Serum IgE response to orally ingested antigen: A novel IgE response model with allergen-specific T-cell receptor transgenic mice

Kan Shida,[a,b] Satoshi Hachimura, PhD,[a] Akio Ametani, PhD,[a] Mina Ishimori,[a] Mei Ling,[a] Masaaki Hashiguchi,[a] Yoshihiro Ueda,[a] Takehito Sato, PhD,[c] Yoshihiro Kumagai, PhD,[d] Kotaro Takamizawa, PhD,[b] Sonoko Habu, MD, PhD,[c] and Shuichi Kaminogawa, PhD[a] *Tokyo, Kunitachi, Isehara, and Yokohama, Japan*

Background: The mechanism by which orally ingested allergens elicit an IgE response remains unclear because there are few animal models available for investigation of this response.
Objective: We tried to develop a murine model suitable for investigation of the IgE response to orally ingested allergens, which would allow us to identify T cells that could promote IgE production.
Methods: Ovalbumin (OVA)-specific T-cell receptor transgenic mice were fed a diet containing OVA, and both the serum antibody response and cytokine production by splenocytes were examined.
Results: Oral administration of OVA to transgenic mice led to an increase in the levels of both antigen-specific IgE and total IgE in the sera. Subsequent intravenous challenge of OVA-fed transgenic mice with OVA resulted in anaphylactic shock. Analysis of cytokine production by splenocytes revealed that high IL-4–producing T cells appeared in the spleen 1 week after the start of feeding the OVA diet. T cells from these mice were found to promote IgE secretion by BALB/c B cells in vitro. This helper activity and the levels of IL-4 secretion were diminished after long-term feeding. These findings suggest the possibility that the orally ingested antigen elicited a response by a subpopulation of T cells that produce high levels of T_{H2}-type cytokines and that promote IgE secretion, and these same T cells were tolerized by the orally ingested antigen.
Conclusion: This experimental model with transgenic mice may be a useful tool for further studies of the cellular and molecular mechanisms of the T-cell and IgE responses to orally ingested antigens. (J Allergy Clin Immunol 2000;105:788-95.)

From [a]the Department of Applied Biological Chemistry, The University of Tokyo; [b]the Yakult Central Institute for Microbiological Research; [c]the Department of Immunology, Tokai University School of Medicine; and [d]the Precursory Research for Embryonic Science and Technology, Research Development Corporation of Japan.
Akio Ametani is currently affiliated with the Division of Immune Regulation, La Jolla Institute for Allergy and Immunology; and Yoshihiro Kumagai is currently affiliated with the Department of Microbiology and Immunology, Nippon Medical School.
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Reprint requests: Kan Shida, Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 186-8650, Japan.

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Food allergy is thought to involve allergic reactions that are triggered by aberrant immune responses to orally ingested antigens. Such harmful immunologic reactions are mediated, at least in part, by IgE antibody specific for the food allergen.[1,2] In general, T_{H2} cells and their cytokines, such as IL-4, IL-5, and IL-6, play a critical role in promoting IgE production. On the other hand, IFN-γ secreted by T_{H1} cells can lead to inhibition of IgE production. Thus a polarized T_{H2} response is considered to favor IgE production, which in turn may induce atopic diseases.[3,4] However, the mechanism of IgE production occurring in response to orally ingested antigens is not fully understood. It remains unclear which types of immunocompetent cells are sensitized by orally ingested antigens and where sensitization, IgE class switching, and IgE production occur in allergic patients, although usually tolerance is established to the orally ingested antigen. A suitable experimental animal model would be helpful to examine these problems.

It can be considered to be very difficult for an orally ingested antigen to elicit a specific IgE response because several mechanisms exist to prevent adverse immune responses to the numerous protein antigens present in food. Enzymatic digestion of antigens, the mucosal barrier restricting antigen uptake, and induction of oral immune tolerance are mechanisms that contribute to prevent adverse immune responses.[5,6] Despite such defense mechanisms, a serum antibody response of the IgG class is sometimes elicited by an orally ingested antigen.[7,8] Difficulty encountered in induction of an IgE response may be also due to rare class switching into the ε-chain in B cells[9,10] and negative feedback regulation of IgE synthesis.[11] Nevertheless, recent reports indicate that oral administration of an allergen, in some cases, is effective to induce IgE production. Intragastric challenge of mice with ovalbumin (OVA) when the animals have been previously sensitized intraperitoneally with OVA induces an IgE response.[12] Furthermore, Ito et al[13] have shown that oral

Abbreviations used
OVA: Ovalbumin
TCR: T cell receptor

administration of milk casein as a constituent of the diet to DBA/2 mice elicited an IgE response. Despite the recent advances in IgE-response models, the available models do not seem to be sufficient to allow examination of the critical contribution of T cells in promoting IgE production. According to previous reports, the T-cell response to orally ingested antigen was not strong, which likely indicates difficulty in examining the T-cell response.

