

Expression of transforming growth factor β 1, transforming growth factor type I and II receptors, and TNF- α in the mucosa of the small intestine in infants with food protein-induced enterocolitis syndrome

Hai Lee Chung, MD,^a Jin Bok Hwang, MD,^a Jeong Ja Park, MD,^b and Sang Gyung Kim, MD^c Taegu, Korea

Background: TNF- α secreted by activated T cells is known to increase intestinal permeability, whereas transforming growth factor (TGF) β has the ability to protect the epithelial barrier. **Objective:** We determined the expression of TGF- β 1, its receptors, and TNF- α on the mucosa of small intestine to investigate their roles in the pathogenesis of food protein-induced enterocolitis syndrome (FPIES).

Methods: Twenty-eight infants diagnosed with FPIES by means of clinical criteria and challenge test results were included. Immunohistochemical stains for TGF- β 1, type I and 2 TGF- β receptors, and TNF- α on duodenal biopsy specimens were performed.

Results: TGF- β 1 expression was generally depressed in patients. Expression of type I TGF- β receptor was significantly lower in the patients who had villous atrophy compared with expression in those patients who did not ($P < .001$) and negatively correlated with the severity of atrophy ($r = -0.59$, $P < .001$). Expression of type 2 TGF- β receptor showed no significant difference between the patients with or without villous atrophy. The immunoreactivity for both TGF- β receptors on lamina propria cells was slight or negative. TNF- α expression was detected on both epithelial and lamina propria cells and was significantly greater in the patients who had villous atrophy compared with that in the patients who did not ($P < .01$). **Conclusion:** Our results suggest that decreased countering activity of TGF- β 1 against T-cell cytokines is implicated in the pathogenesis of FPIES. The significantly lower expression of type I TGF- β receptor compared with type 2 receptor suggests the differential contribution of each receptor to the diverse biologic activities of TGF- β in the intestinal epithelium. (J Allergy Clin Immunol 2002;109:150-4.)

Key words: Food protein-induced enterocolitis syndrome, transforming growth factor β , transforming growth factor β receptors, TNF- α

Abbreviations used

FPIES: Food protein-induced enterocolitis syndrome
TGF: Transforming growth factor

Food protein-induced enterocolitis syndrome (FPIES) is a symptom complex of severe vomiting and diarrhea caused by non-IgE-mediated allergy to cows' milk, soy, or both in infants.¹⁻⁵ These symptoms typically begin in early infancy and, when severe, may lead to lethargy, dehydration, metabolic acidosis, and failure to thrive. Because FPIES is a diagnosis that can be mostly made clinically, there have not been many studies about the histologic findings, and the pathophysiology of the disease has not been clearly defined yet. There is increasing evidence that the immune response to antigen in the gastrointestinal tract may primarily involve T cells. Recent studies demonstrated that TNF- α secreted by circulating milk protein-specific T cells increased intestinal permeability, thus contributing to the influx of antigen into submucosa with further activation of antigen-specific lymphocytes.⁶ Moreover, fecal TNF- α was found to be increased after a positive milk challenge result in patients with enterocolitis.⁷ IFN- γ is known to enhance the action of TNF- α on intestinal epithelial cells.^{6,7} On the contrary, transforming growth factor (TGF) β showed a striking ability to protect the epithelial barrier function from the penetration of foreign antigens by countering the effect of T-cell cytokines.⁸⁻¹¹

In this study we determined the expression of TGF- β 1, type I and 2 TGF- β receptors (TGF- β RI and RII), and TNF- α directly on duodenal biopsy specimens to investigate their roles in the pathogenesis of FPIES.

METHODS

Patients and control subjects

Twenty-eight patients, aged from 7 to 120 days, who were referred to our pediatric department because of recurrent vomiting, diarrhea, or both and were given a diagnosis of FPIES were included in the study. The clinical findings on admission are shown in Table I. All patients fulfilled the following criteria, which were suggested by Sicherer et al⁵: (1) patient was younger

From the Department of ^aPediatrics, ^bPathology, and ^cClinical Pathology, School of Medicine, Catholic University of Taegu, Taegu.

