Original article

Outcome of infants presenting rectal bleeding: A retrospective study in a single institution

Running title: Comparison between FPIP and NTEC

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Abstract

Background: Although rectal bleeding in infancy (RBI) is not a rare phenomenon, the clinical course of RBI is not fully understood.

Methods: To investigate the outcome and pathogenesis of RBI, especially when concomitant with food protein-induced proctocolitis (FPIP) and neonatal transient eosinophilic colitis (NTEC), 22 neonates with rectal bleeding with FPIP and NTEC from January 2008 to June 2012 were enrolled and their clinical course and mechanisms of inflammation were examined.

Results: Thirteen infants showed rectal bleeding after feeding and were diagnosed with FPIP, and 9 infants showed rectal bleeding before feeding and were diagnosed with NTEC. Elevated peripheral white blood cell ($12,685 \pm 3,754/\mu l$ and $30,978 \pm 16,166/\mu l$) and eosinophil ($1,084 \pm 816/\mu l$ and $4,456 \pm 3,341/\mu l$) were confirmed in FPIP and NTEC, respectively. Colonoscopy revealed nodular lymphoid hyperplasia, a pale mucosal surface and oozing with diffuse infiltration of neutrophils, lymphocytes, and eosinophils in both groups. RT-PCR analysis revealed enhanced expression of the IL-6, CCL11, and CXCL13 genes, where CXCL13 expression was more prominent in FPIP. Mucosal infiltration by CD3- and IgA- but not IgE-positive cells was confirmed. Among them, only one infant with FPIP developed milk allergy, whereas none with NTEC had developed milk allergy at the age of 1 year.

Conclusions: FPIP in infancy and NTEC are similar diseases and that IL-6, CCL11, and CXCL13 may play a major role in the pathogenesis of rectal bleeding. Although the involvement of allergic reaction is possible, milk allergy was not a common outcome after 1 year of follow-up.

Introduction

Rectal bleeding in infancy (RBI) is an alarming symptom, and additional investigation is required to differentiate RBI from infection, necrotising enterocolitis, malrotation of the midgut, and other surgical diseases.[1] However, certain infants show no severe symptoms beyond rectal bleeding, as observed in surgical diseases.[2-6] Most of these infants experience rectal bleeding after breast or formula milk ingestion and are diagnosed with food protein-induced proctocolitis (FPIP).[2-6] FPIP is an adverse immunological response to food protein that occurs after the ingestion of the causative food and frequently develops in the first few years of life. We now know that there are relatively few IgE-bearing cells observed in the mucosal lining in patients with FPIP, and a non-IgE-mediated immunological reaction against food antigens is considered to be the cause of adverse events in FPIP, including rectal bleeding.[7,8] Moreover, there is a certain population that shows rectal bleeding before feeding, without any signs of infection or bleeding tendency and without a need for surgical treatment. Because there is a massive eosinophil infiltration of the mucosa in these individuals, we call this condition neonatal transient eosinophilic enterocolitis (NTEC).[9] In contrast to FPIP, eosinophilic inflammation in the colon is always present at the time of bloody stool, and this condition is transient and does not persist for long. However, the precise etiologies of FPIP and NTEC are not known. Antigen specificity is one concern, as certain infants with FPIP exhibit oligoclonal, but not monoclonal, antigen-specific reactions against food proteins, even though they have not been exposed to food antigens other than those in breast or formula milk during early infancy.[10,11] Some investigators believe that infants with FPIP are sensitised *in utero*,[12] but the sensitisation mechanisms that occur during the prenatal period are still unknown. The mechanism of eosinophil migration is

also obscure in both FPIP and NTEC patients. Because infants with NTEC had not received any milk before birth, an antigen-specific immune response is unlikely for these infants. We have previously examined the mucosal expression levels of Th1-, Th2-, Th17-, and Treg-related molecules using microarray analysis and found that these expression levels were high but not significantly elevated.[13] In particular, the expression of CCL11 (eotaxin-1) and CXCL13 was higher in infants with rectal bleeding.

In this study, we examined 22 infants with FPIP and NTEC to investigate the clinical course and pathogenesis of RBI. We also examined the involvement of CCL11 and CXCL13 and analysed the mechanisms of inflammation in FPIP and NTEC.

Methods

Patients

This study was designed as a retrospective analysis at a single institution. All of the study protocols were approved by the institutional ethics committee, and informed consent to participate was obtained from the parents of all of the children prior to enrolment in the study. From January 2008 to June 2012 at Juntendo University Hospital, infants with rectal bleeding who could be followed up for more than 1 year were enrolled in this study. The inclusion criteria were less than 3 months of age, visible rectal bleeding, no signs of infection or bleeding tendency, and no need for surgical treatment.

