



Involvement of interleukin-18 in the pathogenesis of human eosinophilic esophagitis

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Abstract IL-18 is induced in food allergy and EoE is food allergen-induced disease. Therefore, we tested the hypothesis whether IL-18 is involved in food allergen-induced EoE pathogenesis. Accordingly, we examined normal SPT+ and SPT– EoE patient blood and biopsy samples for IL-18, IL-18R α , ICAM and VCAM expression. Herein, we show increased IL-18 level is highly significant in food allergen SPT+ compared to SPT– EoE patients. We also report that IL-18R α + cells and mRNA levels are induced in the esophageal biopsies of EoE patients and blood IL-18 levels correlate with esophageal eosinophilia ($P < 0.01$). Additionally, we report that the levels of esophageal eosinophil and mast cells correlate with ICAM expression in human EoE. Mechanistically, we show that IL-18 in vitro stimulates iNKT cells and endothelial cells and induce eosinophil active cytokines IL-5 and IL-13. We provide the evidence that IL-18 is critical cytokine involved in activation of iNKT cells and ICAM in promoting human EoE.

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Abbreviations: CE, chronic esophagitis; DAPI, 4',6-diamidino-2-phenylindole fluorescent mounting material; EoE, eosinophilic esophagitis; ELISA, enzyme-linked immunosorbent assay; GERD, gastroesophageal reflux disease; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; IL, interleukin; iNKT, invariant natural killer T cell; PCR, polymerase chain reaction; HMVEC, primary human microvascular endothelial cells; SPT+, skin allergen positive test

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1. Introduction

In recent years, major advances have occurred in understanding eosinophil and mast cells (MCs) accumulation [1–3] and remodeling in EoE [4,5]; however, the mechanisms of EoE induction, progression, diagnosis and treatment are still not clearly understood. EoE patients that have <15 eosinophils/hpf in esophageal biopsies are hard to differentiate from gastroesophageal reflux disease (GERD), as some EoE patients may have PPI-responsiveness [6]. Experimental modeling has established that Th2 cytokine signaling is required for induction of EoE. Considerable evidence supports a critical role for the Th2 cytokines interleukin (IL)-5, IL-13 and IL-15 in EoE pathogenesis [7,8]. The gene exhibiting the highest increase of expression in the esophagus of EoE patients is eotaxin-3, an eosinophil chemoattractant and activator produced by esophageal epithelial cells [2]. Additionally, we, and others, reported that IL-15 responsive iNKT cells are induced in EoE and neutralization of iNKT cells ameliorates the severity in pediatric EoE [9–13]. Earlier, our microarray analyses showed increased levels of the IL-18R α transcript in EoE patients [2]. IL-18 activates B cells and natural killer (NK) T cells in an antigen-independent manner [14,15] and this process has been shown to contribute in a number of intestinal allergic responses including celiac disease, a disease that shares features with EoE [16–19]. IL-18 is a pleiotropic cytokine that is elevated in a number of eosinophilic allergic diseases such as food allergy, dermatitis, asthma, and colitis [20–22]. Inflammatory cells involved in innate immunity secrete IL-18 [16,23] and it has been reported that IL-18 stimulates iNKT cells without T cell receptor (TCR) engagement [24,25]. The present study indicates that IL-18 may be involved in the pathogenesis of EoE. Herein, we demonstrate that IL-18 and its specific receptor IL-18R α are increased in the blood and esophagus of EoE patients, respectively. Notably, IL-18R α transcript levels correlate significantly with esophageal eosinophilia in active and treated EoE patients. Mechanistically, we show that IL-18 activate iNKT cells to produce eosinophil active Th2 cytokines (e.g. IL-5, IL-13) that promotes EoE pathogenesis. Taken together, our current findings define a novel role for IL-18 in Th2 responses and provide evidence that IL-18 may have an important role in EoE pathogenesis by activating iNKT cells. Therefore, in the current study we tested the hypothesis that the induction of IL-18 is associated with the esophageal eosinophilia in human EoE. Herein, we report that indeed IL-18 may have a significant role in food allergic (SPT+) EoE patients.

2. Materials and methods

2.1. Selection of EoE and non-EoE patients and tissue collection

Formalin-fixed, paraffin-embedded biopsy samples were obtained from the esophagus of normal individuals or EoE patients as per an Institutional Review Board (IRB)-approved protocol. The comparison control normal subjects (non-EoE), chronic esophagitis (CE) and EoE patients were selected without regard to age, atopic status or gender. Diagnosis was established based on the maximum eosinophil count per high power field (hpf) ($\times 400$). Control individuals (non-EoE or CE)

were defined as having 0–2 esophageal eosinophils/hpf and no basal layer expansion. The normal biopsies were obtained from patients who showed symptoms typical of gastroesophageal reflux disease (GERD) and EoE but were found normal upon microscopic analyses of esophageal biopsies. Typically, these patients had abdominal pain, and some had allergic diseases including asthma and rhinitis. Patients with EoE were defined as having ≥ 15 -esophageal eosinophils/hpf and included other allergic diseases such as asthma and atopic dermatitis. The CE (chronic esophagitis) patients define, as the group of patient does not qualify with the definition of EoE or normal. They have esophageal eosinophilia <15 eosinophil/hpf with basal cell hyperplasia. The hospital pathologist as consistent with reflux esophagitis evaluated the eosinophils and mast cell level in the esophageal biopsies of non-EoE and EoE patients. The fresh biopsy samples were collected in RNA lysis buffer for RNA isolation and blood was drawn from each normal, EoE and non-EoE patients at the time of scheduled biopsy (including before and after treatment of EoE patients) at Cincinnati Children's and Tulane University School of Medicine in a citrate-coated tubes. The blood plasma was isolated by centrifugation and stored at -20°C for cytokine analysis. All samples were used according to the patients' consent and IRB approved protocol of CCHMC, Cincinnati, OH (Year 2011–12) and TUSM, New Orleans, LA (Year 2013–14). The pool EoE and non-EoE patient data generated at both hospital centers are presented and the patient's detailed clinical characteristics are shown in Table 1. Our current study was performed on heterogeneous patient population, as PPI trial was not performed on all EoE patients, and is our limitation.