Received for publication July 16, 2001; revised September 24, 2001; accepted for publication September 26, 2001.

Reprint requests: Hai Lee Chung, MD, Department of Pediatrics, School of Medicine, Catholic University of Taegu, 3056-6 Taemyung 4 Dong Nam-gu, Taegu, Korea 705-034.

Copyright © 2002 by Mosby, Inc.

0091-6749/2002 \$35.00 + 0 1/87/120562

doi:10.1067/mai.2002.120562

than 9 months of age at initial diagnosis; (2) repeated exposure to the incriminated food elicited diarrhea, repetitive vomiting, or both within 24 hours without any other cause; (3) there were only gastrointestinal symptoms; and (4) removal of the offending protein from the diet resulted in resolution of symptoms, and standardized food challenge elicited symptoms within 24 hours after administration of the food. All infants had been fed with cows' milk formula since birth and had negative responses to skin prick tests and RASTs with milk.

Duodenal biopsy specimens were obtained during upper gastrointestinal endoscopy by using a 7.9-mm Olympus GIF XP 20. Sedation with diazepam (0.3 mg/kg) or meperidine (1-2 mg/kg) was required in some infants. Each infant had a biopsy as soon as possible after admission.

Diagnostic food challenges were performed with parental consent. Patients underwent an open challenge consisting of a total of 0.6 g/kg protein in increasing doses over a 4-hour period.⁵ The total amount of protein during challenge was lowered (0.15-0.3 g/kg) if the patient had a history of a severe reaction. The patients were maintained under close observation for 24 hours after challenge.

Ten duodenal biopsy specimens were studied as controls after being diagnosed as normal. Six of them were from the infants who had undergone duodenal endoscopy, and 4 of them were from the infants who had undergone surgical resection. None of them showed evidence of FPIES.

This study was approved by the Catholic University of Taegu Institutional Review Board.

Immunohistochemical study

Biopsy specimens were immediately fixed in 10% neutral-buffered formalin fixative and embedded in paraffin. They were coded and assessed in blinded fashion by 2 observers. One tissue section (approximately 4 μ m) was stained with hematoxyline and eosin and viewed for villous atrophy and infiltration of lymphoplasmic cells, neutrophils, and eosinophils.

For immunohistochemical staining, sections were placed onto organosilane-coated slides (Fisher Biotech) and stained with a microprobe system (Fisher Scientific Co). After deparafinization, the slides were pretreated with Autoblocker (Research Genetics) to block endogenous peroxidase, followed by administration of proteinase K (20 μ g/mL, Sigma) and 3 washes with PBS. Slides were incubated with specific primary antibodies for 60 minutes at 37°C. The primary antibodies used were anti-TGF- β 1 mAb (Serotec), anti-TGF- β RI and anti-TGF- β RII polyclonal antibodies (Santa Cruz), and anti-TNF- α mAb (HyCult Biotechnology). Irrelevant rabbit and mouse IgG1 mAbs were used as negative controls in each procedure. A biotinylated secondary antibody (Immunotech) was added and visualized by using avidine-linked peroxidase. The sections were then counterstained with Harris Hematoxylin, mounted in glycerol, and viewed under light microscopy.

Quantification of immunostains

The expression of TGF- β 1, TGF- β receptors, and TNF- α on intestinal epithelial cells and mononuclear cells in the lamina propria was assessed semiquantitatively on a scale of 0 to 3 in increments of 0.5 according to the extent and intensity of staining (Table II). The extent of staining was assessed as the number of stained cells per 100 epithelial or mononuclear cells at a magnification of 400 $\times$ and graded as follows: 0, 0%; 1, less than 25%; 2, 25% to 50%; and 3, greater than 50%. The intensity of staining was graded as follows: 0, none; 1, weak; 2, moderate; and 3, strong.

The severity of villous atrophy was assessed on a scale of 0 to 3, and the degree of infiltration of inflammatory cells was assessed on a scale of 0 to 3, as described with the Sydney system¹²: 0, none; 1, mild; 2, moderate; and 3, severe.