FPIP is defined as adverse immunological reactions, such as diarrhea and bloody stool against a specific food antigen (mostly breast and/or formula milk) during early infancy.[2-6] Definitive diagnosis of FPIP was made by oral food challenge tests that were performed after complete resolution of the initial symptoms by switching to hydrolysed hypoallergenic formula or elimination of diet. The disappearance of rectal bleeding after changing diet and the recurrence of rectal bleeding after the administration of formula milk was confirmed in all FPIP cases.[14,15] NTEC consists of eosinophilic inflammation in the colon, which was confirmed by endoscopy, and this condition is transient.[9] Once antigen specificity is confirmed, the condition would no longer be NTEC, but rather FPIP. Infants with FPIP show rectal bleeding after feeding, whereas infants with NTEC usually show rectal bleeding before feeding. In this study, clinical courses and laboratory data were compared between infants with FPIP and NTEC.

Five infants with FPIP or NTEC and four normal controls underwent

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colonoscopy with biopsy to evaluate the cause of rectal bleeding, and mucosal samples were collected. Single biopsies from the sigmoid colon were carefully taken from the children and divided in half. One half was used for pathological analysis, and the other half was used for genetic analysis. Mucosal samples were taken from the sigmoid colon in the patients with RBI and the controls. Control patients also underwent colonoscopy to find the cause of visible rectal bleeding. Their final diagnosis was <u>not allergic diseases including FIPE or NTEC but</u> juvenile polyp and normal control mucosal samples were taken from the sigmoid colon of patients who had normal mucosa based on histological examination. .

RNA extraction and real-time RT-PCR

To extract RNA from tissue samples for gene analysis, the mucosal samples from the sigmoid colon were minced and homogenised in Buffer RLT, and RNA was extracted using RNeasy Mini Kit spin columns (Qiagen, Germantown, MD). The quantity and purity of the RNA samples were determined with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, MA) and an Experion RNA StdSens Analysis Kit (Bio-Rad Laboratories, Hercules, CA).

To confirm the expression of the IL-6, CCL11, CXCL13, and CXCR5 genes, real-time RT-PCR was performed using TaqMan PCR Master Mix (Applied Biosystems Inc., Foster City, CA) with an ABI PRISM 7500 Sequence Detection System (Applied Biosystems Inc.). All of the primers were prepared from Assays-on-Demand kits (Applied Biosystems Inc.). PCR was performed as follows: initial denaturation at 95°C for 10 min, 40 amplification cycles with denaturation at 95°C for 15 sec, and annealing and extension at 60°C for 1 min. The expression of each gene was standardised to the expression of the housekeeping gene β-actin using the standard curve method, and

values relative to expression in the control mucosa are shown.

Immunohistochemical analysis

Immunohistochemical analysis was also performed to confirm the expression of CD3, IgA, and IgE in the tissue. Paraffin-embedded sections were used for the immunohistochemical analysis. Deparaffinised sections were incubated with either a monoclonal mouse anti-human CD3 antibody (Invitrogen, Camarillo, CA) or a polyclonal rabbit anti-human IgA/IgE antibody (Dako Cytomation Denmark A/S, Denmark). After washing, the sections were incubated with a biotin-conjugated secondary antibody. The sections were then incubated with avidin peroxidase (Sigma). The peroxidase activity was detected with 3,3'-diaminobenzidine-tetrahydrochloride (Sigma) in Tris-HCl containing 0.01% H_2O_2. Each section was also counterstained with hematoxylin before examination by light microscopy. Nonspecific staining was evaluated in the sections without primary antibody staining.

Statistical analysis

A statistical analysis of the peripheral white blood cell and eosinophil counts was performed using an unpaired *t*-test. Signalling molecule expression relative to β-actin expression was analysed by the Mann-Whitney U test. A p-value <0.05 was considered statistically significant.

Results

Clinical course

Among the 22 infants with RBI studied, 13 were diagnosed with FPIP (mean age, 10.46 ± 19.26 days), and 9 were diagnosed with NTEC (mean age, 0 days) (Tables 1 and 2). The infants with FPIP and the infants with NTEC with rectal bleeding were clinically stable, and their abdomens were soft, with hypoactive bowel sounds, at the time of initial presentation. The skin color was not pale, and there were no petechiae. Abdominal x-rays showed nonspecific bowel gas patterns, with no signs of obstruction, pneumatosis intestinalis, or necrotising enterocolitis.

