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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 IFNλ expression were observed by immune staining. Intestinal epithelial cell line, T84 cells, and a mouse model were employed to observe the effect of IFNλ on the epithelial barrier function and the initiation of skewed T helper (Th)2 polarization in the mouse intestine.
Results: IFNλ 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 IFNλ. Exposure to IFNλ 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 IFNλ that can induce intestinal epithelial barrier dysfunction and promotes the initiation of the aberrant Th2 polarization in the intestine.

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)λ is a newly described cytokine, including IFNλ1 [interleukin (IL)-29], IFNλ2 (IL-28A) and IFNλ3 (IL-28B) three subtypes; the IFNλ1 is only expressed in human. IFNλ 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 IFNλ 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 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 IFNλ in our previous study ([6] data not shown);

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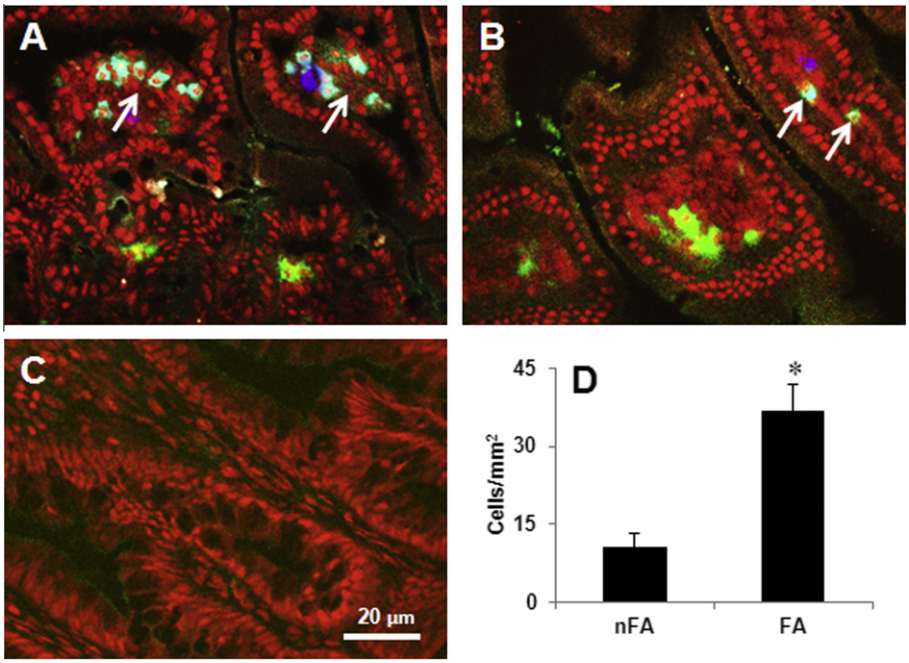

Fig. 1. Eos express IFNλ in the intestine. Duodenal biopsies were processed for immunohistochemistry. The representative confocal images show that the Eo marker, MBP, was stained in green; the IFNλ was stained in blue. The light blue color (pointed by arrows) was merged by the colors of green and blue. 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 ± SD). $^{*}p < 0.01$, compared with food allergy (FA) group. nFA, non-FA. Original magnification: 200×. (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 IFNλ 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 IFNλ; 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 IFNλ 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 °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 IFNλ antibody (1:200) and mouse anti-MBP antibody (1:300), or isotype IgG (using as negative control) overnight at 4 °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×) 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 °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