

Food antigen causes T_H2-dependent enteropathy followed by tissue repair in T-cell receptor transgenic mice

Haruyo Nakajima-Adachi, PhD,^a Ayumi Ebihara, MS,^a Akira Kikuchi, MS,^a Tsuyoshi Ishida, MD,^b Kiyomi Sasaki, BS,^a Kiyomi Hirano,^c Hiroko Watanabe, PhD,^d Kazumi Asai, PhD,^a Yoshimasa Takahashi, PhD,^e Yutaka Kanamori, MD,^f Naoki Shimojo, MD, PhD,^c Hiroshi Matsuda, DVM, PhD,^g Yoichi Kohno, MD, PhD,^c Satoshi Hachimura, PhD,^a and Shuichi Kaminogawa, PhD^h Tokyo, Chiba, and Kanagawa, Japan

Background: Clarification of the mechanisms underlying the development of food-sensitive intestinal inflammation will provide an important clue to combating food allergies.

Objective: To establish a model of intestinal inflammation caused by oral administration of antigen without additional treatments, we focused on the ovalbumin (OVA) 23-3 T-cell receptor transgenic mouse, which had been reported to have high serum antigen-specific IgE responses to the feeding of an egg white diet.

Methods: Changes in body weight of mice fed an egg white diet were monitored throughout the 28-day experimental period. After the 28-day feeding, intestinal tissues were harvested for histologic examination. Endogenous production of cytokines and histamine in the jejunum, and production of cytokines secreted by OVA-specific CD4⁺ T cells purified from mesenteric lymph nodes, were analyzed.

Results: Egg white diet-fed OVA23-3 mice developed weight loss and inflammation with villous atrophy and goblet cell hyperplasia, especially in the jejunum. A further characteristic feature was evidence of weight recovery and tissue repair. Jejunal inflammation was also observed in egg white diet-fed recombination activating gene (RAG)-2-deficient OVA23-3 mice. In addition, tissue sections revealed significant infiltration of specific IgE-positive cells and IgE-positive degranulating mast cells. Higher levels of IL-4 and significant levels of histamine were detected in the tissues. In the supernatant of

OVA-stimulated T cells, IL-10 levels were also markedly elevated.

Conclusion: We report that high-dose and continuous intake of primitive OVA alone induces enteropathy containing regions under repair in OVA23-3 mice. Antigen-specific T cells and inflammatory cells primed by T_H2 responses play important roles in regulation of development and improvement of the disease.

Clinical implications: Long-term antigen intake causes T_H2-dependent and food-sensitive enteropathy followed by tissue repair. (J Allergy Clin Immunol 2006;117:1125-32.)

Key words: T-cell receptor transgenic mouse, food-sensitive intestinal inflammation, ovalbumin, food antigen intake, antigen-specific T cells, IgE, mast cell, T_H2, histamine, tissue repair

Several animal studies have been performed to clarify mechanisms underlying gastrointestinal hypersensitivities or allergies caused by food-derived antigen and to enhance progress toward diagnostic and/or protective measures against them.^{1,2} In mouse models of food-sensitive intestinal inflammation, there are numerous examples of IgE-independent³⁻⁷ and IgE-dependent models⁸⁻¹⁰ but few of mixed models. Until now, almost all models have been established by using adjuvant-associated antigens, injection of drugs concurrent with dietary antigen, or microbial flora-associated antigen to promote systemic or local sensitization initially and to overcome antigen-induced oral tolerance.² However, these procedures do not clarify how the intestinal immune system regulates the induction of tolerance or of food-sensitive intestinal inflammation.

Our previous studies of food allergy prompted us to develop a mouse model of intestinal inflammation.^{11,12} We focused on the transgenic mouse with a T-cell receptor (TCR) recognizing ovalbumin (OVA) residues 323-339¹³ as a means of amplifying immunologic reactions to orally administered antigens without using adjuvant or other agents, because the mouse has been reported to have specific-IgE responses to the 28-day oral feeding with an egg white (EW) diet.¹⁴ We have noticed that these mice had a trend toward loose feces after commencing the EW diet. Thus, we analyzed the histology of their mucosal and immunologic tissues and succeeded in developing a novel and unique mouse model.

From ^athe Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo; ^bthe Department of Diagnostic Pathology, Kanto Medical Center Nippon Telegraph and Telephone East Corporation, Tokyo; ^cthe Department of Pediatrics, Chiba University; ^dthe Chemistry Division, Kanagawa Prefectural Institute of Public Health; ^ethe Department of Immunology, National Institute of Infectious Diseases, Tokyo; ^fthe Department of Pediatric Surgery, University of Tokyo; ^gthe Division of Animal Science, Graduate School, Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology; and ^hthe Department of Food Science and Technology, Nihon University College of Bioresource Sciences, Kanagawa.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication April 11, 2005; revised January 10, 2006; accepted for publication January 11, 2006.

Reprint requests: Haruyo Nakajima-Adachi, PhD, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-Ku, Tokyo 113-8657, Japan. E-mail: aryu@mail.ecc.u-tokyo.ac.jp.