2.2. Quantification of serum IL-18

IL-18 protein concentrations in the serum of normal and EoE patient samples were quantified by using a DuoSet enzyme-linked immunosorbent assay (ELISA) Development kit (R & D Systems). The detection limit was 0.9 pg/ml.

2.3. Quantitative PCR

The RNA samples (500 ng) were subjected to reverse transcription using Bioscript reverse transcriptase (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. IL-18R α , ICAM, VCAM, IL-5 and IL-13 were quantified by real-time PCR using IQ5 (Bio-Rad). Results were then normalized to human or mouse GAPDH amplified from the same cDNA mix and expressed as relative gene expression. cDNAs were amplified using the primers listed in Table 2.

2.4. Immunofluorescence staining of biopsy samples for IL-18R α + cell detection

Cryostat sections from frozen esophageal biopsies of normal subjects (non-EoE) and EoE patients were fixed, blocked with normal goat serum to reduce non-specific binding, and incubated with anti-IL-18R α + antibody (eBioscience). The IL-18R α + immunostained sections were mounted with DAPI mounting material. The images were captured using an Olympus BX51 microscope with appropriate filters. IL-18R α + positive cells were counted on each stained tissue sections per high

Table 1 Patient's clinical and pathological characteristics.

Patients	Age (Year)	Gender	Esophageal disease	Allergic disease	Eosinophil (maximum/hpf)	SPT +	SPT –	Treatment
1	11	F	NL	Rhinitis	0			LTRA
2	9	M	NL	Asthma	0	+		None
3	8	M	NL	Unknown	0			None
4	13	M	NL	None	0			LTRA ^a
5	7	M	NL	Unknown	0			LTRA
6	12	F	NL	None	0			None
7	6	M	NL	Unknown	0			Miralax,
8	4	M	NL	Unknown	0			LTRA, B2RA
9	1	F	NL	Unknown	0		–	None
10	3	M	NL	Unknown	0			LTRA ^a
11	9	F	NL	Unknown	0			PPI
12	3	F	NL	Unknown	0			None
13	11	M	NL	Rhinitis	0			PPI
14	1	M	NL	Unknown	0			mesalamine
15	10	M	NL	Unknown	0			LTRA ^a
16	13	F	EoE	Asthma	30		–	None
17	12	M	EoE	Rhinitis/Asthma/ Food allergy	31	+		Elementary diet
18	2	M	EoE	None	56		–	PPI ^b
19	3	M	EoE	None	79	+		Elementary diet
20	5	F	EoE	Rhinitis	21		–	PPI
21	10	M	EoE	Asthma/Food allergy	205	+		Elementary diet
22	8	F	EoE	Asthma, Atopic dermatitis	43		– (SNS)	PPI
23	9	M	EoE	Unknown	35			None
24	12	M	EoE	Eczema/Rhinitis/ Food allergy	25	+		Elementary diet
25	11	F	EoE	Asthma	48	+		None
26	11	M	EoE	Asthma/Rhinitis	71			INHGC, B2ARA
27	8	M	EoE	Asthma/Eczema	58	+		Elementary diet
28	13	M	EoE	Asthma/Eczema	44			INHGC, LTRA,
29	4	F	EoE	Rhinitis	52		–	PPI
30	12	M	EoE	Asthma/Rhinitis	198		–	INHGC, PPI
31	15	M	EoE	Unknown	48	+		PPI
32	14	F	EoE	Asthma/Eczema	69			INHGC, PPI,
33	5	M	EoE	Eczema	53	+		Elementary diet
34	12	F	EoE	Asthma/Rhinitis	28		–	B2ARA
35	13	M	EoE	Unknown	34	+		Elementary diet
36	10	M	EoE	Asthma/Food allergy/ Rhinitis/Anaphylaxis	236	+		Elementary diet
37	10	F	R	Asthma/Eczema/Rhinitis	0	+		SWGC, Elementary diet
38	6	M	R	Unknown	0			SWGC
39	12	M	R	Asthma/Eczema	0			SWGC, PPI
40	14	M	R	Eczema	0		–	SWGC
41	8	M	R	Asthma/Eczema/ Rhinitis/Food allergy	1			SWGC
42	4	M	R	Unknown	0			SWGC
43	6	F	R	Eczema	2		–	SWGC
44	3	M	R	Asthma/Eczema /Rhinitis	0			SWGC
50	14	M	R	Rhinitis	8 (SNS)	+		Elementary diet
45	5	M	NR	Rhinitis	24			SWGC, PPI
46	4	F	NR	Rhinitis	31		–	SWGC, PPI
47	7	M	NR	Asthma/Eczema / Rhinitis/Food allergy	19	+		Elementary diet
48	7	M	NR	Asthma/Eczema/ Rhinitis/Food allergy	43			SWGC, antibiotic

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Table 1 (continued)