TABLE I. Clinical findings on admission

Clinical findings	n (%)
Diarrhea	19 (67)
Diarrhea and vomiting	4 (15)
Vomiting only	5 (18)
Fever	12 (43)
Abdominal distension	4 (15)
Failure to thrive	9 (32)

TABLE II. Scoring of staining

Score	Intensity	Extent (%)
0	None	0
1	Mild	<25
2	Moderate	25-50
3	Severe	>50

Statistical analysis

Differences between groups were analyzed by using the Mann-Whitney *U* test. Correlation coefficients were calculated with the Spearman rank method. *P* values of less than .05 were accepted as statistically significant.

RESULTS

Duodenal biopsy specimens demonstrated mucosa edema and diffuse infiltration of inflammatory cells in all patients. Villous atrophy was observed in 16 (57%) patients, and 12 patients showed no villous atrophy. The degrees of villous atrophy were mild in 6 patients, moderate in 8 patients, and severe in 2 patients. There was no significant difference in the degree of infiltration among lymphoplasmic cells, neutrophils, and eosinophils.

The expression of TGF- β 1 was generally depressed and not detected in 22 (79%) patients. Among 6 patients who showed expression scores of 1 to 1.5, 4 patients had no villous atrophy, and 2 patients had mild atrophy. TGF- β 1-positive cells were detected along the villi and upper part of the crypt (Fig 1). TGF- β 1 expression was also depressed in control infants.

Both apical and basolateral membranes of the villus and crypt epithelium expressed TGF- β RI and TGF- β RII (Figs 2 and 3). TGF- β RI expression in the patients who had villous atrophy was significantly lower than that in the patients who had no villous atrophy and that in control subjects (0.5 vs 2.0 and 0.5 vs 2.4, respectively, *P* < .001; Fig 4). TGF- β RI expression in the patients who had no villous atrophy was significantly lower than that in control subjects (2 vs 2.4, *P* < .05; Fig 4). TGF- β RI expression was negatively correlated with the severity of villous atrophy (*r* = -0.59, *P* < .001; Fig 5). The expression of TGF- β RII showed no significant difference between the patients and the control subjects. The immunoreactivity for both TGF- β receptors on the cells in the lamina propria was slight or negative in both patients and control subjects.

TNF- α was expressed on both epithelial and lamina propria cells in the mucosa of patients (Fig 6). The degree of expression was significantly greater in the

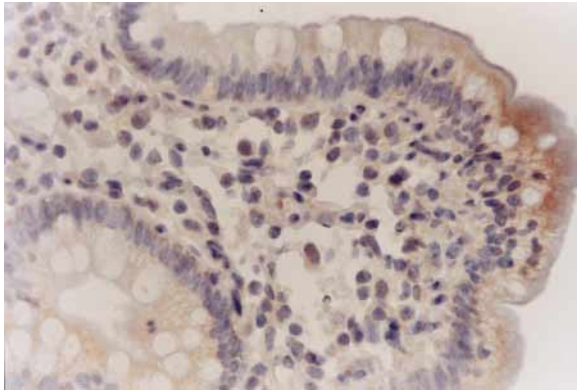


FIG 6. TNF- α expression in both epithelium and mononuclear cells in the lamina propria.