Among the patients, 5 infants with FPIP (mean age, 2.70 ± 3.45 days), 5 infants with NTEC (mean age, 0 days), and 4 normal controls (mean age, 24.75 ± 6.13 months) underwent colonoscopy with biopsy for further analysis (Table 1, 2, 3). By switching to hydrolysed hypoallergenic formula or elimination of enteral feeding with sufficient hydration improved the patients' general condition, as previously reported.[2-6] The colonoscopies revealed oozing and edematous mucosal surfaces, with small or large lymphoid hyperplasia in all cases, and enlarged nodular lymphoid hyperplasia was also confirmed. By concerning the involvement of allergic reactions in the pathogenesis of RBI, a hydrolysed hypoallergenic formula was introduced first after the occult rectal bleeding resolved, followed by breast milk. All of the infants thrived on a diet of breast milk without maternal diet modification and showed no subsequent signs of RBI or other allergic symptoms, except for one infant with FPIP who presented a positive response to a milk challenge test at 1 year of age.

Laboratory and histological findings at the time of initial symptoms

At the time of initial presentation, elevated peripheral white blood cell (12,685

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± 3,754/μl and 30,978 ± 16,166/μl) and eosinophil (1,084 ± 816/μl and 4,456 ±

3,341/μl) counts were confirmed in FPIP and NTEC (Table 1). The numbers of white

blood cells and eosinophils were significantly elevated in the NTEC cases compared

with the FPIP cases (p<0.05). The serum eosinophilic cationic protein (ECP) level was

also elevated in these infants (Table 1). The mucosal biopsy samples showed diffuse

neutrophil, lymphocyte, and eosinophil infiltration, with goblet cell hyperplasia and

disruption of the epithelium, findings that were consistent with those observed in

eosinophilic enterocolitis (Fig. 1). Mucosal infiltration by CD3- and IgA- but not

IgE-positive cells was confirmed by immunohistochemical analysis (Fig. 1). There were

no significant differences in macro- or microscopic findings between FPIP and NTEC,

except that there were more IgA-positive cells in FPIP as seen in a 2year 7month-old

control mucosa.

RT-PCR analysis

Because we have previously reported the involvement of IL-6, CCL11, and

CXCL13 in RBI based on microarray analysis,[13] RT-PCR analysis was performed.

CCL11 and CXCL13 represent an eosinophilic chemoattractant factor and a chemokine

related to lymphoid follicle formation, respectively. IL-6, CCL11, CXCL13, and

CXCR5 (receptor for CXCL13) mRNA expression was significantly enhanced in infants

with FPIP or NTEC relative to the controls (p<0.05 each). The expression of CXCL13

and CXCR5 was significantly enhanced in FPIP compared with NTEC (p<0.05) (Fig.

2).

Discussion

Although the numbers of white blood cells and eosinophils were significantly elevated in the NTEC patients than in the FPIP patients, it is considered that these changes were due to the time course of newborn infants, and their clinical courses were very similar. Both conditions showed oozing and edematous mucosal surfaces with small or large lymphoid hyperplasia by colonoscopy, and their mucosal tissue samples were <u>consistent with</u> those observed in eosinophilic enterocolitis. Rectal bleeding stopped after the discontinuation of enteral feeding, followed by resumption of feeding with breast milk or hypoallergenic formula. Most patients did not develop a milk-specific reaction at the age of 1 year, as previously reported.[2-6] These findings suggest that FPIP in infancy and NTEC are similar conditions.

An RT-PCR analysis was performed to investigate the pathogenesis of FPIP and NTEC and to demonstrate that the enhanced IL-6, CCL11, and CXCL13 expression found in the mucosa is an important feature. Synthesising IgA molecules targeting multiple antigens is an important task for the mucosal immune systems of neonates, which must develop a tolerance to the peptides present in the intestinal lumen.[16] IgA is mainly synthesised in the lymphoid follicles of the intestine, and the enhanced CXCL13 and CXCR5 (receptor for CXCL13) expression observed in neonates is convenient for IgA synthesis because CXCL13 is a chemokine related to lymphoid follicle formation.[17] The immunohistochemical analysis revealed that infiltration by IgA-bearing cells was pronounced in FPIP and NTEC, possibly suggesting that the enhanced immunological reaction against food antigen, including producing IgA, is one of the major elements of the pathogenesis of rectal bleeding in infants. Because lymphoid hyperplasia is one of the characteristic findings in RBI[18] and because IgA synthesis is predominant after birth,

these changes can be favourable immunological findings in infants, including those with RBI.

Meanwhile, the enhanced expression of CXCL13/CXCR5 and increased production of IgA are the most prominent differences between FPIP and NTEC. The rapid generation of secretory immunity is one of the most important tasks for the mucosal immune system in early infancy and this function developed after birth.[16] IgA responses are generated in the lymphoid follicles in the intestine, and the increased CXCL13/CXCR5 expression observed in early infancy is related to the generation of IgA because it is involved in attracting B cells into developing lymphoid tissue.[17] It is considered that the lymphoid hyperplasia was more dominant in FPIP compare to NTEC because of the significantly increased expression of CXCL13/CXCR5 in FPIP compare to NTEC.