Patients	Age (Year)	Gender	Esophageal disease	Allergic disease	Eosinophil (maximum/hpf)	SPT +	SPT –	Treatment
49	10	F	NR	Unknown	24			SWGC
50	4	M	NR	Asthma	28	+		Elementary diet
51	11	M	NR	Food allergy/Anaphylaxis	128		–	SWGC
52	9	F	NR	Unknown	43			SWGC
53	8	M	NR	Rhinitis	16	+		Elementary diet
54	15	M	NR	SWGC, OGC	61			SWGC, OGC
55	6	M	CE	Unknown	5			None
56	11	M	CE	Rhinitis/Food allergy	3	+		SWGC, Elementary diet
57	5	F	CE	Rhinitis/Asthma	8			SWGC, antibiotic
58	8	M	CE	Unknown	4			SWGC, PPI
59	12	F	CE	Asthma	11			PPI/SWGC
60	9	F	CE	Asthma/Eczema	7			SWGC

Abbreviations: B2ARA = beta 2 adrenergic receptor antagonist, EoE = eosinophilic esophagitis, F = female, H1RA = H1 receptor antagonist, INHGC = inhaled glucocorticoid, LTRA = leukotriene receptor antagonist, M = male, NL = normal, NR = FP Non-responder, OGC = oral glucocorticoid, (prednisone), PPI = proton pump inhibitor, R = FP responder, SWGC = swallowed glucocorticoid (FP), SNS = Samples not sufficient to use.

power field (400×) and expressed as “IL-18Rα + cells/hpf.” Total 4–5 high power fields in each esophageal sections were evaluated in EoE or normal (non-EoE) control individual biopsy sections.

2.5. Analysis of ICAM and VCAM expression on HMVEC by a flowcytometer

The primary microvascular endothelial cells (HMVEC, Cat # CC-2527, Lonza Group, Ltd., USA) were obtained and were exposed to 0, 5 and 50 nM IL-18 for 24 hrs. These cells were stained with cell surface molecule-specific antibodies for flowcytometer analysis. The following reagents were used for specific antigen analysis: anti-ICAM-1, anti-VCAM-1 and anti-TNFα antibodies (eBiosciences). The cells were incubated for specific antigens with the required combination of antibodies at 4 °C for 45 min followed by two washes. The

flowcytometric analysis was performed using a FACSCalibur (BD Biosciences) and analyzed using Flow J software.

2.6. IL-18 treatment to human iNKT cell line

The non-immortalized human iNKT cell line [26] was provided by Dr. Mattner (CCHMC, Cincinnati, OH) and was grown in RPMI-1640 (HyClone Laboratories, Logan, UT) and Click's Medium (Sigma Aldrich, St. Louis, MO) containing 10% fetal calf serum (FCS, Gibco BRL), 1% 200 mM L-Glutamine (Invitrogen, Grand Island, NY) and penicillin–streptomycin (Gibco BRL). The cells were grown and split into 6-well plates starting with a concentration of 1×10^6 cells in 2 ml media per well. Subsequently, different concentrations of IL-18 (0, 100, 500, 100 ng/ml) were added to the culture protein levels using a DuoSet ELISA set (R&D Systems) as per the manufacturer's protocol.

2.7. Statistical analysis

For all cell counts, stained slides were analyzed randomly and in a blinded fashion. The nonparametric Mann–Whitney *U*-test was employed for comparison of data between two groups, and Mann–Whitney test for comparison of more than two groups. Parametric data were compared using *t*-tests or analysis of variance. Values are reported as mean ± S.D. *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. IL-18 is induced and correlates with esophageal eosinophilia in EoE

We examined the blood levels of IL-18 in normal (non-EoE) and EoE patients. The analysis revealed that indeed IL-18

Table 2 List of primer sequences.

Genes	Sense and anti-sense primer sequence
hIL-5	5'-GCTTTGCATTTGAGTTTGCTAGCT3' 5'-TGGCCGTCAATGTATTTCTTTATTAAG3'
hIL-13	5'-ACAGCCCTCAGGGAGCTCAT3' 5'-TCAGTTGATGCTCCATACCAT3'
hIL-18Rα	5'-CGCTGCTTCTCAGTACTTT3' 5'-AAAGGTTCCCTTCAACCAC3'
hVCAM	5'-ACCACATCTACGCTGACAAT3' 5'-AACACTTGACTGTGATCGGC3'
hICAM	5'-AGCGCTATAAAGGATCACGC3' 5'-GGATGACTTTTGAAGGGGAC3'
hGADPH	5'-TGGAAATCCCATCACCATCT3' 5'-GTCTTCTGGGTGGCAGTGA T3'

levels are >3-fold higher in active EoE patients compared to the normal (non-EoE) patients (Fig. 1 A). Second, we tested whether food allergy is responsible for the elevated IL-18 in EoE patients by examining the levels of IL-18 in food allergen skin positive (SPT+) EoE patients. We found that SPT+ EoE patients have higher levels of IL-18 compared to the food

allergen SPT- EoE patients (Fig. 1B). Notably, the differences of IL-18 levels in between SPT+ and SPT- are significant but due to the small sample size these data may not be very conclusive and need more work. Currently the sample size is our limitation. Interestingly, the blood IL-18 levels correlated to the eosinophil counts in the esophageal biopsies of EoE