0091-6749/\$32.00

© 2006 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2006.01.016

Abbreviations used

AB:	Alcian blue
BALB-mice:	BALB/c mice
CN:	Casein
CNB:	Casein diet-fed BALB/c mice
DFS:	Direct fast scarlet
EW:	Egg white
EWB:	Egg white diet-fed BALB/c mice
EWR:	Egg white diet-fed RAG-2-deficient BALB/c mice
FITC:	Fluorescein isothiocyanate
MLN:	Mesenteric lymph nodes
OVA:	Ovalbumin
PAS:	Periodic acid-Schiff
TB:	Toluidine blue
TCR:	T-cell receptor
Tg-mice:	Ovalbumin 23-3 mice

METHODS**Animals**

Ovalbumin 23-3 mice transgenic for OVA323-339-specific and I-A^d-restricted TCR- $\alpha\beta$ (V α 3/V β 15) on a BALB/c genetic background were kindly provided by S. Habu (Tokai University School of Medicine).¹³ These mice were crossed with recombination activating gene (RAG)-2-deficient BALB/c-mice (BALB-mice) to establish RAG-2-deficient OVA23-3 mice (Tg-mice). Animals were maintained in the Hoshino Animal Center (Chiba, Japan). Male mice were used because female mice did not show clear weight changes. Male BALB-mice were purchased from Clea Japan Inc (Tokyo, Japan). They were housed under specific pathogen-free conditions. Guidelines formulated by the University of Tokyo were followed for the care and use of animals.

Ovalbumin administration

Seven-week-old male Tg-mice, RAG-2-deficient BALB-mice, RAG-2-deficient Tg-mice, and BALB-mice were divided into experimental groups as indicated both in figures and figure legends and administered for 28 days with a range of experimental diets: CE2-diet (Clea Japan Inc), EW diet, OVA diet, or casein (CN) diet (Funabashi Farm, Funabashi, Japan). The composition of experimental diets is indicated in the Online Repository at www.jacionline.org. By feeding EW diet and OVA diet, it is calculated that a mouse consumes about 250 mg OVA per day. During the experimental period, the weight of each mouse was measured every 4 or 7 days. After the 28-day feeding, tissues were taken from each mouse for further analysis. Sera were obtained before and after feeding and stored at -80°C . Concentrations of serum total IgE and OVA-specific IgE, IgG₁, and IgG_{2a} were analyzed by ELISA and estimated as described previously.¹⁴

Histologic analysis

Longitudinal sections of intestinal tissue (3 cm) were taken from the duodenum, the jejunum (11 cm distal to duodenum), the ileum (the superior part of cecum), and the colon (proximal and distal), respectively. Tissues were opened longitudinally, fixed in 10% formalin and embedded in paraffin. Sections 3 or 5 μm thick were prepared and stained with hematoxylin and eosin for morphologic analysis, direct fast scarlet (DFS) to analyze eosinophils, periodic acid-Schiff (PAS)-Alcian blue (AB) for visualization of goblet cells,

and Toluidine blue (TB) to detect mast cells. Parameters and numbers of mice used for quantification of intestinal tissue inflammation are indicated in the Online Repository at www.jacionline.org. For analysis of inflammation by scanning electron microscopy, jejunal sections were fixed in 2.5% glutaraldehyde and coated with gold. Electron micrographs of these specimens were scanned by using an S-3500N scanning electron microscope (Hitachi, Tokyo, Japan) and stored for subsequent observation and analysis.

Immunohistochemical staining

Jejunum sections, 1 cm in length and 4 cm distal to duodenum, were mounted in optimal cutting temperature compound (Tissue-tek; Sakura, Tokyo, Japan). Cryosections (6 μm thick) were fixed with acetone at -20°C . After rehydration with PBS, sections were blocked overnight with antimouse CD16/CD32 Ab (2.4G2; Pharmingen, San Diego, Calif) at 4°C and stained overnight with fluorescein isothiocyanate (FITC)-conjugated rat antimouse IgE (R35-72; Pharmingen) or biotinylated rat antimouse IgE (R35-72; Pharmingen) at 4°C . Biotinylated sections were incubated with streptavidin-horseradish peroxidase (ZYMED, South San Francisco, Calif) followed by DAB (Sigma, St Louis, Mo). IgE-positive mast cells were detected by further counterstaining with TB. To detect ovalbumin-specific IgE, FITC-labeled sections were further stained with biotinylated OVA followed by streptavidin-Texas red (Roche, Tokyo, Japan).

Histamine and cytokine levels in whole gut tissue homogenates

Four-centimeter sections of the jejunum immediately distal to the duodenum were collected and processed according to the method of van Halteren et al.¹⁵ Homogenates were centrifuged and supernatants used for determination of histamine levels by HPLC (Post-Column Reaction Systems; Waters Co, Milford, Mass).¹⁶ To study the endogenous production of IFN- γ , IL-4, and TNF- α , 6-cm sections of the jejunum located 5 cm distal to the duodenum were collected and processed. Protein lysates of the jejunum were prepared with a cocktail of proteinase inhibitors and centrifuged. Supernatants were collected and concentrations of IL-4 and IFN- γ assessed by ELISA as described previously.¹⁴ The OptEIA ELISA Set was used for TNF- α analysis according to the manufacturer's instructions (Pharmingen). Histamine and cytokine contents were standardized to the weight of jejunum tissue in each sample.

Cell culture and cytokine measurements

Mesenteric lymph node (MLN) lymphocytes isolated from each mouse fed with the CN diet or EW diet were pooled for each experimental group. MLN CD4⁺ T cells were positively identified by using anti-CD4 mAb conjugated to immunomagnetic beads and selected using a MACS cell separation system (Miltenyi Biotec, Bergish Gladbach, Germany) according to the manufacturer's protocol. The proportion of MLN CD4⁺ T cells was more than 94% in each analysis. The MLN CD4⁺ T cells (1×10^5 cells/well) were added to 96-well flat-bottom plates and cultured in the absence or presence of OVA (1 mg/mL) with mitomycin-treated splenocytes as antigen-presenting cells (4×10^5 cells/well) in complete RPMI 1640. Supernatants were collected 48 hours later and cytokine concentrations measured by ELISA (IFN- γ , IL-4, IL-2, and IL-5). The OptEIA ELISA Set was used for TNF- α and IL-10 analysis as described.

