly dropped and the permeability to HRP was significantly increased, which could be blocked by addition of the anti-IFN $\lambda$  antibody ([Fig. 4A and B](#)). The data imply that the Eo-derived IFN $\lambda$  can compromise the intestinal epithelial barrier function.

To further confirm the results in [Fig. 4A](#), we induced the eosinophilia in mouse intestine by infecting mice with parasites. The mice with eosinophilia were injected with LPS or PGN via i.p. to induce the Eos to release IFN $\lambda$ ; the mice were concurrently gavaged with OVA to determine the epithelial barrier permeability. As shown by Western blotting ([Fig. 4C and D](#)), the contents of OVA in the protein extracts of intestinal tissue were significantly increased in a TLR ligand dose-dependent manner, which could be blocked by pretreatment with the anti-IFN $\lambda$  antibody, but not by pretreatment with an isotype antibody (data not shown). The results indicate that Eo-derived IFN $\lambda$  can cause intestinal epithelial barrier dysfunction.

To find the mechanism by which Eo-derived IFN $\lambda$  induces intestinal epithelial barrier dysfunction, we examined the T84 cells in [Fig. 3](#) and the intestinal epithelial cells of mice in [Fig. 4](#). The results showed that abundant apoptotic cells were detected among the

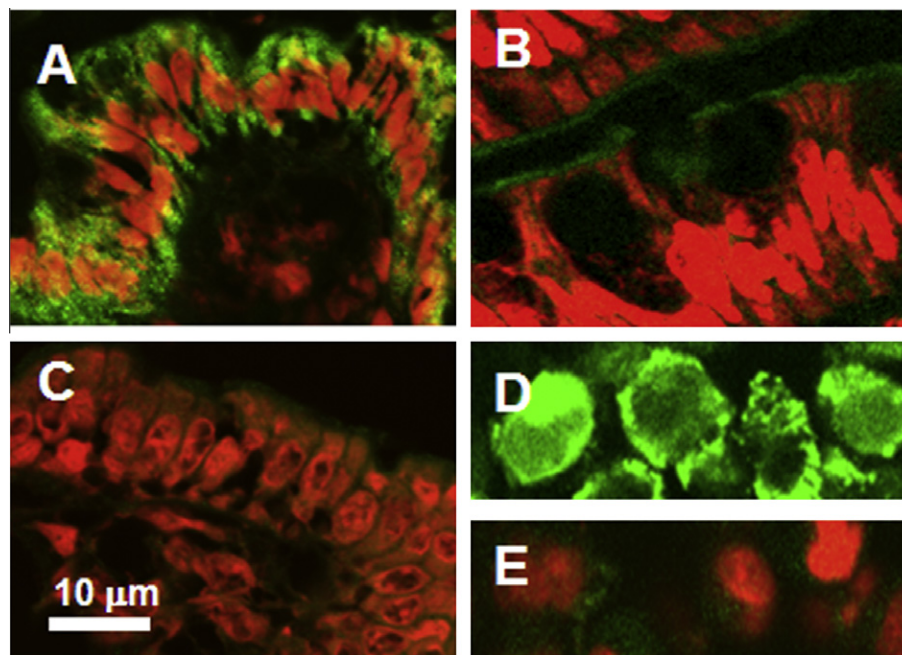


**Fig. 3.** Intestinal epithelial cells express IFN $\lambda$ R. Human duodenal biopsies (see Fig. 1) and T84 cells were processed for immune staining. IFN $\lambda$ R was stained in green. The nuclei were stained in red. Sample annotation: (A) food allergy; (B) non-food allergy; (C) isotype control; (D) T84 cells; (E) isotype control. The data represent three experiments. Original magnification: 630 $\times$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

T84 cells and the intestinal epithelial layer. The results indicate that the Eo-derived IFN $\lambda$  induces the intestinal epithelial barrier dysfunction via causing the intestinal epithelial cell apoptosis.