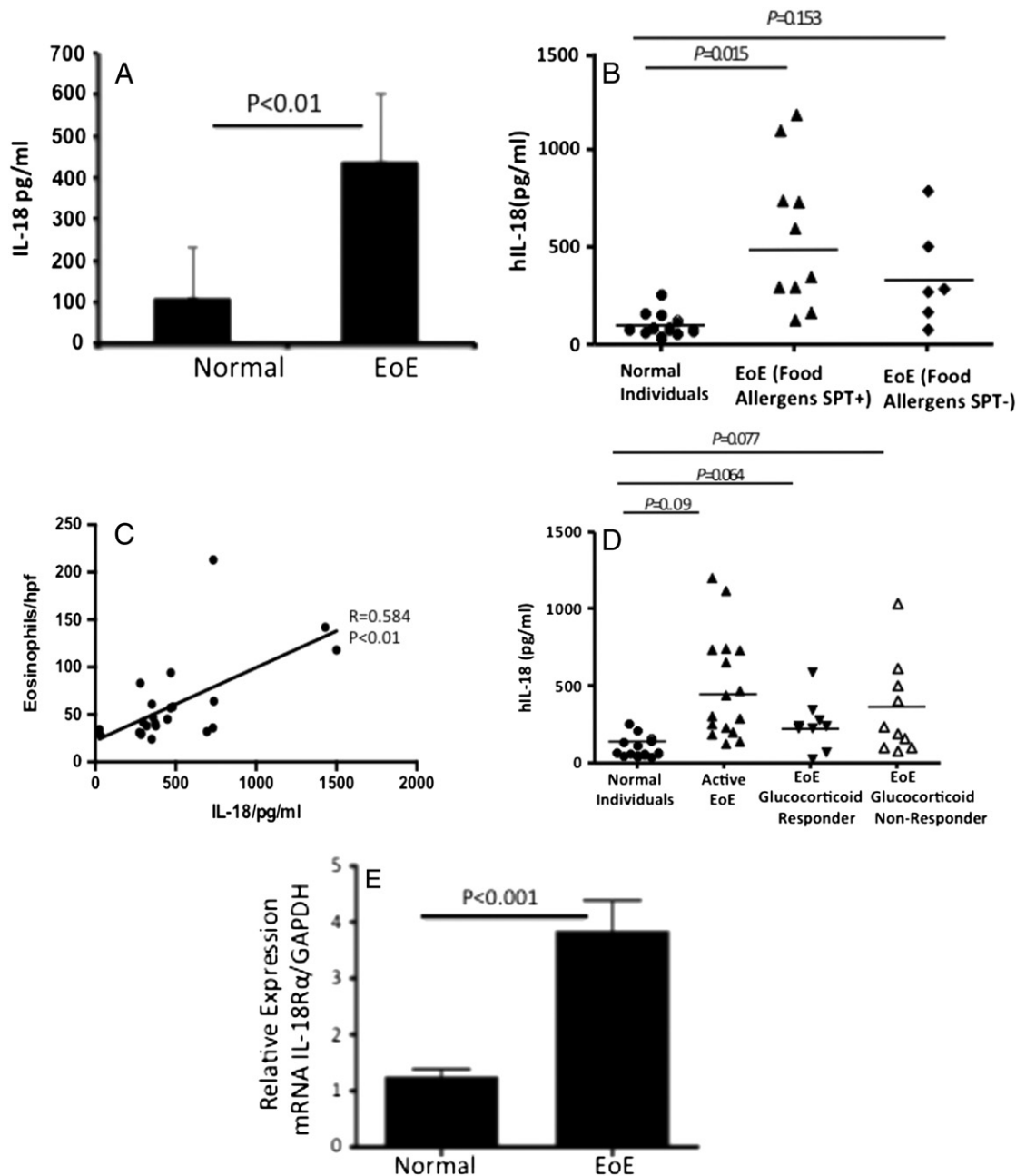


Figure 1 Induced expression of blood IL-18 and esophageal IL-18Rα+ cells in non-EoE, and active EoE patients. Blood IL-18 was measured in non-EoE (normal) individuals and EoE patient samples by performing ELISA analysis. Additionally the patients are divided to skin test positive (SPT+) and (SPT-) for food allergens. Their blood IL-18 levels are also analyzed and shown (A, B). A correlation between the maximum eosinophil number/hpf of EoE patients with blood IL-18 levels is statistically analyzed in human EoE and is found $r = 0.58$ and $P < 0.01$ (C). The blood IL-18 levels in non-EoE (normal) individuals, active EoE patients (>24 eosinophils/hpf); patients responsive to glucocorticoid treatment or non-responsive to the treatment groups are analyzed and are shown (D). The relative expression of IL-18Rα mRNA of normal (non-EoE) and EoE patients biopsies are analyzed by real time PCR analysis and is shown (E). Each data point represents an individual patient. The data is expressed as mean ± SD. P-values in between each group are calculated using Kreskas-Wallis test followed by Dunn's multiple comparison tests.

patients (Fig. 1 C). Further, we also found that the patients with improved EoE following oral glucocorticoid treatment had a significantly reduced IL-18 levels (Fig. 1 D).

3.2. IL-18 R α expressing cells increase in human EoE

Notably, our previously reported microarray data showed an increase IL-18R α transcript levels in EoE patients compared

to the normal individuals [2]. Therefore, we further validated these preliminary findings by quantifying IL-18R α mRNA expression in the esophagus of EoE patients compared to control individuals. An ~4-fold increase in levels of IL-18R α mRNA in esophageal biopsies of EoE patients was found compared to the normal (non-EoE) patients (Fig. 1 E). Next, our interest was to examine and quantitate the IL-18R α + cells in the esophageal biopsies of normal