In this study, in an effort to develop a novel murine model for examining the IgE response to an orally ingested antigen, we used T-cell receptor (TCR) transgenic mice expressing OVA-specific TCR-αβ. This model should allow us to study both B-cell and T-cell responses to oral antigen. The mice were expected to display not only enhanced T-cell responses but also antibody production in response to orally ingested OVA because the OVA-reactive $CD4^+$ T-cell population is very large in these mice compared with that found in conventional mice. We have developed a novel murine model in which an orally ingested allergen can elicit a specific IgE response, as well as a T-cell response and tolerance. This model is expected to be a useful means to study the basic mechanisms of both the IgE response to orally ingested antigen and food allergy.

METHODS

Animals

Female OVA23-3 mice[14] (7-13 weeks old) transgenic for $OVA_{323-339}$–specific and $I\text{-}A^d$–restricted TCR-αβ (Vα3/Vβ15) with a BALB/c genetic background were used in the experiments. The percentage of transgene-expressing cells among peripheral T cells was greater than 90%. Female BALB/c mice (7-10 weeks old) were purchased from Clea Japan Inc (Tokyo, Japan). All experiments were performed in accordance with the guidelines for the care and use of laboratory animals of The University of Tokyo.

Culture medium

RPMI-1640 medium (Nikken Bio Medical Lab, Kuze, Japan) supplemented with 10% heat-inactivated FCS, 100 U/mL penicillin, and 100 μg/mL streptomycin was used to culture lymphocytes.

Administration of OVA

For oral administration of OVA, mice were fed a pelleted diet containing egg-white protein at the concentration of 20% (OVA diet; Funabashi Farm, Funabashi, Japan). The daily intake of OVA was approximately 250 mg per mouse. For intraperitoneal sensitization to OVA, the mice were intraperitoneally administered 50 μg of OVA adsorbed onto 1 mg of alum in 0.1 mL of sterile saline. The intraperitoneal injection was performed twice with a 2-week interval, and the mice were bled 5 days after the second injection.

Induction of an anaphylactic reaction

To induce an anaphylactic reaction, 1 mg of OVA dissolved in 0.1 mL of PBS was injected into the tail vein of mice fed the OVA diet for various periods. The mice were observed for a 30-minute period after OVA injection. The anaphylactic reaction was evaluated and rated according to 4 scores, as described by Poulsen et al.[15] The scores were as follows: –, no reaction observed; +, the mice are slow and mobile if provoked; ++, the mice are stationary even if provoked; and +++, the mice lie down and have severe anaphylactic shock. The mice with a score of +++ were immediately killed. The experiments were carried out twice (experiment 1 and experiment 2). In experiment 2 serum was collected just before intravenous OVA challenge to analyze the OVA-specific IgE level.

Cell cultures for cytokine production

Splenocytes were isolated as a single-cell suspension from OVA-fed transgenic mice. After depletion of erythrocytes, the cells (2.5×10^6/mL) were cultured with OVA (100 μg/mL) in 1 mL of medium in a 48-well culture plate (Costar, Cambridge, Mass). Supernatants were collected after 44 hours for determination of cytokine levels.

Peyer's patch cells were isolated as a single-cell suspension by means of mechanical dissociation of Peyer's patches in Jaklik-modified minimal essential medium (Sigma, St Louis, Mo). The cells (2.5×10^6/mL) were cultured with OVA (100 μg/mL) in 1 mL of medium in a 48-well culture plate, and supernatants were collected after 44 hours for determination of cytokine levels.

Assay for IgE helper activity

$CD4^+$ T cells were purified from splenocytes of OVA-fed transgenic mice by means of magnetic cell sorting (Miltenyi Biotech GmbH, Bergish Gladbach, Germany) with anti-CD4 beads. B cells were also purified from splenocytes of unprimed BALB/c mice by means of magnetic cell sorting with anti-B220 beads. The purity of each of the cell preparations obtained was more than 97%, as indicated by flow cytometry. The $CD4^+$ T cells (5×10^5/mL) were cultured with BALB/c B cells (2×10^6/mL) and OVA (100 μg/mL) in 1 mL of medium in a 48-well plate. Supernatants were collected on day 7 for the determination of total IgE levels.