In our study the expression of TGF- β RI and TGF- β RII was demonstrated in the epithelial cells of both the villus and crypt of the small intestinal mucosa. The degree of expression of these two TGF- β receptors, however, was different. The expression of TGF- β RI was significantly lower in the patients who had severe mucosal damage with villous atrophy and negatively correlated with the severity of villous atrophy. In contrast, the expression of TGF- β RII showed no difference between patients and control subjects. In addition to its growth-inhibitory effect, TGF- β is known to stimulate the synthesis of extracellular matrix proteins, collagen, and fibronectin¹⁷ and enhance the binding between cells and matrix protein by altering the expression of integrins.¹⁸ For the intestinal epithelial cell, these events have important implications for the function of its intercellular tight junctions, for its growth on matrix proteins comprising the basement membrane, and, ultimately, for its barrier function. Of 3 distinct TGF- β receptors, TGF- β RI and TGF- β RII are transmembrane serine-threonine kinases, which are essential for TGF- β signaling.^{13,19} It is known that TGF- β RIII (β -glycan) is not directly involved in signaling but presents TGF- β to its signaling receptors.¹⁹ Previous studies suggested a differential contribution of the two signaling receptors to the diverse biologic actions of TGF- β : TGF- β RII may mediate growth inhibition, and TGF- β RI may mediate extracellular matrix deposition.^{20,21} In our patients the significantly lower expression of TGF- β RI compared with that of TGF- β RII was observed. It suggests that the impaired epithelial barrier function caused by the decreased TGF- β RI activity is implicated in the pathogenesis of FPIES. The expression of TGF- β receptors was detected in both the villus and crypt epithelium in our study. It was different from the results of the previous studies in adults, which showed the expression only in the upper parts of crypts or the villous tip.²²⁻²⁴ There have been no studies about the expression of TGF- β receptors in the mucosa of the small intestine in young infants. Our results are consistent with findings in the study with postnatal rats²⁵ and suggest a different distribution of TGF- β receptors in young infants compared with that in adults.

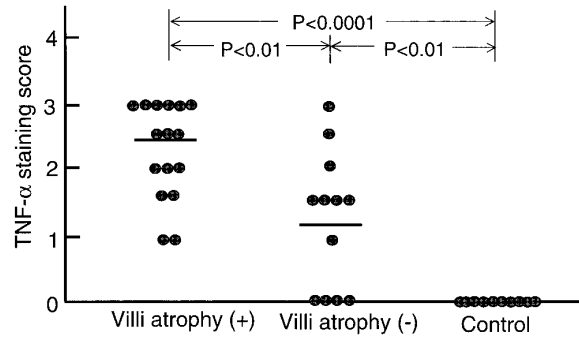


FIG 7. Mucosa TNF- α expression is significantly higher in patients with villous atrophy than in patients without villous atrophy and control subjects.

Our study showed significantly increased TNF- α expression in the epithelial cells and mononuclear cells in the lamina propria. The degree of expression was greater in the patients with villous atrophy than in the patients without villous atrophy. These results are consistent with the previous studies that demonstrated TNF- α released from milk-specific T cells altered intestinal barrier capacity and increased intestinal permeability.⁶

In conclusion, our results suggest that TNF- α released by lamina propria T cells and decreased activity of TGF- β 1 are implicated in the pathogenesis of FPIES in young infants. The significantly lower expression of TGF- β RI compared with that of TGF- β RII in FPIES suggests the differential contribution of each receptor to the diverse biologic activities of TGF- β in the intestinal epithelium.

We thank John H. Kwon, MD, PhD, for his helpful discussion and critical reading of the manuscript.

REFERENCES

1. Powell GK. Milk- and soy-induced enterocolitis of infancy. *J Pediatr* 1978;93:553-60.
2. Powell GK. Food protein-induced enterocolitis syndrome of infancy: differential diagnosis and management. *Compr Ther* 1986;12:28-37.
3. Powell GK. Enterocolitis in low-birth-weight infants associated with milk and soy protein intolerance. *J Pediatr* 1976;88:840-4.
4. Murray K, Christie D. Dietary protein intolerance in infants with transient methemoglobinemia and diarrhea. *J Pediatr* 1993;122:90-2.
5. Sicherer S, Eigenmann PA, Sampson HA. Clinical features of food protein-induced enterocolitis syndrome. *J Pediatr* 1998;133:214-9.
6. Heyman M, Daron N, Dupon C, Dugas B, Hirribaren A, Blaton MA, et al. Mononuclear cells from infants allergic to cow's milk secrete tumor necrosis factor α , altering intestinal function. *Gastroenterology* 1994;106:1514-23.
7. Madara JL, Stafford J. Interferon- γ directly affects barrier function of cultured intestinal epithelial monolayers. *J Clin Invest* 1989;83:724-7.
8. Majamaa H, Miettinen A, Laine S, Isolauri E. Intestinal inflammation in children with atopic eczema: faecal eosinophil cationic protein and tumor necrosis factor- α as non-invasive indicators of food allergy. *Clin Exp allergy* 1996;26:181-7.
9. Planchon SM, Martins CAP, Guerrant RL, Roche JK. Regulation of intestinal barrier function by TGF- β 1. Evidence for its role in abrogating the effect of a T cell cytokine. *J Immunol* 1994;153:5730-9.
10. Planchon S, Fiocchi C, Takafuji V, Roche JK. Transforming growth factor- β 1 preserves epithelial barrier function: identification of receptors, biochemical intermediates, and cytokine antagonists. *J Cell Physiol* 1999;181:55-66.