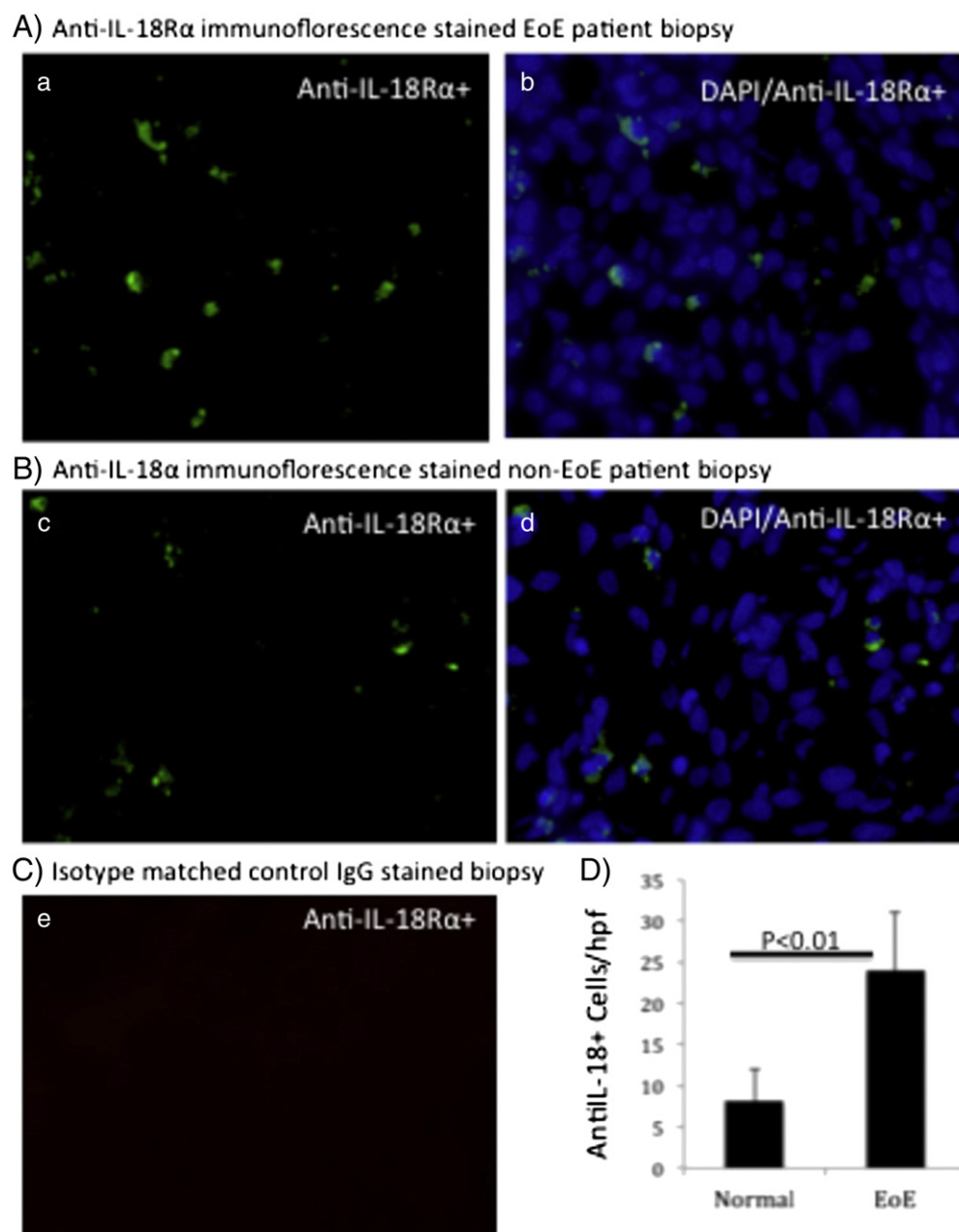


Figure 2 Immunofluorescence analysis of esophageal IL-18R α + cells in active EoE patients and non-EoE individual. A representative photomicrograph of anti-IL-18R α FITC conjugated and DAPI mounted esophageal biopsy section of EoE patient and non-EoE individual is shown (A, B). The analysis detected IL-18R α + cells (stained green) in EoE patient esophageal biopsy [A (a)]. The overlapped merged figure of DAPI/IL-18R α + cells is shown [A, (b)]. A similar analysis was performed in the biopsy of non-EoE individual and shown [B, (c-d)]. The isotype matched control antibody has not detected any staining of IL-18R α + cells in EoE patient biopsy [C, (e)]. All photomicrograph shown has original magnification in 400 $\times$. The quantitation of IL-18R α + cells in normal (comparison control) individuals and EoE patients are shown in [D]. The level of significance was calculated by using the Mann–Whitney test, $P < 0.01$, $n = 7-8$.

individual (non-EoE patient) and EoE patients. Therefore, we examined IL-18R α positive cells by performing anti-IL-18R α immunofluorescence staining in the frozen proximal and distal esophageal biopsies. Our analysis detected a high level of anti-IL-18R α positive cells in the epithelial mucosa of EoE patient esophageal biopsies [Fig. 2A (a–b)]. In contrast, a few numbers of anti-IL-18R α positive cells were detected in the epithelial mucosa of non-EoE patients [Fig. 2B (c–d)]. The isotype matched negative control IgG did not detect immunoreactive cells in the esophageal mucosa of EoE patient biopsies [Fig. 2C (e)]. Quantification of the IL-18R α -positive cells in esophageal biopsies indicated that $\sim 24 \pm 7$ IL-18R α + cells/hpf, $n = 8$ accumulated in the

esophageal biopsies of EoE patients compared to $\sim 8 \pm 4$ IL-18R α + cells/hpf, $n = 7$ in the non-EoE control group (Fig. 2D).