### 3.5. Eo-derived IFN $\lambda$ plays an important role in the initiation of food allergen-related Th2 polarization in the intestine

Finally we examined the role of Eo-derived IFN $\lambda$  in the pathogenesis of aberrant Th2 polarization. With the same mouse model in Fig. 3, we induced eosinophilia in the intestine. The mice were gavage-fed with a food allergen together with i.p. injection with LPS or PGN. As shown in Fig. 5, the high levels of IL-4 and OVA-specific IgE were detected in the intestinal protein extracts; high frequencies of Eos and mast cells were observed in the intestinal sections; and high frequencies of the OVA-specific Th2 cells were also detected in the intestine. On the other hand, the serum levels of IFN $\lambda$  were increased upon the treatment with LPS or PGN; the frequencies of Th1 cells and Tregs in the intestine were suppressed. Mice pretreated with anti-IFN $\lambda$  antibody, were not induced the aberrant Th2 polarization in the intestine; pretreatment with isotype IgG antibody did not block the aberrant Th2 polarization in the intestine. Mice with eosinophil depleted did not show any signs of Th2 inflammation in the intestine.

## 4. Discussion

The present paper reports a novel finding that the intestinal Eos express IFN $\lambda$ . Microbial products, LPS and PGN, can activate Eos to release the IFN $\lambda$ . Intestinal epithelial cells express the IFN $\lambda$ R; the Eo-derived IFN $\lambda$  can compromise the intestinal epithelial barrier function by inducing the intestinal epithelial cell apoptosis. Concurrent exposure to microbial products and food antigens can induce the aberrant Th2 polarization in the intestine of mice that can be blocked by administration with anti-IFN $\lambda$  antibody.

The integrated intestinal epithelial barrier plays an important role in maintaining the homeostasis in the intestine. A number of intestinal disorders are related with the epithelial barrier dysfunction, such as food allergy and inflammatory bowel disease [15]. Our previous study revealed that the Th2 cytokine IL-4 could compromise intestinal epithelial barrier function [16]. Others found that IL-13 also induced intestinal epithelial barrier dysfunction [17]. In addition, other cytokines, such as IFN $\gamma$  [18], tumor necrosis factor alpha [19], and many others [20,21], could also affect the epithelial barrier function. The present study has revealed a previous undescribed factor, the IFN $\lambda$ , which also can cause intestinal epithelial barrier dysfunction. This cytokine exists in normal Eos, even under non-allergy conditions.

Most of the previously reported factors, such as tumor necrosis factor alpha, involved in damaging the epithelial barrier function are also inflammatory mediators [22]; whether these factors are the results from epithelial barrier dysfunction-induced inflammation, or the pre-existed inflammation produces the cytokines, the latter in turn induces the epithelial barrier dysfunction, is not fully understood. In the present study, we found that mice with eosinophilia, which was caused by parasite infections, still maintained the normal intestinal epithelial barrier function. The mice had epithelial barrier dysfunction only occurred on exposing microbial products, abundant IFN $\lambda$  was released from Eos that caused the epithelial barrier permeability to increase. Of course, there are a number of other chemical mediators in Eos apart from IFN $\lambda$ ; whether those mediators are also involved in the epithelial barrier dysfunction observed in the present study? The present data show that the increase in epithelial barrier permeability can be blocked by anti-IFN $\lambda$  antibody, demonstrating that IFN $\lambda$  is responsible for the epithelial barrier dysfunction in this experimental system. We also used microbial products, LPS and PGN, in the present study; these TLR ligands may not damage the epithelial barrier function directly because the intestinal epithelia are tolerant to these commensal bacterial products under the physiological condition [23,24]. Furthermore, upon exposure to