Statistical analysis

Results are presented as the means $\pm$ SDs. Weight change was analyzed by using the Fischer's Protect Least Significant Difference test of multiple comparisons. The Mann-Whitney *U* test was used for nonparametric analysis of levels of serum antibodies, histamine or

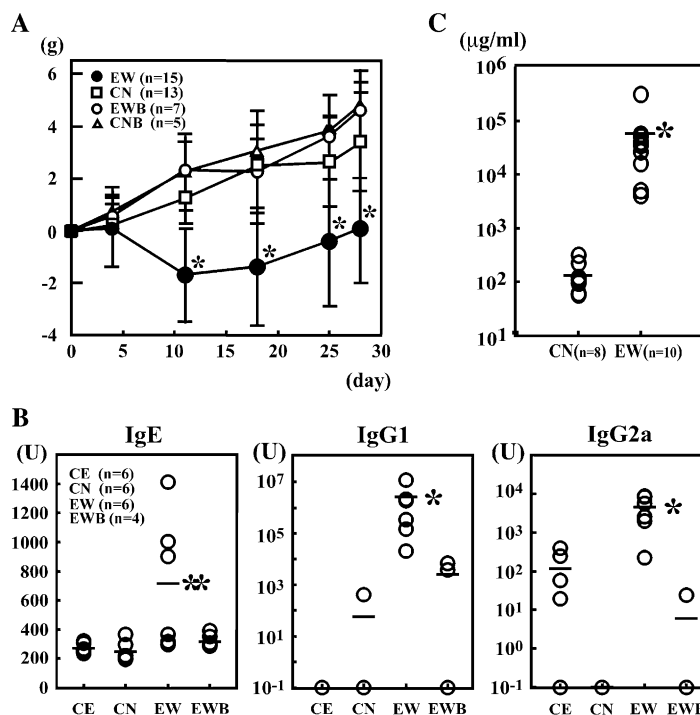


FIG 1. **A**, Weight loss in EW diet-fed Tg-mice. (* $P < .05$). **B**, Increase in serum OVA-specific antibodies in EW diet-fed Tg-mice. These data represent 4 independent experiments containing 3 to 6 mice in each group. **C**, Increase in total IgE in EW diet-fed Tg-mice. In **B** and **C**, ○ and bars indicate antibody titers for each mouse and the means, respectively. (* $P < .01$).

cytokine production, and the numbers of inflammatory cells in the intestine. Differences were considered statistically significant for values of $P < .05$.

RESULTS

Feeding of EW diet induces wasting diseases and hyperproduction of serum antibody in Tg-mice

Feeding Tg-mice with the EW diet caused loose feces (see this article's Fig E1 in the Online Repository at www.jacionline.org). Body weight changes from day 0 revealed that EW diet-fed Tg-mice (Fig 1, A: EW) failed to thrive from day 4, whereas from day 10 through day 28, weight loss was significant compared with control groups showing normal growth. However, body weights showed a tendency to recover after the initial loss. Control groups were composed of CE2 diet-fed Tg-mice (data not shown), CN diet-fed Tg-mice (Fig 1, A: CN), EW diet-fed BALB-mice (Fig 1, A: EWB), and CN diet-fed BALB-mice (Fig 1, A: CNB), respectively. There were no significant differences in volumes of experimental diet consumed by mice in each group (data not shown).

As shown in Fig 1, B, OVA-specific IgE was detected in some (but not all) EW diet-fed Tg-mice (Fig 1, B: EW). Extensive induction of OVA-specific IgG1 and high levels of OVA-specific IgG2a were detected in EW diet-fed Tg-mice compared with control groups (Fig 1, B: CN, EWB, and CE, CE2-diet-fed Tg-mice).

All of the EW diet-fed Tg-mice (Fig 1, C: EW) showed significant total IgE responses compared with control group (Fig 1, C: CN).

Villous morphologic changes in the jejunum of EW diet-fed Tg-mice

Analysis of intestinal histology indicated that inflammation associated with morphologic changes was particularly noticeable in sections of the jejunum from EW diet-fed Tg-mice (Fig 2, A: EW). Changes to the jejunal tissue consisted of a thickened muscular layer, crypt elongation, and villous atrophy, associated with goblet cell hyperplasia and increased numbers of Paneth cells. However, the severity of inflammation varied from animal to animal in the 28-day feedings: inflammatory regions were identified on all sequential segments in some Tg-mice (Fig 2, A: EW-Jejunum; and Fig 2, B: EW1), and in discrete areas of sequential segments in others (partial villous atrophy, Fig 2, B: EW2). The severity of inflammation showing partial villous atrophy was moderate in general. A characteristic histologic feature in this inflammatory process is villous blunting of the mucosa. Villous blunting without significant inflammatory cell infiltration as indicated in these panels is considered to represent a moderate degree of inflammation under mucosal repair. Thus, these panels provide morphologic evidence of repair (Fig 2, B: EW3). PAS-AB staining confirmed excess mucus in EW diet-fed Tg-mice (Fig 2, B: EW1-PAS, PAS-AB-stained EW1).