3.3. Intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 is induced in human EoE

Eosinophils and mast cells express intracellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) and their induction has been implicated in the adherence of these cells in a number of allergic diseases

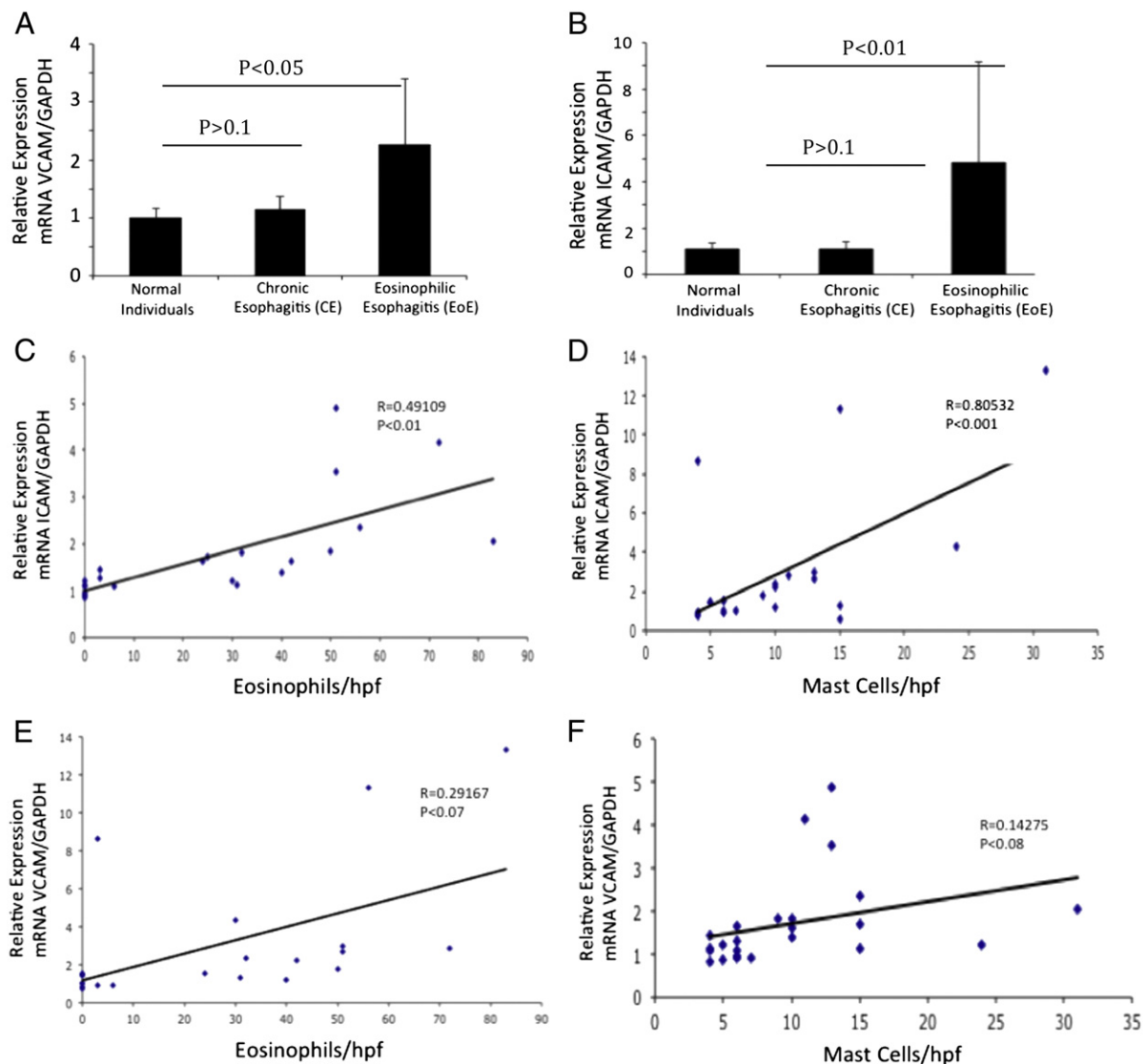


Figure 3 Analysis of IL-18-induced ICAM-1 and VCAM-1 genes expression in human esophageal biopsies. The mRNA levels of ICAM-1 and VCAM-1 were examined by performing quantitative real-time PCR analysis on the biopsy samples of normal individuals, CE and EoE patients. The mRNA expression was normalized to GAPDH mRNA and expressed as relative expression. Statistical significance was calculated using the Mann–Whitney test. P-values for each experiment are provided in the figure (A–B). Correlations between the peak eosinophil and mast cell numbers/hpf vs. ICAM-1 and VCAM-1 mRNA expression normalized with GAPDH mRNA in human EoE is shown (C and F). The r -value was calculated using the Spearman correlation test ($n = 17$ – 20). Statistical significance was calculated using both the Mann–Whitney and Kruskal–Wallis test.

[27,28]. Therefore, we next examined the expression of ICAM-1 and VCAM-1 in the esophageal biopsies of normal individuals, chronic esophagitis (CE) and EoE patients. Our analyses revealed that the expression of both ICAM-1 and VCAM-1 are induced in EoE compared to CE or normal individuals (Fig. 3 A, B). Further, we tested the hypothesis that ICAM-1 and VCAM-1 expression correlated with the number of esophageal eosinophils and mast cells in EoE patients. Interestingly, our analysis indicated that the expression of ICAM-1 correlated well with the esophageal eosinophils and mast cells (Fig. 3 C, D); however a very weak correlation of esophageal eosinophils and mast cells with VCAM-1 levels was observed in EoE patients (Fig. 3, E, F).

3.4. IL-18 Induces ICAM-1 and VCAM-1 expression in endothelial cells (HMVEC)

Next, we tested the hypothesis that IL-18 is responsible for the induction of ICAM-1 and VCAM-1 expression. Accordingly,

we performed in vitro experimentation using rIL-18 and HMVEC. Our experimentation indicated that rIL-18 induces ICAM-1 and VCAM-1 expression in primary microvascular endothelial cells (HMVEC, Cat # CC-2527, Lonza Group, Ltd., USA) were exposed to 0, 5 and 50 nM IL-18 for 24 hrs. Induction of ICAM-1 and VCAM-1 to a lesser extent by both 5 and 50 nM IL-18 was demonstrated by a flowcytometer, TNF α was used as a positive control that induces both ICAM-1 and VCAM-1 expression on endothelial cells (Fig. 4 A–D).