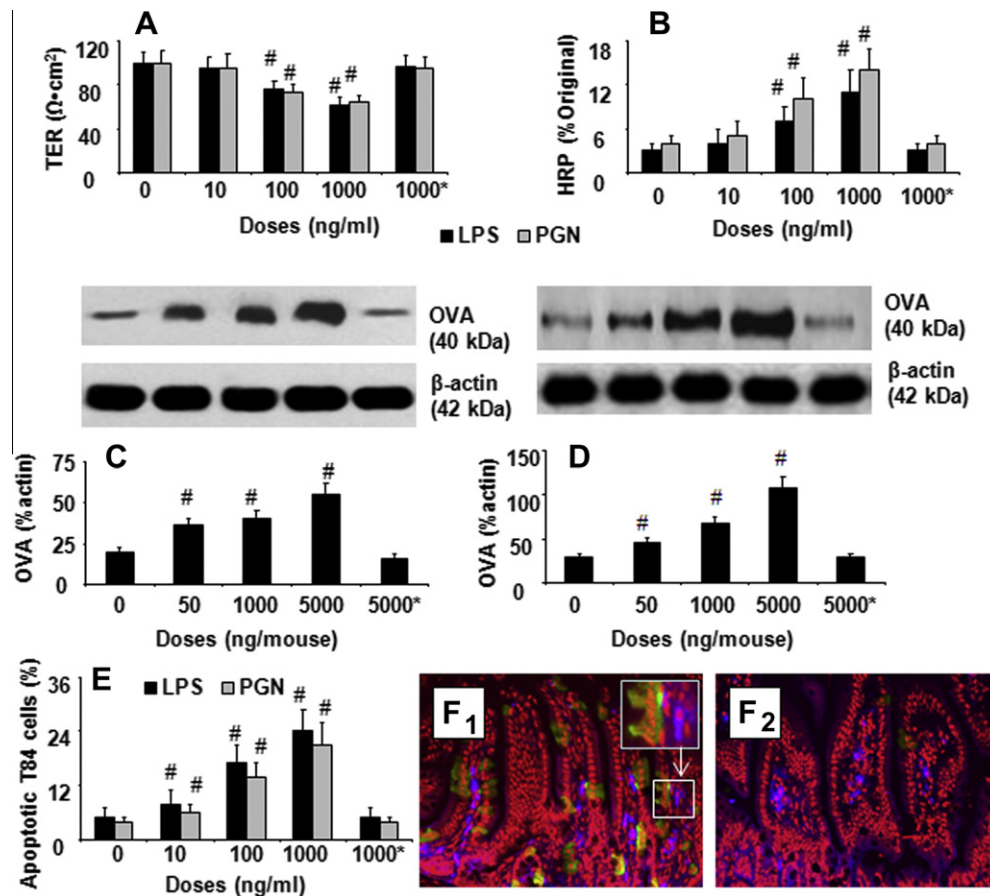


**Fig. 4.** Eo-derived IFN $\lambda$  compromises the intestinal epithelial barrier integrity. T84 monolayers were cultured in transwells with Eos in the basal chambers in the presence of LPS or PGN. (A) Bars indicate the transepithelial electric resistance (TER) of T84 monolayers in response to LPS or PGN stimulation. (B) Bars indicate levels of horseradish peroxidase (HRP; using as a permeability tracer) in the culture medium in the basal chambers of transwells. (C and D) Bars indicate the levels of OVA (using as a tracer for assessing the epithelial permeability *in vivo*) in the intestinal tissue of mice treated with OVA/LPS (C) or OVA/PGN (D). (E) Bars indicate the frequency of the apoptotic T84 cells in (A) and (B), which was determined by flow cytometry. (F) The representative confocal images show apoptotic epithelial cells (F<sub>1</sub>, in green) and Eos (in blue) in the intestine of mice in (C) and (D). F<sub>2</sub> is an isotype control. Original magnification: 200 $\times$ . Each experimental group consisted of six mice. The T84 cell experiments were performed three times. \*Pretreatment with anti-IFN $\lambda$  antibody (500 ng/ml). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PGN, a ubiquitin editing E3 enzyme, the A20, is upregulated in the cells; the A20 in turn promotes tolerance to LPS in enterocytes [25,26].