Although there was an increase in the expression of IL-6-mRNA in the FPIP mucosa, it was not significant in our previous study.[13] As the number of patients increased in this study, we could confirm the significant increase in the expression of IL-6-mRNA level both in FPIP and NTEC when compared to controls.

We have examined more breastfed infants showing rectal bleeding than formula-fed infants, and certain researchers have suggested that RBI is often observed in exclusively breastfed infants.[11] Interestingly, these children tend to exhibit lymphoid hyperplasia. Because TGF-β, which is present in breast milk, stimulates antigen-specific IgA synthesis, it is possible that the enhanced CXCL13 expression and the TGF-β in breast milk stimulate lymphoid follicle formation and induce lymphoid hyperplasia, leading to rectal bleeding.

A food allergy is defined as an adverse immunological reaction in response to a

specific food antigen.[2-6] Although the infants in this study showed rectal bleeding, most infants are immunologically active enough to develop a tolerance to oligoclonal antigens by producing IgA. Confirming an allergic reaction during the neonatal period can be challenging because breast or formula milk is the only substance ingested by infants. A challenge test is necessary to confirm the involvement of allergic reactions; however, this procedure is often omitted because infants consume only breast or formula milk during early infancy. Most infants with FPIP are diagnosed with milk allergies based on confirmed adverse events that occur after a milk challenge, but they have not experienced antigenic peptides derived from other foods, such as egg whites and wheat. The antigen specificity in these infants is not clear because some cases exhibit rectal bleeding without any antigen ingestion, and others show oligoclonal reactions.[5,9,11] Although our study revealed the involvement of CD3-positive cells in rectal bleeding, these reactions seem premature because migrated lymphocytes are usually not specifically characterised as well-differentiated Th1, Th2, or Th17 cells, as we have previously reported.[13] Because none of our patients with NTEC was positive and only one infant overall was positive in a later milk challenge test, further analysis is necessary to confirm the involvement of allergic reactions in RBI.

Eosinophil migration is the major characteristic of FPIP and NTEC.[17,18] Among the types of eosinophilic enterocolitis, the frequency of eosinophilic esophagitis is increasing in Western countries; however, the condition's etiology seems to be different from that of RBI. *Blanchard et al.* suggested that CCL26 (eotaxin-3) and IL-13 may be critical mediators of eosinophilic oesophagitis.[21-23] Because IL-13 is the key cytokine for Th2 cells, the immunological reactions observed in eosinophilic esophagitis are most likely allergic reactions. In contrast to eosinophilic esophagitis, however, the levels of

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CCL26, IL-13, and other Th2-related molecules are less prominent than the levels of CCL11 and CXCL13 in RBI.[13] Our findings suggest that the pathogeneses of both FPIP and NTEC may not be closely related to allergic reactions as we see in eosinophilic esophagitis. Although peripheral white blood cell counts and eosinophil counts were elevated in NTEC than in FPIP, there was no significant difference in a mucosal expression of CCL11-mRNA level between FPIP and NTEC. Since there are several mediators to chemoattract eosinophils, CCL11 may not be the key molecules for elevation of peripheral white blood cell and eosinophil counts in early infancy. Precise bone marrow and peripheral blood analysis should be performed to understand the mechanism of this phenomenon.

In conclusion, this study suggests that the etiologies of FPIP and NTEC are very similar, and the rectal bleeding in both is related to lymphoid hyperplasia with eosinophil infiltration into the colonic mucosa facilitated by CCL11 and CXCL13. Moreover, the rectal bleeding may not be related to allergic reactions against specific antigens, as demonstrated by the fact that the migrated lymphocytes were mostly IgA-bearing cells and milk allergy was not a common outcome after 1 year of follow-up.

Contributions Authorship contributions is as follows; M. M., K. J., E. I., Y. A., and T. K. performed experiments, and analyzed data; A. I., and S. Y. performed experiments; R. S., and T. S. contributed to discussions; Y. O. designed research, performed experiments, and wrote the first draft of the paper.

Competing interests   All authors declare no conflicts of interest.

References

1. Lawrence WW, Wright JI. Causes of rectal bleeding in children. *Pediatr. Rev.* 2001; 22: 394-95.

2. Lake AM. Food-induced eosinophilic proctocolitis. *J. Pediatr. Gastroenterol. Nutr.* 2000; 30(suppl): S58-60.

3. Lake AM, WhitingtonPF, Hamilton SR. Dietary protein-induced colitis in breast-fed infants. *J. Pediatr.* 1982; 101: 906-10.

4. Pittschieler K. Cow's milk protein-induced colitis in the breast-fed infant. *J. Peditar. Gastroenterol. Nutr.* 1990; 10: 548-9.