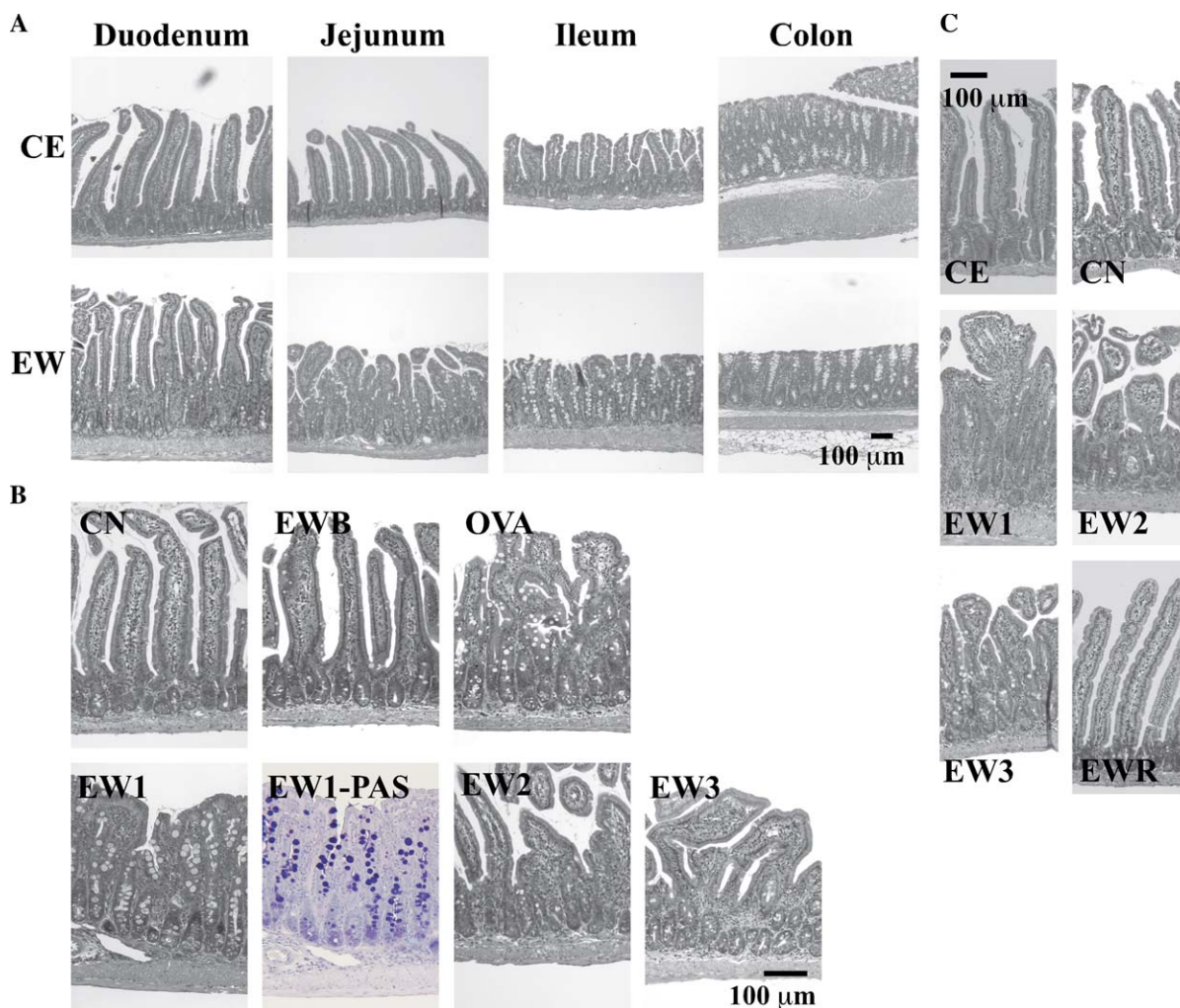


FIG 2. **A**, Intestinal inflammation detected in EW diet-fed Tg-mice. **B**, Detailed jejunal inflammation. EW1, EW2, and EW3 are panels from 3 different mice. **C**, Jejunal inflammation detected in EW diet-fed RAG-2-deficient Tg-mice (EW). EW1, EW2, and EW3 are panels from 3 different mice.

The duodenum and ileum from Tg-mice with severe inflammation in the jejunum also exhibited inflammation, which in most cases was less severe than that of the jejunum (Fig 2, A). Severe morphologic changes were not observed in the large intestine, although half of the sections from EW diet-fed Tg-mice (7 of 14 animals) showed polymorphonuclear leukocyte infiltration into the villous site of the lamina propria, particularly in the distal colon.

In OVA diet-fed Tg-mice, similar weight loss, increases in serum specific antibody titers, and inflammatory responses were observed (Fig 2, B: OVA; see also Figs E2, A, and E2, B, in the Online Repository at www.jacionline.org). This result indicated that the OVA diet has the same effects on the mouse as the EW diet, thereby showing that purified OVA, and not other components contained in EW, was the cause of the inflammation. Alterations to the intestinal mucosa could not be found in control groups (Fig 2, A: CE, CE2 diet-fed Tg-mice; Fig 2, B: CN, CN diet-fed Tg-mice; EWB, EW

diet-fed BALB-mice, ovalbumin and CN diet-fed BALB-mice, data not shown).

We further analyzed the histology of EW diet-fed RAG-2-deficient Tg-mice. In these mice, severe weight loss (data not shown) along with jejunal inflammation were also observed (Fig 2, C: EW1, EW2, and EW3), but not in control groups (Fig 2, C: CE, CE2 diet-fed RAG-2-deficient Tg-mice; CN, CN diet-fed RAG-2-deficient Tg-mice; EWR, EW diet-fed RAG-2-deficient BALB-mice). RAG-2-deficient Tg-mice with developed disease are defective in local and serum IgE responses because of lack of mature B cells (data not shown),¹⁷ and thereby have only OVA-specific T cells. On the other hand, RAG-2-deficient BALB-mice with undeveloped disease lack both mature B cells and T cells. The results thus indicate that specific T cells are indispensable in development of wasting disease and its pathogenesis in EW diet-fed Tg-mice.

The severity of the morphologic changes was reflected by the significant decrease in the villous height to crypt

depth in EW diet-fed Tg-mice. Scanning electron micrographs confirmed the results of hematoxylin and eosin staining experiments (see this article's Figs E3 and E4 in the Online Repository at www.jacionline.org).