The present data indicate that Eo-derived IFN $\lambda$  can compromise the epithelial barrier's integrity via inducing epithelial cell apoptosis. The results are in line with previous observations in which we found that IFN $\lambda$  could cause CD3<sup>+</sup> cell apoptosis [6]; others indicated that IFN $\lambda$  could induce cancer cell death via apoptotic pathway [7]. The epithelial cell apoptosis results in epithelial barrier defects and increase in epithelial barrier permeability to macromolecular antigens, such as OVA or HRP (their molecular weight is over 40 kDa), as shown by the sharply down-regulated TER of T84 monolayers and increase in HRP flux in the present data. Thus, the absorbed specific antigen, OVA, as shown by the *in vivo* data, is assumed being absorbed via the defect of epithelial layer caused by apoptosis.

Under the physiological conditions, the ingested proteins are digested by enzymes in the intestine before they are absorbed into the intestinal tissue. The integral epithelial barrier restricts the absorption of macromolecular protein and large peptides; the absorbed nutrients usually have the least antigenicity. Upon exposure to Eo-derived IFN $\lambda$ , a large quantity of macromolecular protein passed the epithelial barrier and got into the intestinal tissue as shown by

the present data. The antigens then have the opportunity to contact the immune cells to initiate immune responses. The concurrently presented-microbial products, LPS or PGN, can speed up the immune response, or skew the immune response to aberrant Th2 polarization, as we recently observed that PGN could increase the T cell immunoglobulin mucin domain 4 (TIM-4) in dendritic cells and macrophages (data not shown); TIM-4 can induce Th2 polarization [27,28]. Indeed, employing a mouse model with eosinophilia, after exposed the mice to microbial products LPS or PGN, we did observe the skewed antigen-specific Th2 polarization and Th2 pattern inflammation in the intestine.

Based on the present data and literature review, we may envisage a scenario like this: Under the condition of eosinophilia, such as the parasite infections, the eosinophils deposit a large quantity of IFN $\lambda$  in the body, as shown by the present data that normal Eos also have detectable IFN $\lambda$ . When the Eos encounter microbial stimuli, such as under psychological stress as we reported before [1,2], microbial products, such as LPS or PGN, cross the damaged epithelial barrier to enter deep tissues where the Eos disperse everywhere. The microbial products activate the TLRs on Eos to cause Eos to release IFN $\lambda$ , the latter causes epithelial barrier dysfunction resulting in proteins or large peptides with good antigenicity to be absorbed. The absorbed antigens and microbial