5. Ravelli A, Villanacci V, Chiappa S, Bolognini S, Manenti S, Fuoti M. Dietary protein-induced proctocolitis in childhood. *Am. J. Gastroenterol.* 2008; 103: 2605-12.

6. Jang HJ, Kim AS, Hwang JB. The etiology of small and fresh rectal bleeding in not-sick neonates: should we initially suspect food protein-induced proctocolitis? *Eur. J. Pediatr.* 2012; 171: 1845-9.

7. Sicherer SH, Sampson HA. Food allergy. *J. Allergy Clin. Immunol.* 2010; 125 (suppl): S116-25.

8. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). *J. Allergy. Clin. Immunol.* 2004; 113: 11–28.

9. Ohtsuka Y, Shimizu T, Shoji H *et al.* Neonatal transient eosinophilic colitis causes rectal bleeding in early infancy. *J. Pediatr. Gastro. Hepato. Nutr.* 2007; 44: 501-5.

10. Juvonen P, Månsson M, Kjellman NI, Björkstén B, Jakobsson I. Development of immunoglobulin G and immunoglobulin E antibodies to cow's milk proteins and ovalbumin after a temporary neonatal exposure to hydrolyzed and whole cow's milk proteins. *Pediatr. Allergy Immunol.* 1999; 10: 191-8.

11. Arvola T, Ruuska T, Keränen J, Hyöty H, Salminen S, Isolauri E. Rectal bleeding in infancy: clinical, allergological, and microbiological examination. *Pediatrics* 2006; 117: e760-8.

12. Bønnelykke K, Pipper CB, Bisgaard H. Sensitization does not develop in utero. *J. Allergy Clin. Immunol.* 2008; 121: 646-51.

13. Ohtsuka Y, Jimbo K, Inage E *et al.* Microarray analysis of mucosal biopsy specimens in neonates with rectal bleeding: Is it really an allergic disease? *J. Allergy Clin. Immunol.* 2012; 129:1676-8.

14. Winter HS, Antonioli DA, Fukagawa N, Marcial M, Goldman H. Allergy-related proctocolitis in infants: diagnostic usefulness of rectal biopsy. *Mod. Pathol.* 1990; 3: 5-10.

15. Xanthakos SA, Schwimmer JB, Melin-Aldana H, Rothenberg ME, Witte DP, Cohen MB. Prevalence and outcome of allergic colitis in healthy infants with rectal bleeding: a prospective cohort study. *J. Pediatr. Gastroenterol. Nutr.* 2005; 41: 16-22.

16. Kukkonen K, Kuitunen M, Haahtela T, Korpela R, Poussa T, Savilahti E. High intestinal IgA associates with reduced risk of IgE-associated allergic diseases. *Pediatr. Allergy Immunol.* 2010; 21: 67-73.

17. Carlsen HS, Baekkevold ES, Johansen FE, Haraldsen G, Brandtzaeg P. B cell attracting chemokine 1 (CXCL13) and its receptor CXCR5 are expressed in normal and aberrant gut associated lymphoid tissue. *Gut* 2002; 51: 364-71.

18. Kaplan B, Benson J, Rothstein F, Dahms B, Halpin T. Lymphonodular hyperplasia of the colon as a pathologic finding in children with lower gastrointestinal bleeding. *J. Pediatr. Gastroenterol. Nutr.* 1984; 3: 704-8.

19. Goldman H, Proujansky R. Allergic proctitis and gastroenteritis in children. Clinical

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and mucosal biopsy features in 53 cases. *Am. J. Surg. Pathol.* 1986; 10: 75–86.

20. Odze RD, Bines J, Leichtner AM, Goldman H, Antonioli DA. Allergic proctocolitis in infants: A prospective clinicopathologic biopsy study. *Hum. Pathol.* 1993; 24: 668–74.

21. Blanchard C, Wang N, Stringer KF *et al.* Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J. Clin. Invest.* 2006; 116: 536-47.

22. Blanchard C, Mingler MK, Vicario M *et al.* IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J. Allergy Clin. Immunol.* 2007; 120: 1292-300.

23. Blanchard C, Stucke EM, Rodriguez-Jimenez B *et al.* A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J. Allergy Clin. Immunol.* 2011; 127: 208-17.

Figure legends

Fig 1. Histological images of two representative samples from infants with NTEC or FPIP and control. The hematoxylin and eosin (HE)-stained mucosa showed diffuse neutrophil, lymphocyte, and eosinophil infiltration, with goblet cell hyperplasia and epithelial disruption. The immunohistochemical analysis was performed using anti-CD3, IgA, and IgE antibodies. The migration of anti-CD3 antibody-labelled cells was noted in mucosa from patients with RBI. IgA-positive (but not IgE-positive) cells were present in these mucosal samples. Control: Mucosal sample taken from 2year 7month-old polyp patient. FPIP: food protein-induced proctocolitis. NTEC: neonatal transient eosinophilic colitis. Magnification × 400.