Predominant T_H2 responses in EW diet-fed Tg-mice

Local cytokine production analysis in the jejunum showed higher levels of IL-4 but undetectable levels of IFN- γ (data not shown) in EW diet-fed Tg-mice (Fig 3, A: EW). IFN- γ was also not detected in the control group (CN diet-fed Tg-mice; data not shown). Significant differences in TNF- α production were not observed between EW diet-fed and CN diet-fed Tg-mice (Fig 3, A: CN).

The cytokines of OVA-specific CD4⁺ T cells from MLN indicated significantly higher levels of IL-4 and IL-10 but significantly lower levels of IFN- γ production were detected in EW diet-fed Tg-mice (Fig 3, B: EW) compared with CN diet-fed Tg-mice (Fig 3, B: CN). IL-5 was below levels of detection in both groups. TNF- α and IL-2 production was significantly induced in CN diet-fed Tg-mice, whereas in EW diet-fed Tg-mice, both cytokines were at lower levels. Similar but lower production of cytokines of whole lymphocytes and undetectable levels in cells other than CD4⁺ T cells obtained from MLN (data not shown) suggested that secretion of these cytokines depends on OVA-specific CD4⁺ T cells in MLN.

Predominant infiltration of IgE-positive mast cells into the jejunum of EW diet-fed Tg-mice

We next investigated whether the higher titer of serum IgE responses in EW diet-fed Tg-mice was reflected in the prominent jejunal responses. IgE-positive mast cells, as well as other IgE-positive cells, predominantly infiltrated into the lamina propria near crypts or into submucosa of the jejunum of these mice (Fig 4, A: EW). Extensive degranulation was detected in most IgE-positive mast cells. Many of the IgE-positive cells in the villous lamina propria were predicted to be IgE-secreting plasma cells by further staining with antibody for CD138 (data not shown). An important result was detection of OVA-specific IgE-positive cells in the jejunum of EW-diet-fed Tg-mice (Fig 4B), whereas IgE-positive cells and degranulation of mast cells were absent in the jejunum of control group (Fig 4, A and C: CN, CN diet-fed Tg-mice). Furthermore, local histamine levels in jejunal tissue of EW diet-fed Tg-mice (Fig 4, C: EW) were significantly increased compared with those of CN diet-fed Tg-mice, even there was considerable variation within each group. Thus, orally administered OVA induced mast cell degranulation triggered by binding of specific IgE to mast cells. The IgE-positive mast cells may therefore function by secreting large amounts of histamine. Analysis of paraffin embedded sections confirmed predominant infiltration of mast cells into the jejunum and distal colon of EW diet-fed Tg-mice compared with controls (see this article's Figs E5 and E6 and Results in Online Repository at

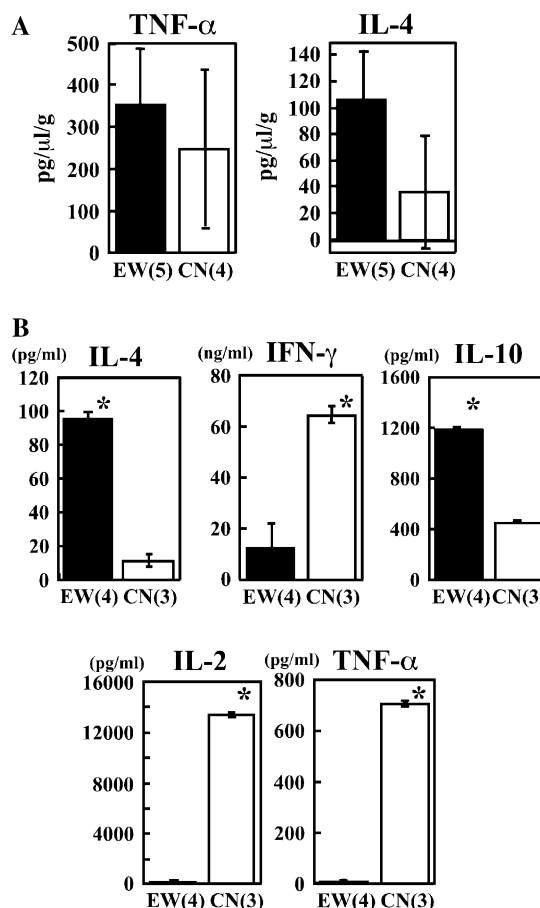


FIG 3. A, T_H2 polarized endogenous cytokine levels detected in the jejunum tissue from EW diet-fed Tg-mice. B, T_H2 polarized cytokines secreted by OVA-specific MLN CD4⁺ T cells from EW diet-fed Tg-mice (*P < .05). The number of mice used in each group is indicated in brackets; results are representative of 2 independent experiments, respectively.

www.jacionline.org), and the numbers were quantified (Fig 5).

Other inflammatory responses in EW diet-fed Tg-mice

Participation of eosinophils or polymorphonuclear leukocytes in the inflammation process is indicated in the section "Aggregation of eosinophils in the lamina propria of the jejunum and distal colon and crypt abscess formation in the jejunum observed only in EW diet-fed Tg-mice" (see this article's Results and Figs E7 and E8 in Online Repository at www.jacionline.org). The average number of eosinophils aggregated in the villous lamina propria of the jejunum of EW diet-fed Tg-mice (Fig 6: EW) was significantly greater than that in control groups (Fig 6: CN, CN diet-fed Tg-mice; EWB, EW diet-fed BALB-mice). Inflammatory responses in other immunologic tissues and in tracheal tissue from EW diet-fed Tg-mice are described in the Methods and Fig E9 in the Online Repository at www.jacionline.org.