3.5. IL-18 stimulates iNKT cells to produce eosinophil active Th2 cytokines

Lastly, we focused our studies on the mechanism by which IL-18 promotes EoE in humans. We tested the hypothesis that IL-18 activates iNKT cells, as induced iNKT cells in EoE patients were earlier reported by us and others [9–13] and neutralization of iNKT cells protects against EoE in mice [10]. Accordingly, we tested the hypothesis that IL-18

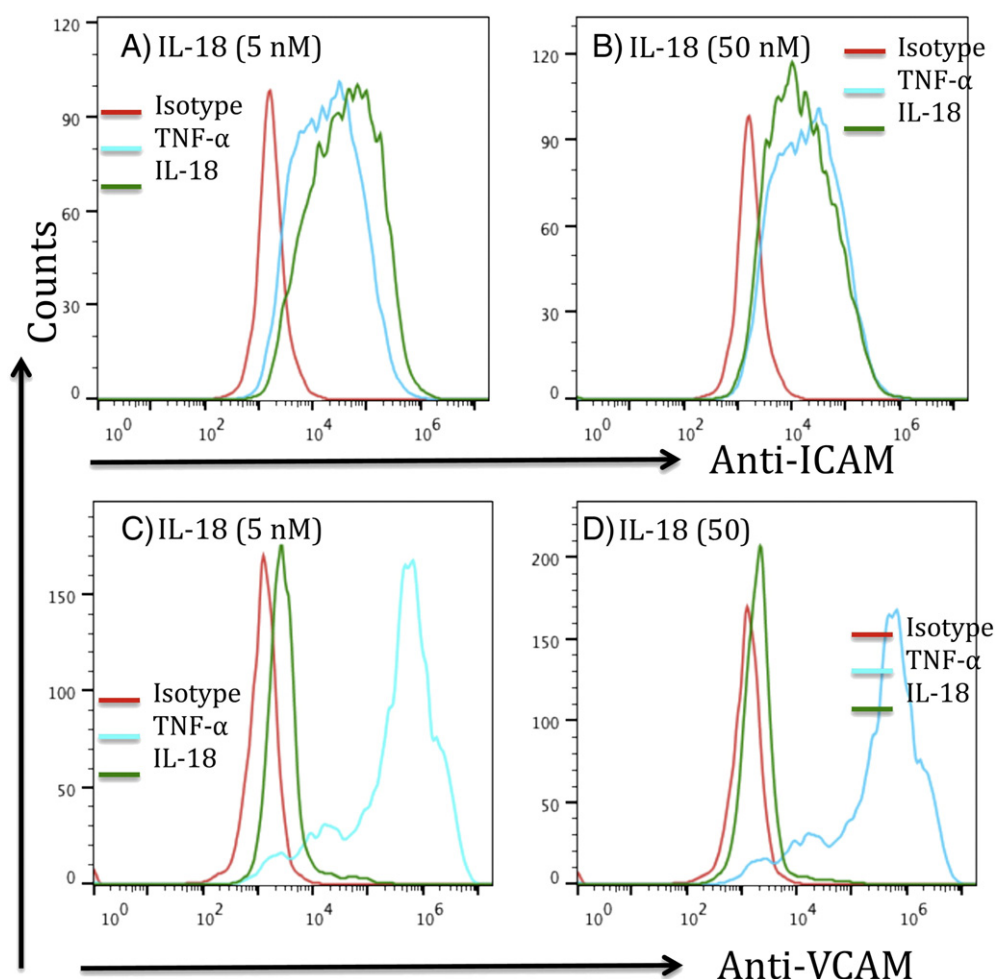


Figure 4 IL-18 induces ICAM-1 and VCAM-1 on endothelial cells; but VCAM-1-deficient mice are not protected from allergen-induced EoE. A flowcytometric analysis was performed to examine ICAM-1 and VCAM-1 expression on IL-18 treated (5 and 50 nM) and a positive control TNF- α (10 nM) on endothelial cells. The histogram showed that both doses of IL-18 treatment of endothelial cells show a significant induction in the expression of ICAM-1 (A, B) and also induces some expression of VCAM-1 (C, D). The positive control TNF- α also confirms the induced expression of both adhesion molecules on endothelial cells. The histogram is representative of three independent experiments.