Fig 2. RT-PCR analysis of IL-6, CCL11, CXCL13, and CXCR5 (receptor for CXCL13) expression in the NTEC (n=5) and FPIP (n=5) mucosa. The gene expression levels were normalised to β-actin expression, and values relative to the expression in control mucosa are shown. Central box, interquartile range; line, median; whiskers, minimum and maximum values. *p<0.05. FPIP: food protein-induced proctocolitis. NTEC: neonatal transient eosinophilic colitis.

Figure 1
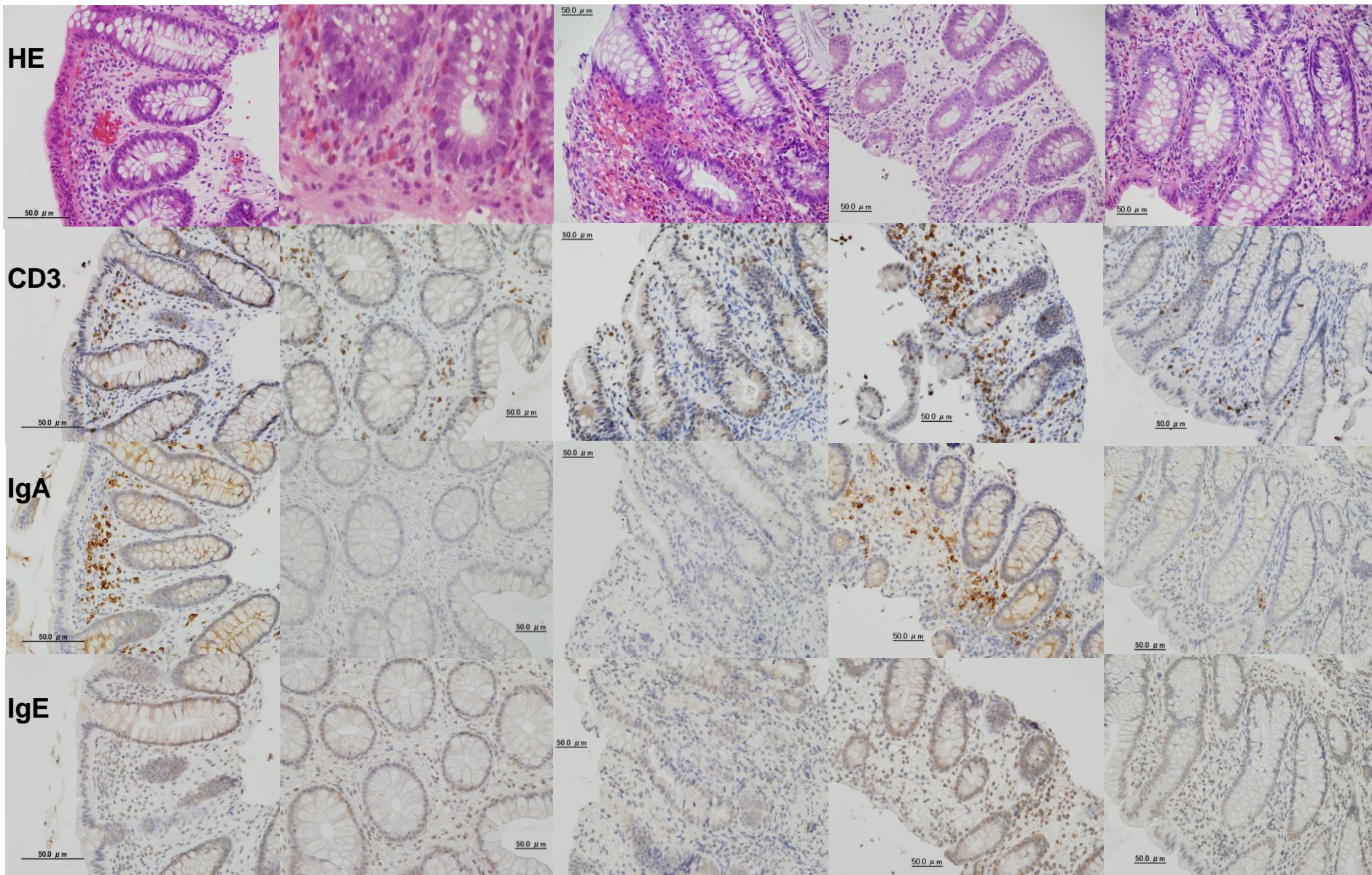
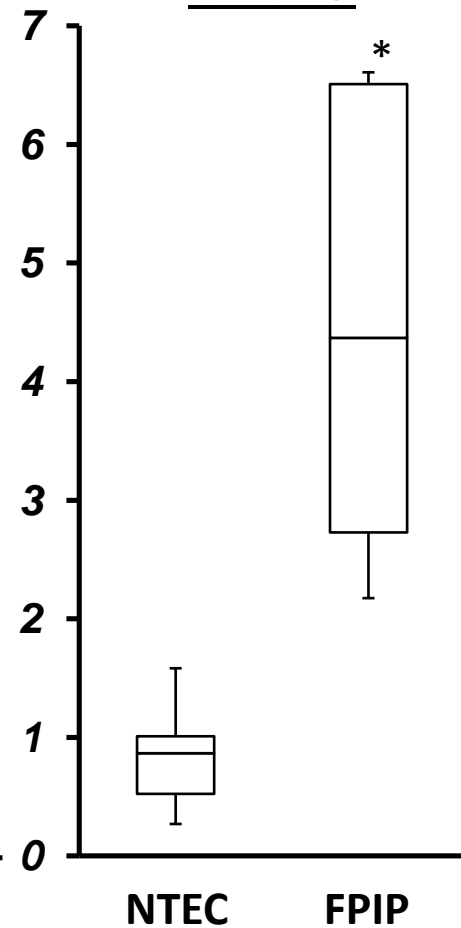**Figure 2**
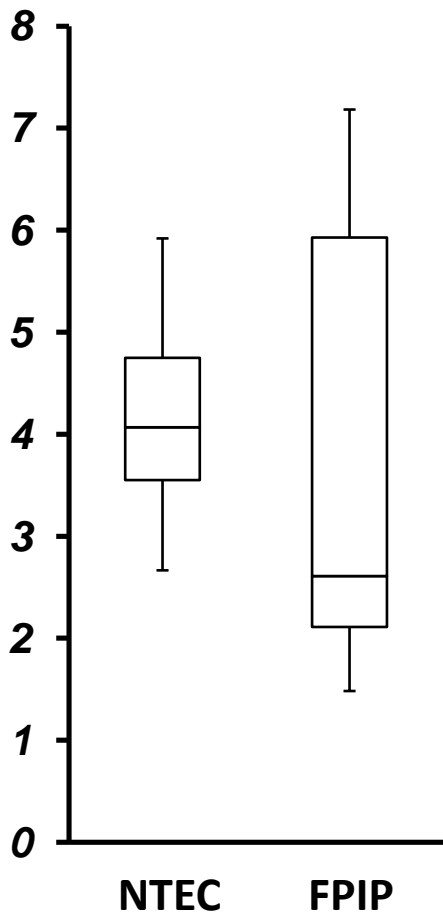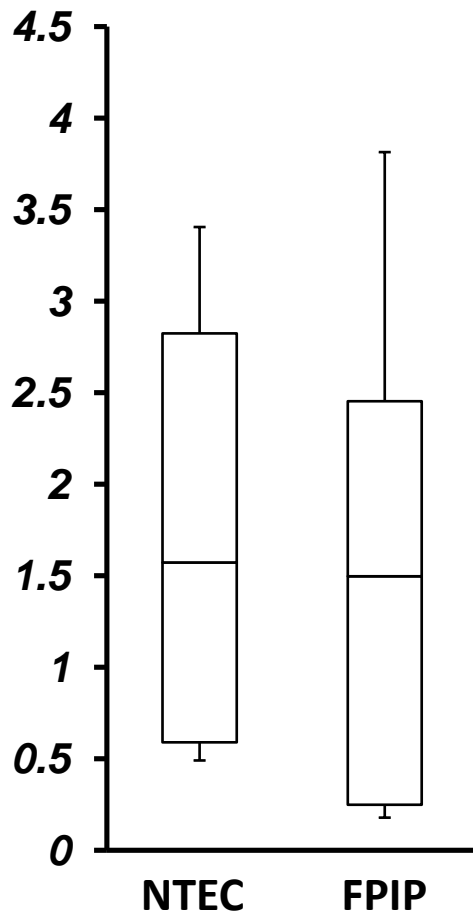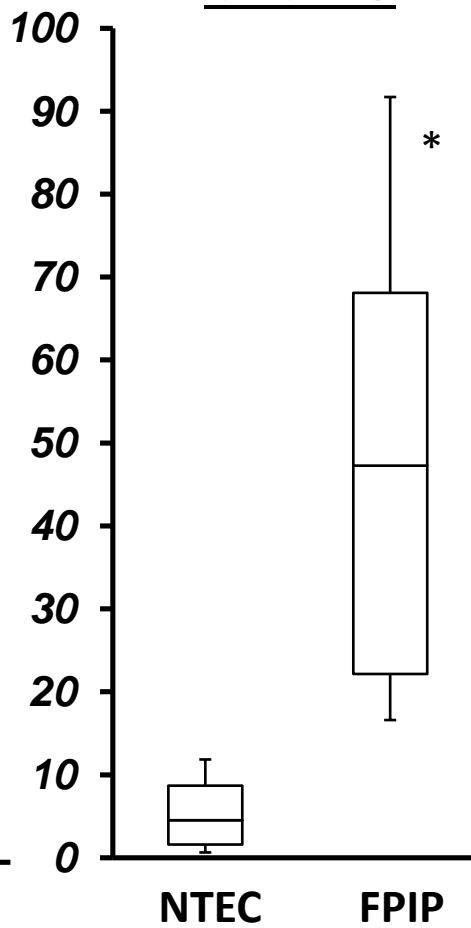
Table 1