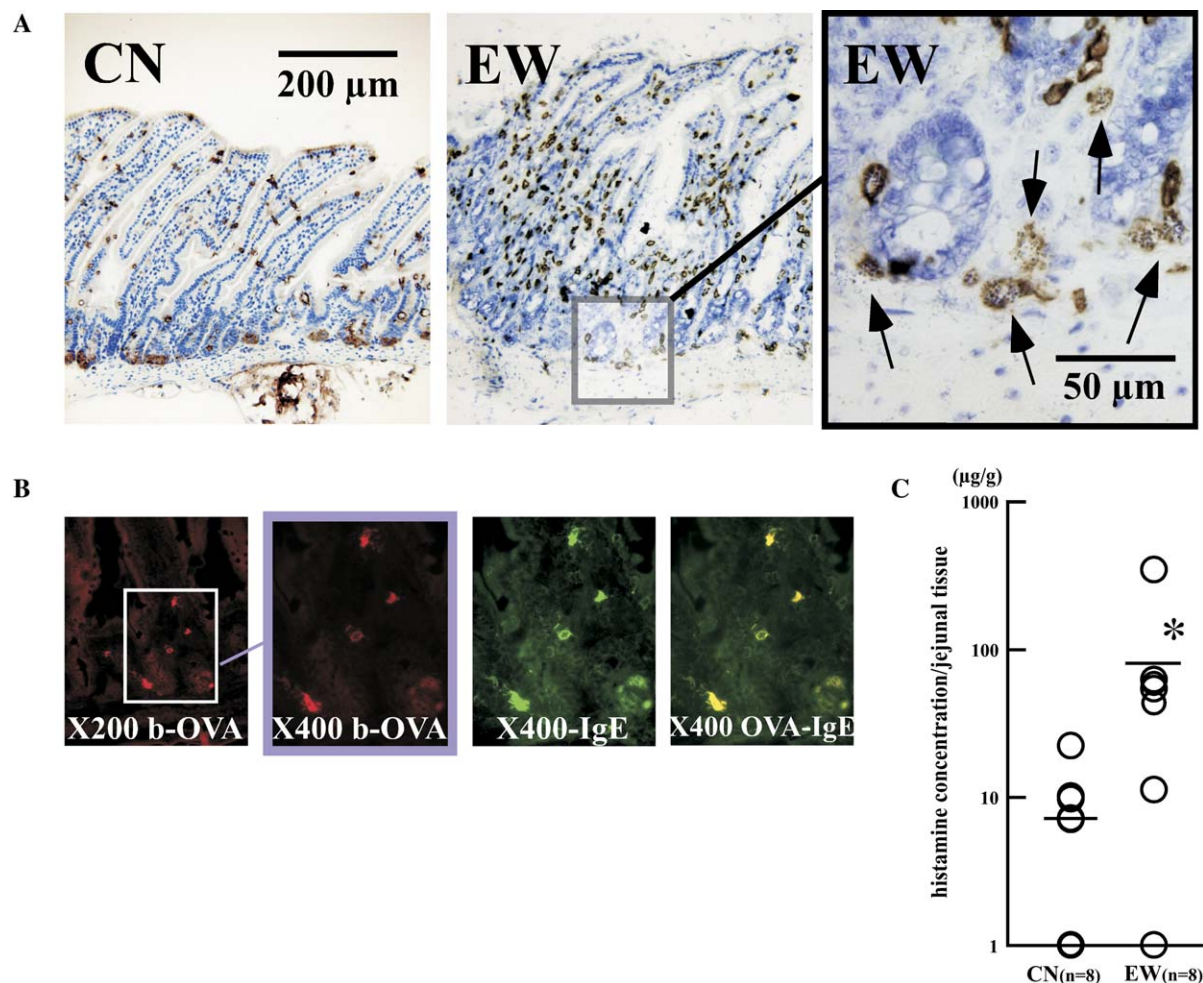


FIG 4. **A**, Mast cells bind IgE and degranulate in EW diet-fed Tg-mice. Arrows indicate IgE-positive and degranulating mast cells. **B**, Intestinal OVA-specific IgE detection in the jejunum of EW diet-fed Tg-mice. *b*-OVA, biotinylated OVA stained; *IgE*, FITC-labeled IgE-stained; *OVA-IgE*, double-stained with *b*-OVA and FITC-IgE. **C**, Jejunal histamine levels. ○, values of each mouse; —, means of the data. * $P < .05$.

DISCUSSION

We have successfully developed a unique animal model that undergoes weight loss in response to oral administration of OVA alone. The intestinal tissue of this animal model exhibits loss of villous architecture together with a characteristic feature of tissue repair, where specific IgE and degranulating IgE-positive mast cells, as well as antigen-specific T cells, were functional. Pathological changes were similar to results of studies on tissues collected from patients with food-sensitive enteropathy,¹⁸⁻²¹ in that an increase in mucosal IgE-secreting plasma cells and degranulating mast cells in the jejunum were described.²⁰ In addition, in relation to the large intestine, although this model lacked features such as diarrhea and morphologic changes observed in other models of food-induced colitis,⁵⁻⁷ the pathology was consistent with that examined in tissues from patients with intestinal food allergies.²¹ The deficiency in our model may be the absence of bacterial stimulation

to initiate severe inflammation in the large intestinal immune system.