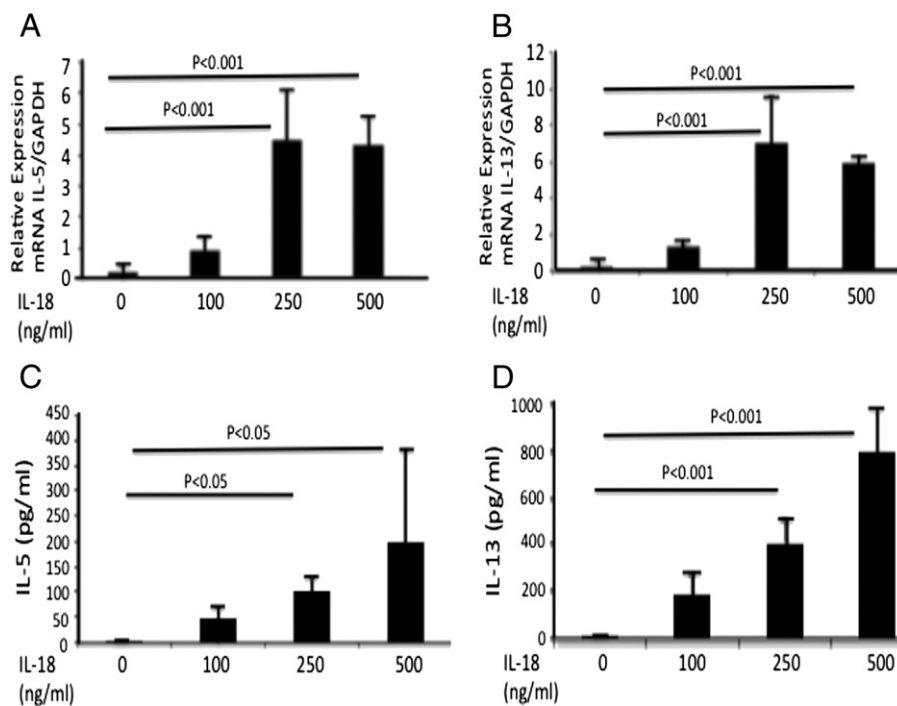


Figure 5 iNKT cells express IL-18R α in experimental EoE and generate Th2 cytokine from iNKT cell line following exposure with IL-18. iNKT cell line was exposed to IL-18 (0, 50 and 100, 250 and 500 ng/ml) for 48 hrs. IL-18 activates iNKT cells and induces eosinophil active cytokines IL-5 and IL-13 mRNA (A, C), and protein (B, D). The cytokines mRNA expression was normalized with GAPDH and shown as a relative expression of each cytokine.

stimulated a human iNKT cell line [26] to express eosinophil active Th2-type cytokines. iNKT cells (5×10^5) exposed to 0, 100, 250, and 500 ng/ml recombinant IL-18 for 48 hrs increased IL-5 and IL-13 transcripts (Fig. 5 A, C) and proteins (Fig. 5 B and D). Notably, addition of IL-18 to conventional CD4 $^+$ T cells (isolated from mouse spleen) did not produce IL-5 or IL-13 following 24, 48 and 72 hrs in vitro incubation (data not shown). All cytokine transcript data obtained were normalized with GAPDH and expressed as relative expression, the cytokine protein data is expressed as pg/ml of media. The data is expressed as mean $\pm$ SD; n = 3 independent experiments.

4. Discussion

Esophageal eosinophilia occurs in a variety of clinical disorders including GERD, eosinophilic gastroenteritis and EoE [29,30]. A number of clinical studies suggest that these disorders (especially EoE) are occurring with increasing frequency [31–33]. Our current study was designed to understand the molecular processes involved in iNKT cell-mediated EoE pathogenesis. A number of studies implicated iNKT cell accumulation in human EoE [9–13]; however, it is not yet clear what mechanism accounts for activation of iNKT cells in human EoE. Our previous microarray analyses indicated that the IL-18R α transcript was induced in EoE patients [2]. In the present study, we show that the blood IL-18 and IL-18R α $^+$ cells in esophageal biopsies are greatly induced in EoE patients. Interestingly, the level of blood IL-18 is found significantly increased in food allergen SPT+ EoE patients while SPT– EoE patients does not have elevated IL-18 in the blood.

Furthermore, the levels of IL-18 strongly correlate with esophageal eosinophils in active EoE patients and the levels of blood IL-18 significantly decrease in resolving EoE patients compared to active EoE patients. Of note, our analysis is on the smaller sample size, therefore, we recommend to establish that elevated serum levels of IL-18 as a non-invasive biomarker for the diagnosis of SPT+ EoE patients, more samples have to be analyzed for confirmed conclusion.

Additionally, our studies also found that EoE patients have increased expression ICAM-1 and VCAM-1 and that the levels of ICAM-1 significantly correlate with esophageal eosinophil and mast cell numbers. Notably, ICAM-1 is found induced in a number of allergic diseases [34] and implicated in the tissue recruitment of eosinophils and mast cells [27,28,35,36]. The ICAM-1 and VCAM-1 are expressed by both eosinophils and mast cells [37,38] and our data indicates that both are induced in EoE patients. Therefore, we further tested induction of ICAM-1 and VCAM-1 expression by IL-18. Treatment of human endothelial cells with IL-18 induced both ICAM-1 and VCAM-1, with the former more significantly induced that is in accordance with EoE patient data that ICAM-1 significantly correlates with tissue eosinophils and mast cells in human EoE. Another mechanistic possibility is that induced IL-18 activates iNKT cells to release eosinophil active cytokines that promote EoE in humans. Earlier, others and we reported that iNKT cells are induced and critical in EoE [9–13]. Of note, the mechanism by which iNKT cells promote EoE is not well understood. Herein, we provide the data that show IL-18 stimulates iNKT cells to produce eosinophil active cytokines like IL-5 and IL-13. This observation is in accordance with earlier reports that mature and activated iNKT cells are a prominent source

of Th1 and Th2 cytokines [15,39,40]. The present and prior studies now provide evidence that IL-18 activation causes IL-15-responsive iNKT cells to promote EoE in humans. In summary, we demonstrate that EoE patients have increased levels of IL-18 and IL-18R α . Induction of IL-18 significantly correlates with esophageal eosinophilia in humans and, following treatment, IL-18 levels decline in resolving EoE patients. IL-18 induces ICAM-1 in esophageal endothelial cells to facilitate eosinophil and mast cell recruitment to the esophagus. More importantly, we show that IL-18 stimulates iNKT cells to produce abundant eosinophil active cytokines, IL-5 and IL-13 that are shown to be critical in EoE pathogenesis [8,41,42].

Taken together, the current study provides a rationale and a better understanding on the significance of the earlier findings on the significance of IL15 [15,43] and CD1d-restricted iNKT cells in human EoE [9–13]. Most importantly, the current study identifies a novel role for IL-18 in stimulating IL-15 responsive iNKT cells and B cells leading to induction of Th2 cytokines and IgE-mediated food allergen-induced EoE. Most importantly, our current study highlights the significance of IL-18 as a future diagnostic and therapeutic target molecule for human EoE.

Conflict of interest statement

The author(s) declare that there are no conflicts of interest.

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