

Letter to the Editor

Microarray analysis of mucosal biopsy specimens in neonates with rectal bleeding: Is it really an allergic disease?

To the Editor:

Food protein-induced proctocolitis (FPIP), also known as allergic proctocolitis, is a significant cause of lower gastrointestinal bleeding (LGB) in infants.¹ Because most patients with FPIP are infants and young children with eosinophilia and exhibit LGB after milk ingestion, it is believed that an allergic reaction against milk is involved in the pathogenesis. Because there are relatively few IgE-bearing cells in the guts of these children, non-IgE-mediated allergic reactions have also been proposed.²

However, LGB can be seen in the absence of any apparent allergic reactions. For instance, infants with neonatal transient eosinophilic colitis exhibit LGB before milk ingestion.³ Some infants with LGB exhibit oligoclonal antigen-specific reactions against food proteins, even though they have not been exposed to food antigens except through breast milk in early infancy.^{4,5} It is thought that these infants are sensitized *in utero*.⁶

The aim of this small study was to study the transcriptome of mucosal biopsy specimens of children with FPIP. Inflammatory gene expression was compared with that seen in control children and children with Crohn disease and ulcerative colitis (Table I).⁷

Children with FPIP had increased white blood cell and eosinophil counts. Sigmoidoscopy revealed nodular lymphoid

hyperplasia with an oozing and edematous mucosal surface. Histology of mucosal biopsy samples showed mucosal damage and goblet cell hyperplasia with diffuse infiltration of mononuclear cells and eosinophils similar to what is seen in patients with eosinophilic proctocolitis.

A microarray assay was performed according to the manufacturer's instructions for the Ambion WT Expression Kit (Applied Biosystems, Foster City, Calif) and the GeneChip WT Terminal Labeling and Controls Kit (Affymetrix, Santa Clara, Calif) to analyze gene expression. Total RNA was reverse transcribed into cDNA with random primers and hybridized to a GeneChip Human Gene 1.0 ST Array (Affymetrix) at 45°C for 17 hours. The chips were washed, stained with streptavidin-phycoerythrin, and scanned with a GeneChip Scanner 3000 (Affymetrix). Scanned images were converted into CEL files, and data analysis was performed with GeneSpring GX v1.1 software (Agilent Technologies, Santa Clara, Calif). The raw intensity values from each chip were normalized by using the RMA16 algorithm, and the genes exhibiting significant differences in signal intensities compared with the control samples ($P < .05$, t test) were considered to be upregulated or downregulated. The heat map displays the log summarized value with color-intensity view.

Microarray analysis revealed enhanced expression of CCL11 in the very young neonates and CXCL13 in the older children. The expression of T_H1 -, T_H2 -, and T_H17 -signaling transcripts was not significantly enhanced compared with that of the control

TABLE I. Clinical features of patients enrolled in this study

Disease	Active phase		Remission phase		Sample	Comment
Ulcerative colitis	F	15 y 1 mo	F	20 y 2 mo	Sigmoid colon	
	F	14 y 7 mo	M	6 y 10 mo		
	M	14 y 0 mo	F	13 y 6 mo		
Crohn disease	M	13 y 6 mo	F	7 y 6 mo	Cecum	
	M	12 y 1 mo	M	11 y 10 mo		
	F	14 y 3 mo	F	4 y 11 mo		
FPIP	F	2 d			Sigmoid colon	WBCs: 22,100/ μ L, eosinophils: 4,641/ μ L; IgE RIST: <3, IgE milk: (–), none
	M	10 d				WBC: 14,200/ μ L, eosinophils: 3,053/ μ L, IgE RIST: <3, IgE milk: (–), formula fed
	F	6 mo				WBC: 16,100/ μ L, eosinophils: 3,462/ μ L, IgE RIST: 4, IgE milk: (–), exclusively breast-fed
	F	7 mo				WBC: 15,900/ μ L, eosinophils: 1749/ μ L, IgE RIST: <3, IgE milk: (–), exclusively breast-fed
Control	M	11 y 10 mo			Cecum	Polyp
	M	14 y 4 mo			Sigmoid colon	Irritable bowel syndrome
	M	12 y 6 mo				

Patients with FPIP fulfilled 3 elements of the Powell criteria,⁷ but oral challenge tests were not performed.

F, Female; M, male; RIST, radio immunosorbent test; WBC, white blood cells.