Pathological changes similar to those described by us showing loss of villous architecture in tissue from the ileum have been reported by Dohi et al,²² who used a TCR-deficient mouse model to which IFN- γ -deficient CD45RB^{high} T cells were transferred. In our model, established in an antigen-specific manner, it is unlikely that CD45RB^{high} T cells would play a role in the development of inflammation. Also, inflammation in the model by Dohi et al²² was detected at a different site from ours. However, wasting disease and villous atrophy are common results, and Dohi et al²² further elucidated the significance of T_H2 cell responses. Thus, for our model, it is also considered that wasting disease and villous atrophy induced by feeding the EW diet would probably be mediated by significant T_H2-polarized responses. In food allergy reactions, T_H2 skewing has been demonstrated.¹ However it remains unclear whether the T_H2-type immune response stimulated by feeding with food allergens could

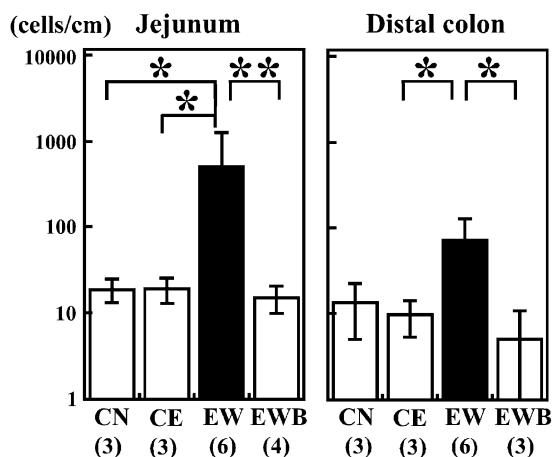


FIG 5. Quantification of mast cells predominantly infiltrating into the intestinal tissues of EW diet-fed Tg-mice (EW) compared with controls (CN or CE, CN diet-fed or CE2 diet-fed Tg-mice, respectively; EWB, EW diet-fed BALB-mice, * $P < .05$, ** $P < .01$). The number of mice used in each group is indicated in parentheses.

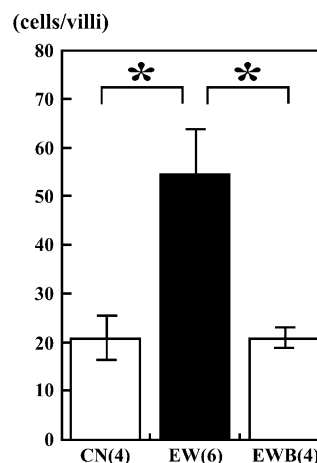


FIG 6. Quantification of eosinophils aggregated in the villous lamina propria of the jejunum from EW diet-fed Tg-mice. The number of mice used in each group is indicated in parentheses. (* $P < .01$).

explain the development of enteropathy observed in patients.¹⁹ Although this is a mouse model, the current study is significant in showing that food-sensitive enteropathy relates to T_H2 -dominant responses. Further detailed studies are needed to examine the involvement of IL-5 and IL-13 in our model, considering the eosinophil aggregation in villi and changes detected in the trachea.²³

The most significant novel aspect of our model was that the histologic changes demonstrated a more complex pathology, as indicated particularly by the observation of the unsuccessful repair of injured tissues with marked infiltration of degranulating IgE-positive mast cells. The results of IgE-positive mast cell responses induced by food antigen intake can also be compared with findings reported by Brandt et al.¹⁰ In their model, upregulation of OVA-specific T_H2 -type responses by using aluminum hydroxide after oral administration of ovalbumin induced transient diarrhea dependent on IgE responses. This effect was induced by serotonin, not by histamine produced by mast cells. In contrast, elevated responses of serum and local IgE and the marked manifestation of mast cells in EW diet-fed Tg-mice in our study did not correlate with symptoms of diarrhea or the severity of villous atrophy. Furthermore, we have observed that even after an allergen-free period, oral readministration of either the EW diet or OVA itself to mice having higher levels of serum OVA-specific IgE did not cause diarrhea (data not shown). In addition, 10-day feeding with EW diet seemed to induce severe crypt elongation and goblet cell hyperplasia in all EW diet-fed Tg-mice (see this article's Fig E10: EW1 and EW2, in the Online Repository at www.jacionline.org). During this period, serum total IgE showed significantly low levels compared with those of the 28-day feeding.¹⁴ Therefore, in contrast with the study by Brandt et al,¹⁰ the dramatic infiltration of mast cells and IgE in our model for the 28-day feeding seemed to involve either the regulatory effect of histamine on T_H2 cells, or

the predictive roles of IgE for oral tolerance binding to high expression of FcεRI on oral Langerhans cells,^{24,25} rather than the injurious responses.

Actually in our system, the protective cytokine IL-10 was markedly produced in MLN $CD4^+$ T cells. Also, induction of oral tolerance has paradoxically been reported in spleen T cells from Tg-mice fed the EW diet for 28 days.²⁶ Thus, it is possible that the intestinal tissue, after exposure to an EW diet for 28 days, reflects a state of partial suppression of the injurious T_H2 responses, thereby causing the villous blunting or partial villous atrophy and a tendency to weight recovery. However, feeding times longer than 28 days, during the period serum IgE maintained higher levels, did not completely resolve inflammation in all mice, although the reason remained unclear (see this article's Fig E10: EW3 and EW4, in the Online Repository at www.jacionline.org). Further study of this animal model is expected to shed light on the mechanisms of T_H2 -associated humoral and cellular activation in food-sensitive intestinal inflammation, thereby opening new possibilities for the treatment of food allergies involving tolerance induction.

We thank S. Habu and T. Sato at Tokai University for providing animals and K. Shida and S. Matsumoto at Yakult Central Institute for their technical support and for providing experimental systems and animals. We thank S. Fukuda at the University of Tokyo Hospital and K. Matsumoto at Hitachi High-Technologies Corp for their technical support with scanning electron microscopy. We thank C. Ogawa at Ochanomizu University for her technical support.

REFERENCES

1. Sampson HA, Sicherer SH, Birnbaum AH. AGA technical review on the evaluation of food allergy in gastrointestinal disorders. American Gastroenterological Association. *Gastroenterology* 2001;120:1026-40.
2. Helm RM. Food allergy animal models: an overview. *Ann N Y Acad Sci* 2002;964:139-50.