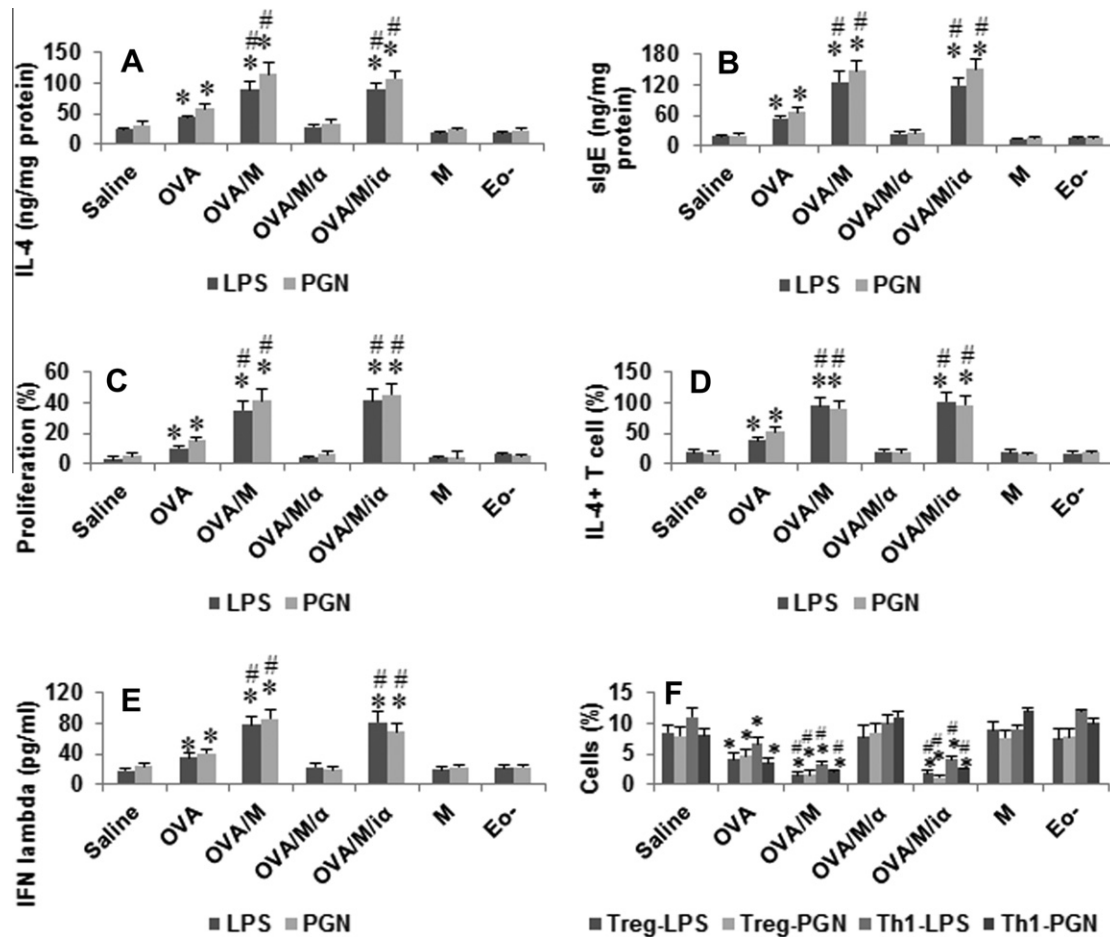


**Fig. 5.** Eo-derived IFN $\lambda$  facilitates the aberrant Th2 polarization in the intestine. Mice with eosinophilia and OVA-sensitization were gavaged with OVA together with LPS or PGN i.p. as described in [Supplementary materials](#). (A and B) Bars indicate the levels of IL-4 (A) OVA specific IgE (sIgE) (B) in the protein extracts of the jejunum. (C) Bars indicate the rate of jejunal T cell proliferation that was assessed by the CFSE dilution. (D) Bars indicate the frequency of IL-4+ T cells among the proliferated cells in (C). (E) The bars indicate the serum levels of IFN $\lambda$ . (F) The LPMC were prepared and analyzed by flow cytometry. The bars indicate the frequencies of Treg or Th1 cells in the LPMC of mice treated with LPS or PGN. Each group consisted of six mice. M, mice were treated with microbial product (LPS or PGN, 1  $\mu$ g/mouse, i.p.).  $\alpha$  (or  $\alpha$ ), mice were pretreated with anti-IFN $\lambda$  antibody (500 ng/mouse, i.p.) (or isotype IgG) 30 min prior to each challenge with antigen. Eo-, mice with eosinophil depleted. The data were presented as mean  $\pm$  SD. \* $p$  < 0.05, compared with the saline group. # $p$  < 0.05, compared with the OVA-group.

products collaborate together to initiate the aberrant Th2 polarization and to further develop into allergic disorders such as food allergy.

#### Author contributions

S.-H.H., Z.L., C.-H.S., H.Z., W.M., L.-F.Z. and T.M. collected samples, performed experiments and revised the manuscript. S.-H.H. and P.-C.Y. designed the project, analyzed experimental data and wrote the manuscript.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.cyto.2012.01.003](https://doi.org/10.1016/j.cyto.2012.01.003).