ELISA for antibodies

Determination of total and OVA-specific IgE levels was carried out by sandwich ELISA, as described previously.[16] For determining total IgE levels, the wells of a Maxisorp immunoplate (Nunc, Roskilde, Denmark) were coated with rat anti-mouse IgE mAb (Pharmingen, San Diego, Calif; clone R35-92). After blocking the unoccupied sites on the plastic with BSA, the test samples and standard mouse IgE (Pharmingen) were added. Subsequently, bound IgE was detected by sequential incubation with biotinylated rat anti-mouse IgE (Serotec, Oxford, England; clone LO-ME-2), streptavidin-alkaline phosphatase conjugate (Genzyme, Cambridge, Mass), and enzyme substrate (*p*-nitrophenylphosphate). For the assay of OVA-specific IgE, biotinylated OVA (5 μg/mL) was used instead of biotinylated rat anti-mouse IgE antibody as the second antibody. The level of OVA-specific IgE was either expressed as the optical density at 405 nm or expressed as a relative value compared with the value displayed by hyperimmunized mouse serum. The hyperimmunized mouse serum was obtained from transgenic mice sensitized by means of intraperitoneal administration of 3 doses of OVA adsorbed onto alum, and the OVA-specific IgE level of this control serum was arbitrarily taken to be 1000 units/mL. The specific IgE levels were calculated as units per milliliter by comparing the ELISA values obtained for the diluted samples of serum with a standard curve prepared by using control serum. Biotinylation of OVA was performed by using the ECL protein biotinylation module (Amersham, Buckinghamshire, UK).

To assay OVA-specific IgG1, IgG2a, IgA, and IgM, the wells of Maxisorp immunoplates were coated with OVA. After blocking the unoccupied sites on the plastic, the test samples were added. Sub-

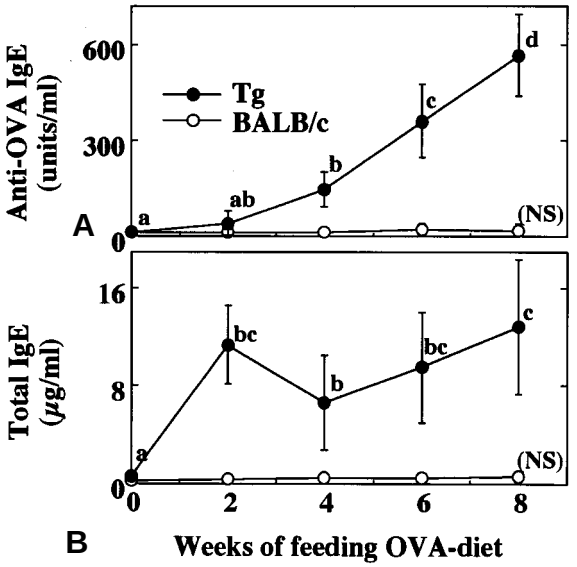

FIG 1. Levels of OVA-specific IgE and total IgE in the sera of transgenic *(Tg)* mice fed the OVA diet. Age-matched transgenic and BALB/c mice were fed the OVA diet for the indicated period, and the levels of OVA-specific IgE **(A)** and total IgE **(B)** in sera were determined by using ELISA. OVA specific IgE level was expressed as relative units compared with hyperimmunized mouse serum (1000 U/mL). Data are expressed as means ± SD of 8 (transgenic) or 6 (BALB/c) mice. Points not sharing a common letter are significantly different. In BALB/c mice ANOVA did not show a significant difference between levels of IgE at each time point. Experiments were repeated 3 times with similar results.