	Male/Female	Gestational Age	Birth weight (g)	Onset	WBC (/µL)	Eosinophil (/µL)	IgE (IU/ml)	Milk-IgE (IU/ml)	ECP <14.7 (µg/L)	ALST	Feeding	Milk challenge test at age of 1
*1	M	38w2d	3,015	day 10	12,400	1,364	< 3	＜0.34	35.8		breastfed	-
2	F	41w1d	2,778	day 2	13,900	1,529	6	＜0.34	15		breastfed	-
*3	M	39w4d	3,296	day 3	6,300	347	< 3	＜0.34	33.2		breastfed	-
4	F	39w0d	3,098	day 3	8,600	344	< 3	＜0.34			breastfed	-
*5	M	39w0d	3,248	day 2	14,200	3,053	< 3	＜0.34	33		formula-fed	-
*6	F	39w2d	3,386	day3	12,200	732	5	＜0.34	34.2		breastfed	-
*7	M	37w2d	2,912	day 8	13,000	1,166	< 3	＜0.34			breastfed	-
8	F	29w2d	744	day 74	6,200	341	37	＜0.34			breastfed	-
9	M	38w4d	3,422	day 6	14,800	1,998	< 3	＜0.34		+	formula-fed	+
10	M	39w3d	3,166	day 7	17,900	448	< 3	＜0.34		+	breastfed	-
11	F	39w5d	3,154	day 6	16,600	498	< 3	＜0.34			breastfed	-
12	M	36w4d	2,655	day 4	16,900	1,622	< 3	＜0.34			mixed	-
13	F	37w0d	2,468	day 8	11,900	654	< 3	＜0.34		-	breastfed	-

TABLE 1. The clinical features of the FPIP enrolled in this study.

ALST, antigen (milk) -specific lymphocyte stimulating test; ECP, eosinophilic cationic protein; F, female; M, male

* Samples used for the RT-PCR analysis.

Table 2

	Male/Female	Gestational Age	Birth weight (g)	Onset	WBC (/μL)	Eosinophil (/μL)	IgE (IU/ml)	Milk-IgE (IU/ml)	ECP <14.7 (μg/L)	ALST	Feeding	Milk challenge test at age of 1
*1	F	37w4d	2,400	day 0	22,100	4,641	4	＜0.34	24.2		none	-
*2	M	37w1d	2,564	day 0	21,000	2,211	<3	＜0.34	123		none	-
*3	F	35w1d	2,504	day 0	28,100	10,397	8.3	0.38			none	-
*4	M	39w5d	2,654	day 0	23,400	1,755	<3	＜0.34	150		none	-
*5	F	39w4d	3,296	day 0	28,100	2,282	<3	＜0.34	33.2		none	-
6	F	35w0d	2,512	day 0	33,000	3,696	<3	＜0.34			none	-
7	F	34w4d	2,140	day 0	59,300	9,014	<3	＜0.34			none	-
8	F	34w3d	1,678	day 0	54,600	5,460	<3	＜0.34	21.1		none	-
9	F	37w0d	2,118	day 0	9,200	644	<3	＜0.34	21.8	+	none	-

TABLE 2. The clinical features of the NTEC enrolled in this study.

ALST, antigen (milk) -specific lymphocyte stimulating test; ECP, eosinophilic cationic protein; F, female; M, male

* Samples used for the RT-PCR analysis.

Table 3

	Male/Female	Age	WBC (/μL)	Eosinophil (/μL)	IgE (IU/ml)	Food allergy	Diagnosis
*1	F	2y7m	6,600	198	3	none	polyp
*2	M	2y4m	5,800	261	<3	none	polyp
*3	F	1y5m	10,400	146	8	none	polyp
*4	M	1y11m	10,800	540	<3	none	polyp

TABLE 3. The clinical features of the control patients.

F, female; M, male.

* Samples used for the RT-PCR analysis.