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Supplemental materials

**Eosinophil-derived interferon- $\lambda$  contributes to induction of skewed T helper 2 polarization in the intestine**

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Running title: IFN $\lambda$  compromises gut epithelial barrier function

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## Reagents

Interferon (IFN) $\lambda$  ELISA kit was purchased from eBiosciences (San Diego, CA; sensitivity = 8 pg/ml). Antibodies of IFN $\lambda$  (C-19), IFN $\lambda$  receptor (H-150), MBP (H-9), OVA (094-06), CCR3 (H-52), TLR2 (TL23) and TLR4 (C-18) were purchased from Santa Cruz Biotech (Santa Cruz, CA). IL-4 ELISA kit was purchased from R&D Systems (Shanghai, China; the sensitivity is 2pg/ml). Apop Tag Plus Fluorescein *in situ* Apoptosis Detection Kit was purchased from Chemicon International (Temecula, CA). OVA-specific IgE ELISA kit was purchased from AbD Serotec (Raleigh, NC; sensitivity = 8 ng/ml). Annexin V (FITC and PE) was purchased from Abcam (MA). Endotoxin levels in all reagents were detected using the limulus assay (Limulus amebocyte lysate QCL 1000, Bio Whittaker, Walkersville, MD, USA). LPS-contaminated reagents were treated by polymyxin B-agarose to eliminate the LPS. The reagents used in this study contained <0.2U endotoxin/10  $\mu$ g reagents.

## Mice

BALB/c mice (6-8 week old) were purchased from the Beijing Experimental Animal Institute. Mice were maintained in a specific pathogen free condition. All studies were conducted under a protocol approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

## Eo cell lines

The eosinophil cell line, EoL1 cells, generated from a patient with acute myeloid leukaemia following hypereosinophilic syndrome, was purchased from the RIKEN Cell Bank (Tsukuba, Japan). EoL1 cells were cultured with RPMI 1640 medium under a routine cell culture condition.

### **Diagnosis of food allergy:**

***Case history:*** The case history of food allergy was collected from each patient.

Symptoms typically appear within minutes to two hours after the ingestion of the food to which he or she is allergic, which included a tingling sensation in the mouth, swelling of the tongue and the throat, difficulty breathing, hives, vomiting, abdominal cramps, diarrhea, drop in blood pressure, loss of consciousness, etc.

***Skin prick test:*** One drop of extract of each fresh food (purchased from Beijing Xiehe Hospital, Beijing, China) was applied to the patients' forearm. The skin covered by the drop of food antigen was pricked with 1 mm single-peak lancets. Positive control was performed using 10 mg/ml histamine dihydrochloride; the negative control using saline solution. The results were read after 15 min. The outline of each wheal was documented using a piece of transparent paper to copy the wheal. The skin wheal areas were determined with scanned digital files using the software ImageJ. Skin prick tests were regarded positive when the area was larger than 7 mm<sup>2</sup>. Subjects with a wheal area below 3 mm<sup>2</sup> in positive controls were excluded from this study.

***Oral challenge with food antigens:*** Oral challenges were performed in all the subjects in this study using one of the positive food allergens (revealed by skin test; usually chose the one had the largest wheal area). The food allergens in the skin prick test

to screen the offending allergens in these patients are listed in Table S1 although some patients might also sensitize to other food antigens. The oral challenge was performed in a double blind manner. The antigen extracts or placebos were contained in coded capsules. The patients took 3 capsules of antigen or placebo; they were asked to take another 3 capsules (placebo or antigens) one week later. The study subjects were closely monitored for 4 hours following oral challenge in our department where treatment of anaphylaxis was readily available. The healthy controls were also oral challenged with a mixture of the 10 antigens.

***Serum levels of cytokine and allergen-specific IgE:*** Blood samples were collected from each patient and healthy controls. The sera were separated immediately after withdrawn and stored at -70°C until use. The levels of total IgE, antigen specific IgE, IL-4, IL-13 and IFN- $\gamma$  in the sera were measured by ELISA following the manufacturer's instructions. Negative controls included omitting the primary antibodies and using isotype IgG instead of the primary antibodies for each parameter.

***Counting eosinophil and mast cell in the intestinal mucosa:*** Numbers of eosinophils and mast cells in the intestine were counted. Intestinal segments or biopsies were fixed with 4% paraformaldehyde (for Eo counting) or Carnoy solution (for mast cell counting) overnight and processed for paraffin sections. The sections were stained with hematoxylin (for Eo counting) or 0.5% toluidine blue (for mast cell counting) and observed under a light microscope.