3. Ohtsuka Y, Yamashiro Y, Maeda M, Oguchi S, Shimizu T, Nagata S, et al. Food antigen activates intraepithelial and lamina propria lymphocytes in food-sensitive enteropathy in mice. *Pediatr Res* 1996;39:862-6.
4. Newberry RD, Stenson WF, Lorenz RG. Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. *Nat Med* 1999;5:900-6.
5. Iqbal N, Oliver JR, Wagner FH, Lazenby AS, Elson CO, Weaver CT. T helper 1 and T helper 2 cells are pathogenic in an antigen-specific model of colitis. *J Exp Med* 2002;195:71-84.
6. Yoshida M, Shirai Y, Watanabe T, Yamori M, Iwakura Y, Chiba T, et al. Differential localization of colitogenic Th1 and Th2 cells monospecific to a microflora-associated antigen in mice. *Gastroenterology* 2002;123:1949-61.
7. Kweon MN, Yamamoto M, Kajiki M, Takahashi I, Kiyono H. Systemically derived large intestinal CD4(+) Th2 cells play a central role in STAT6-mediated allergic diarrhea. *J Clin Invest* 2000;106:199-206.
8. Ohtsuka Y, Naito K, Yamashiro Y, Yabuta K, Okumura K, Ra C. Induction of anaphylaxis in mouse intestine by orally administered antigen and its prevention with soluble high affinity receptor for IgE. *Pediatr Res* 1999;45:300-5.
9. Li XM, Schofield BH, Huang CK, Kleiner GI, Sampson HA. A murine model of IgE-mediated cow's milk hypersensitivity. *J Allergy Clin Immunol* 1999;103:206-14.
10. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest* 2003;112:1666-77.
11. Nakajima H, Hachimura S, Nishiwaki S, Katsuki T, Shimojo N, Ametani A, et al. Establishment and characterization of alpha s1-casein-specific T-cell lines from patients allergic to cow's milk: unexpected higher frequency of CD8+ T-cell lines. *J Allergy Clin Immunol* 1996;97:1342-9.
12. Nakajima-Adachi H, Hachimura S, Ise W, Honma K, Nishiwaki S, Hirota M, et al. Determinant analysis of IgE and IgG4 antibodies and T cells specific for bovine alpha(s)1-casein from the same patients allergic to cow's milk: existence of alpha(s)1-casein-specific B cells and T cells characteristic in cow's-milk allergy. *J Allergy Clin Immunol* 1998;101:660-71.
13. Sato T, Sasahara T, Nakamura Y, Osaki T, Hasegawa T, Tadakuma T, et al. Naive T cells can mediate delayed-type hypersensitivity response in T cell receptor transgenic mice. *Eur J Immunol* 1994;24:1512-6.
14. Shida K, Hachimura S, Ametani A, Ishimori M, Ling M, Hashiguchi M, et al. Serum IgE response to orally ingested antigen: a novel IgE response model with allergen-specific T-cell receptor transgenic mice. *J Allergy Clin Immunol* 2000;105:788-95.
15. van Halteren AG, van der Cammen MJ, Biewenga J, Savelkoul HF, Kraal G. IgE and mast cell response on intestinal allergen exposure: a murine model to study the onset of food allergy. *J Allergy Clin Immunol* 1997;99:94-9.
16. Yamanaka H, Matsumoto M. Simultaneous determination of polyamines in red meat fishes by high performance liquid chromatography and evaluation of freshness. *J Food Hyg Soc Japan* 1989;30:396-400.
17. Shinkai Y, Rathbun G, Lam KP, Oltz EM, Stewart V, Mendelsohn M, et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* 1992;68:855-67.
18. Maluenda C, Phillips AD, Briddon A, Walker-Smith JA. Quantitative analysis of small intestinal mucosa in cow's milk-sensitive enteropathy. *J Pediatr Gastroenterol Nutr* 1984;3:349-56.
19. Hauer AC, Breese EJ, Walker-Smith JA, MacDonald TT. The frequency of cells secreting interferon-gamma and interleukin-4, -5, and -10 in the blood and duodenal mucosa of children with cow's milk hypersensitivity. *Pediatr Res* 1997;42:629-38.
20. Shiner M, Ballard J, Smith ME. The small-intestinal mucosa in cow's milk allergy. *Lancet* 1975;1:136-4.
21. Dupont C, Heyman M. Food protein-induced enterocolitis syndrome: laboratory perspectives. *J Pediatr Gastroenterol Nutr* 2000;30:S50-7.
22. Dohi T, Fujihashi K, Koga T, Shirai Y, Kawamura YI, Ejima C, et al. T helper type-2 cells induce ileal villous atrophy, goblet cell metaplasia, and wasting disease in T cell-deficient mice. *Gastroenterology* 2003;124:672-82.
23. Forbes E, Smart VE, D'Aprile A, Henry P, Yang M, Matthaie KI, et al. T helper-2 immunity regulates bronchial hyperresponsiveness in eosinophil-associated gastrointestinal disease in mice. *Gastroenterology* 2004;127:105-18.
24. Akdis CA, Blaser K. Histamine in the immune regulation of allergic inflammation. *J Allergy Clin Immunol* 2003;112:15-22.
25. Allam JP, Novak N, Fuchs C, Asen S, Berge S, Appel T, et al. Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcεRI expression. *J Allergy Clin Immunol* 2003;112:141-8.
26. Asai K, Hachimura S, Kimura M, Toraya T, Yamashita M, Nakayama T, et al. T cell hyporesponsiveness induced by oral administration of ovalbumin is associated with impaired NFAT nuclear translocation and p27kip1 degradation. *J Immunol* 2002;169:4723-31.