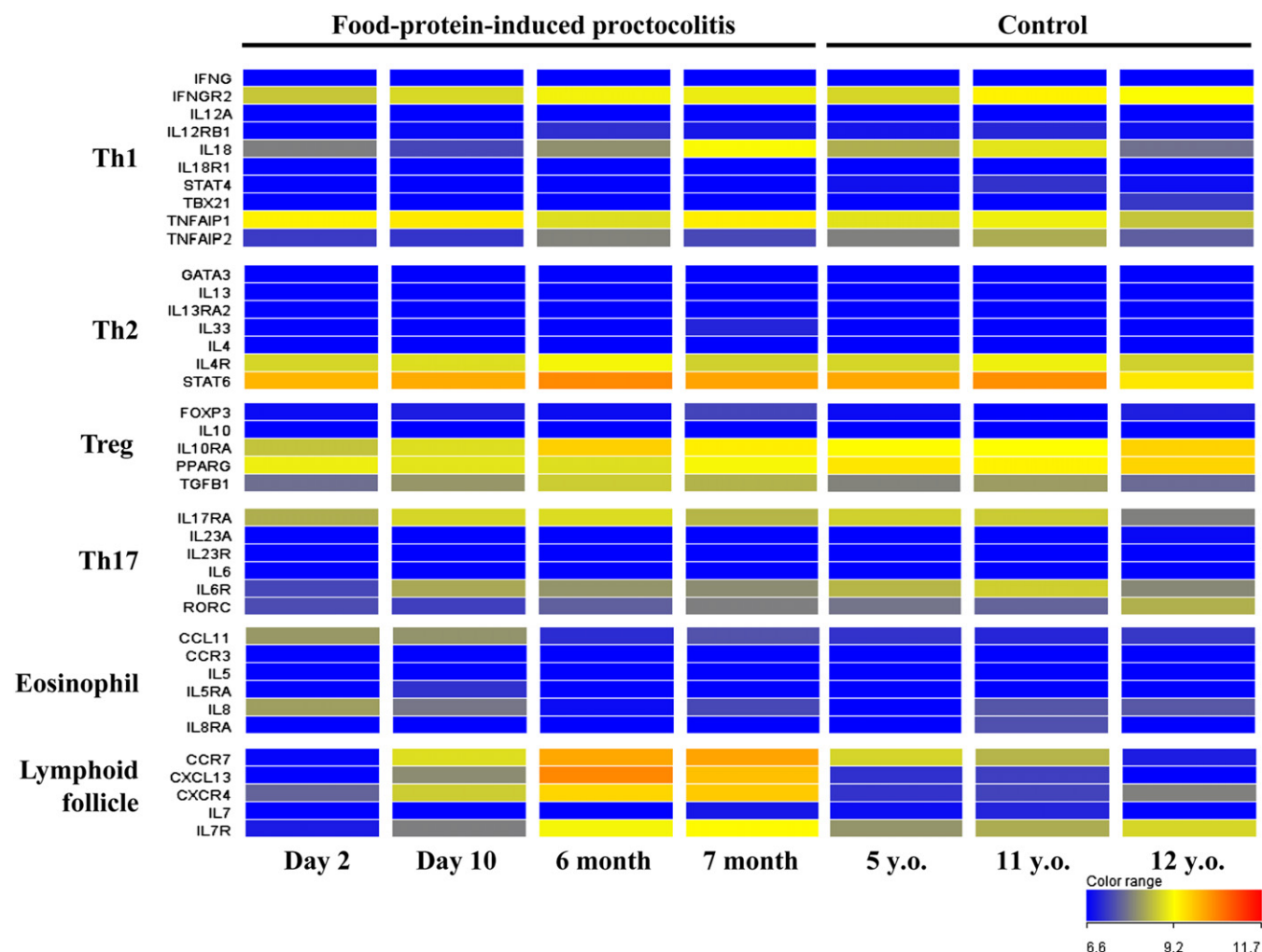


FIG 1. Microarray analysis of T_H1 -, T_H2 -, T_H17 -, regulatory T cell-, neutrophil-, eosinophil-, and lymphoid follicle formation-related molecules in the mucosa. The expression of CCL11 and CXCL13, but not T_H1 -, T_H2 -, and T_H17 -related molecules, was significantly enhanced in patients with FPIP compared with that seen in control subjects. The mean signal intensity of each group is shown by heat maps. *FOXP3*, Forkhead box protein 3; *IFNG*, IFN- γ ; *IFNGR2*, IFN- γ receptor 2; *IL3RA2*, IL-3 receptor α 2; *IL4R*, IL-4 receptor; *IL5RA*, IL-5 receptor α ; *IL6R*, IL-6 receptor; *IL7R*, IL-7 receptor; *IL8RA*, IL-8 receptor α ; *IL10RA*, IL-10 receptor α ; *IL12RB1*, IL-12 receptor β 1; *IL17RA*, IL-17 receptor A; *IL23R*, IL-23 receptor; *PPARG*, peroxisome proliferator-activated receptor γ ; *RORC*, retinoic acid-related orphan receptor C; *STAT*, signal transducer and activator of transcription; *TBX21*, T-box 21; *TGFB1*, TGF- β 1; *TNFAIP1*, TNF- α -induced protein 1; *Treg*, regulatory T cell.

subjects. Likewise, regulatory T cell-related transcripts were also expressed at high levels in all children (Fig 1).

Allergic reactions against milk are considered the cause of the LGB in patients with FPIP. However, the question remains as to whether the LGB is really the result of an allergic reaction. A food allergy is defined as an adverse immunologic reaction against a specific food antigen. A challenge test is necessary to prove the involvement of allergic reactions; however, this procedure is often omitted because infants only consume milk during early infancy. Most infants with FPIP have been given a diagnosis of milk allergy based on an adverse event after challenge with milk, but some infants have never been exposed to cow's milk or even breast milk.^{3,5}

The rapid generation of secretory immunity is one of the most important tasks for the mucosal immune system in neonates.⁸ IgA responses are generated in the lymphoid follicles in the intestine,

and it might be that the increased CXCL13 expression observed in neonates is related to the generation of IgA because it is involved in attracting B cells into developing lymphoid tissue.⁹ Lymphoid hyperplasia is one of the characteristic findings of LGB in early infancy.¹⁰ The observation that CCL11 (eotaxin-1) was highly expressed in the guts of these very young neonates is consistent with mucosal eosinophilia. Further studies are needed to identify the cellular source of CCL11.

One potential confounder in this work is the control tissues, which came from much older children. It is well known that the immunologic composition of the gut mucosa changes with age. However, because it is impossible to obtain normal neonatal mucosa, it is difficult to circumvent this problem.

In summary, our preliminary microarray data do not support the concept that FPIP has an allergic cause, and more studies need to be done.

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