**Western blotting:** The homogenates of intestinal tissue were centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was collected; the levels of proteins were determined by Bio-Rad protein assay. A total of 50 µg denatured protein were separated on 12.5% SDS-PAGE gel and transferred onto nitrocellulose membrane. The membranes were then incubated with anti-OVA antibody (1:1000) or isotype IgG (using as negative control) overnight at 4°C. Reactions were developed using the Pierce ECL chemiluminescence substrate kit. Results were recorded with X ray film. Bands were semi-quantified by densitometry. The intensity of the specific band was normalized to the intensity of β-actin band.

### **Flow cytometry**

Cells were collected and incubated with fluorescence-labeled primary antibodies on ice for 30 min (For the intracellular staining, cells were fixed with 1% paraformaldehyde on ice for 30 min and incubated with permeabilization reagents for 30 min on ice). The stained cells were analyzed using a flow cytometer (BD FACSCanto; BD Bioscience, San Jose, CA). To detect the frequency of apoptotic T84 cells, the cells were stained with Annexin V with a commercial reagent kit following the manufacturer's instruction.

### **CFSE dilution**

T cell proliferation was assessed by CFSE dilution. The lamina propria mononuclear cells (LPMC) were isolated by MACS and labeled with CFSE. The LPMC were cultured in the presence of a specific antigen, OVA (20 ng/ml), for 4 days. The cells were collected, washed, stained with anti-IL-4 and CD3 antibodies, and analyzed by flow cytometry. The

proliferated cell population was gated, the percentage of proliferated cells was calculated. Then, the percentage of CD3<sup>+</sup> IL-4<sup>+</sup> T cells was further calculated regarding as proliferated Th2 cells.

### **Intestinal eosinophilia model**

A parasite infection model was adopted to induce eosinophilia in the intestine. The maintenance of the *T. muris* parasite and methods used for infection and large intestinal worm burden assessment were as previously described (Motomura Y, et al. Mechanisms underlying gut dysfunction in a murine model of chronic parasitic infection. *Am J Physiol Gastrointest Liver Physiol.* 2010;299:G1354-60.). Mice were infected by oral gavage with 120–150 infective eggs and sacrificed or used for further experiments 45 days later. The cecum was removed and opened to count the number of parasite. The results showed the parasites were found in the cecum of all infected mice (5-11 parasites were found in each cecum. The jejunum was excised and processed for paraffin sections and stained with H&E method. The number of Eos was counted under a light microscope. The results showed that the number of Eos was  $46.4 \pm 8.6/\text{mm}^2$  in infected mice in contrast to  $11.5 \pm 3.4/\text{mm}^2$  in saline control mice ( $p < 0.01$ ).

### **T84 cell culture**

The human colon-derived crypt-like cell line, T84 cells, was purchased from ATCC (Manassas, VA, USA) as a passage of 30. Only passages 30–35 were used for the experiments. Cells were grown in 5% CO<sub>2</sub> at 37°C and maintained in Dulbecco's modified eagle's medium supplemented with 10% heat inactivated fetal bovine serum,

1% nonessential amino acids, 1% glutamine, and 2% penicillin-streptomycin. Upon 80–90% confluence, cells were split using 0.05% trypsin plus 1 mmol/L EDTA. T84 cells ( $10^6$  cells in 1 mL) were seeded on tissue culture transwell system (0.4  $\mu$ m pore size, 1.2 cm<sup>2</sup> surface area of the filter support) and grown for 5–7 days and used for experimentation. The media were changed every day.

### **T84 monolayer electrical resistance measurements**

Electrical resistance is a measure of the epithelial barrier function of passive ion movement. Transepithelial electric resistance (TER) was measured to quantify changes in epithelial cell culture integrity and confluence with a Millicell-ERS apparatus (Millipore, Danvers, MA, USA). Experiments were carried out using differentiated T84 cells with a TER of  $>1000 \Omega \cdot \text{cm}^2$ .