11. Roche JK, Martins CAP, Cosme R, Fayer R, Guerrant RL. Transforming growth factor- β 1 ameliorates intestinal epithelial barrier disruption by *Cryptosporidium parvum* in vitro in the absence of mucosa T lymphocytes. *Infect Immun* 2000;68:5635-44.
12. Dixon MF, Genta RM, Yardly JH, Correa P. International workshop on the histopathology of gastritis, Houston 1994. Classification and grading of gastritis: the updated Sydney system. *Am J Surg Pathol* 1996;20:1161-81.
13. Attisano L, Wrana JL, Lopez-Casillas F, Massague J. TGF- β receptors and actions. *Biochim Biophys Acta* 1994;1222:71-80.
14. Penttila IA, Spriel ABV, Zhang MF, Xian CJ, Steeb CB, Cummins AG, et al. Transforming growth factor- β levels in maternal milk and expression in postnatal rat duodenum and ileum. *Pediatr Res* 1998;44:524-31.
15. Saito S, Yoshida M, Ichijo M, Ishizaka S, Tsujii T. Transforming growth factor- β in human milk. *Clin Exp Immunol* 1993;94:220-4.
16. Lionetti P, Pazzaglia A, Moriondo M, Resti M, Andrea A, Vierucci A. Differing patterns of transforming growth factor- β expression in normal intestinal mucosa and in active celiac disease. *J Pediatr Gastroenterol Nutr* 1999;29:308-13.
17. Igotz RA, Massague J. Transforming growth factor- β stimulates the expression of fibronectin and collagen and their incorporation into the cellular matrix. *J Biol Chem* 1986;261:4337-45.
18. Heino J, Igotz RA, Helmer ME, Crouse C, Massague J. Regulation of cell adhesion receptors by transforming growth factor- β . Concomitant regulation of integrins that share a common β 1 subunit. *J Biol Chem* 1989;264:380-8.
19. Massague J, Andres J, Attisano L, Cheifetz S, Lopez-Casillas F, Ohtsuki M, et al. TGF- β receptors. *Mol Reprod Dev* 1992;32:99-104.
20. Chen RH, Ebner R, Derynck R. Inactivation of the type 2 receptor reveals two receptor pathways for the diverse TGF- β activities. *Science* 1993;260:1335-8.
21. Miettinen PJ, Ebner R, Lopez AR, Derynck R. TGF- β induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type 1 receptors. *J Cell Biol* 1994;127:2021-36.
22. Winesett M, Ramsey GW, Barnard JA. Type II TGF- β receptor expression in intestinal cell lines and in the intestinal tract. *Carcinogenesis* 1996;17:989-95.
23. Ohtani H, Kagaya H, Nagura H. Immunohistochemical localization of transforming growth factor- β receptors I and II in inflammatory bowel disease. *J Gastroenterol* 1995;30:76-7.
24. Eskinazi R, Resibois A, Svoboda M, Peny MO, Alder M, Robberecht P, et al. Expression of transforming growth factor β receptors in normal human colon and sporadic adenocarcinoma. *Gastroenterology* 1998;114:1211-20.
25. Zhang MF, Zola H, Rad LC, Penttila IA. Localization of transforming growth factor- β receptor I, II, and III in the postnatal rat small intestine. *Pediatr Res* 1999;46:657-65.