### **Horseradish peroxidase flux of T84 monolayer**

Horseradish peroxidase (HRP; 10  $\mu$ mol/L) was added to the apical chamber of transwells. Samples were obtained from the basal chambers 2 h later. Samples were assayed for HRP by kinetic enzymatic assay. Briefly, the sample was added to phosphate buffer containing H<sub>2</sub>O<sub>2</sub> and o-dianisidine. Enzyme activity was determined from the rate of increase in optical density at 460 nm. HRP transport was calculated as recovery of the original amounts of HRP.

### **Observation of the effect of the Eo/IFN $\lambda$ -induced intestinal epithelial barrier dysfunction on inducing the skewed Th2 polarization in the intestine**

BALB/c mice with or without eosinophilia were immunized with OVA 0.1 mg mixed in 0.3 ml alum via subcutaneous injection on day 0, day 7 and day 14. The mice were gavage-fed with OVA (0.1 mg/mouse) together with LPS (1 $\mu$ g/mouse, ip) or PGN (1  $\mu$ g/mouse, ip) on day 28, day 30 and day 32 respectively. The mice were sacrificed on day 33. Some mice were ip injected with anti-IFN $\lambda$  (500 ng/mouse, 30 min prior to each exposure to OVA) antibody or isotype IgG (using as a control). The total proteins were extracted from a piece of the jejunum and subjected to analyses of OVA-specific IgE and IL-4; the lamina propria mononuclear cells were isolated from the jejunum and subjected to the analysis of OVA-specific Th2 cell proliferation. The experimental procedures were referred to our previous reports (Yang, et al. *Gastroenterology* 2007; 133(5):1522-33. He, et al. *Gastroenterology* 2011; 141:249-258).

Table S1. Demographic data of FA patients (pt)

| Pt# | Age | Sex | Case History (years) | Food allergens (determined by skin test) | Other allergies | Specific IgE (ng/ml) | Total IgE (ng/ml) |
|-----|-----|-----|----------------------|------------------------------------------|-----------------|----------------------|-------------------|
| 1   | 32  | M   | 5                    | Milk                                     | Asthma          | 61.2                 | 91.2              |
| 2   | 22  | M   | 4                    | Wheat, milk, egg                         | Dermatitis      | 58.3                 | 94.5              |
| 3   | 43  | M   | 12                   | Egg, sea food, wheat                     |                 | 45.2                 | 71.3              |
| 4   | 42  | M   | 8                    | Egg, soy bean, peanut                    | Asthma          | 61.2                 | 84.6              |
| 5   | 26  | M   | 6                    | wheat sea food, sesame                   |                 | 84.2                 | 124.3             |
| 6   | 53  | M   | 8                    | Egg, peanut, tree nuts                   |                 | 52.1                 | 61.2              |
| 7   | 62  | M   | 6                    | soy bean, peanut                         | Rhinitis        | 48.6                 | 85.2              |
| 8   | 35  | M   | 11                   | Wheat, Peanut, sea food,                 |                 | 47.3                 | 85.4              |
| 9   | 33  | M   | 5                    | Tree nut, wheat, egg,                    | Rhinitis        | 61.3                 | 79.2              |
| 10  | 43  | M   | 6                    | Milk                                     |                 | 43.5                 | 77.8              |
| 11  | 19  | F   | 14                   | Sea food, sesame                         |                 | 35.6                 | 118.6             |
| 12  | 62  | F   | 5                    | Egg, milk                                | Dermatitis      | 84.6                 | 142.5             |
| 13  | 44  | F   | 4                    | Milk, wheat                              |                 | 54.8                 | 84.2              |

|    |    |   |   |                            |          |      |      |
|----|----|---|---|----------------------------|----------|------|------|
| 14 | 53 | F | 8 | Milk, wheat                |          | 57.8 | 81.5 |
| 15 | 26 | F | 6 | Peanut, tree nut,<br>wheat | Rhinitis | 71.5 | 94.6 |