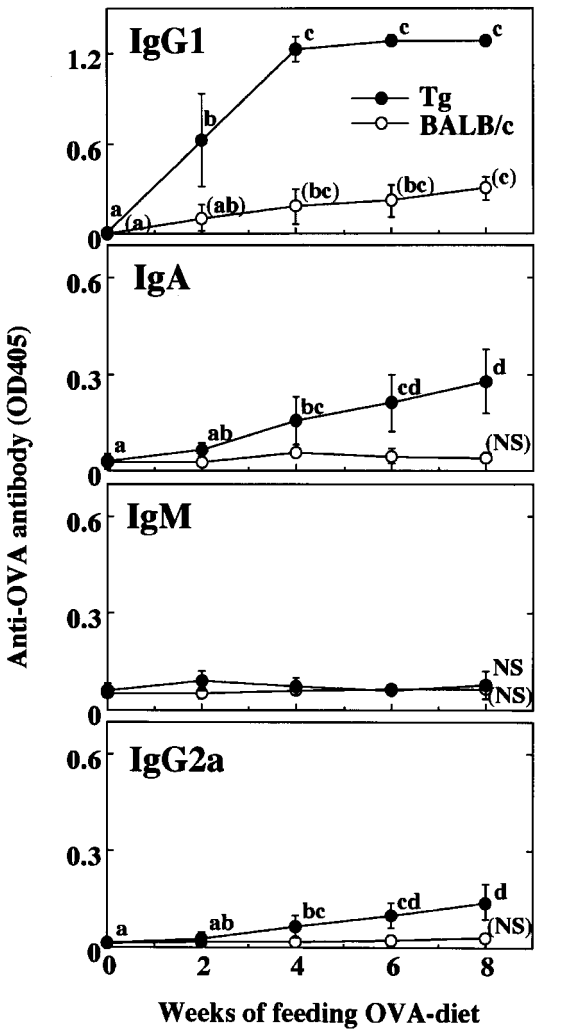

FIG 2. OVA-specific antibody responses in terms of IgG1, IgG2a, IgA, and IgM classes in transgenic *(Tg)* mice fed the OVA diet. Transgenic and BALB/c mice were fed the OVA diet for the indicated period, and OVA-specific antibody levels in sera were determined by using ELISA. OVA-specific antibody level was expressed as optical density at 405 nm. Data are expressed as means ± SD of 8 (transgenic) or 6 (BALB/c) mice. Points not sharing a common letter are significantly different. *NS,* Not significant. Letters in parentheses are for BALB/c mice. Experiments were repeated 3 times with similar results.

sequently, bound antibodies were detected by means of alkaline phosphatase–conjugated anti-mouse IgG1, IgG2a, IgA, and IgM antibodies (Zymed, South San Francisco, Calif). After incubation with the enzyme substrate, the absorbance at 405 nm was measured. The antibody level was expressed as optical density.

ELISA for cytokines

Determination of IFN-γ, IL-2, IL-4, and IL-5 levels was done by using a sandwich ELISA. Rat anti-mouse IFN-γ (clone R4-6A2), IL-2 (clone JES6-1A12), IL-4 (clone BVD4-1D11), and IL-5 (clone TRFK5) mAbs were used as the capture antibody, with biotinylated rat anti-mouse IFN-γ (clone XMG1.2), IL-2 (clone JES6-5H4), IL-4 (clone BVD6-24G2), and IL-5 (clone TRFK4) mAbs, respectively, as the detection antibody. These antibodies, except for R4-6A2 and XMG1.2, were obtained from Pharmingen, and R4-6A2 and XMG1.2 were purified from fluid ascites of mice inoculated intraperitoneally with these B-cell hybridoma clones (gifts of T Tada). Standard recombinant mouse IFN-γ, IL-2, and IL-4 were purchased from Genzyme, and IL-5 was obtained from Pharmingen.

Statistical analysis

Results are expressed as means ± SD. Differences in levels of antibodies or cytokines between time points were analyzed by using 1-way ANOVA followed by the Tukey multiple comparison test where appropriate. The Student *t* test was used to compare the antibody levels between oral and intraperitoneal administration groups. The relationship between the severity of the anaphylactic reaction and the serum IgE level was analyzed by using Spearmen rank correlation. A *P* value of less than .05 was considered significant.

RESULTS

Serum antibody response elicited by the OVA diet

Transgenic mice were orally administered OVA as a constituent of the diet in an attempt to induce a serum IgE response to this antigen. As shown in Fig 1, OVA-specific IgE antibody was first detected in 5 of 8 transgenic mice 2 weeks after the start of feeding the OVA diet, and the IgE levels increased with time according to the length of the feeding period. The pattern of the increase in total IgE was different from that of specific IgE. A high level of total IgE could be detected even at week 2, and this continued during the period of OVA feeding. No increase

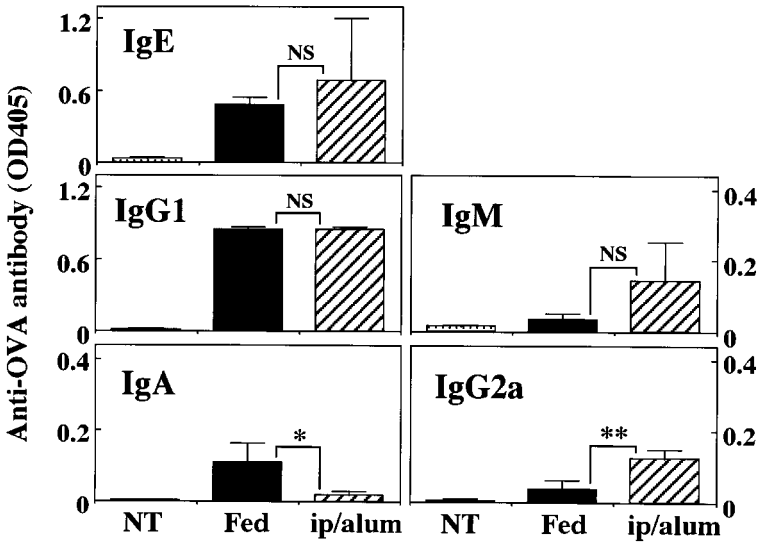

FIG 3. Comparison of the antibody response elicited by oral feeding of OVA and that elicited by intraperitoneal injection of OVA. Transgenic mice were either fed the OVA diet for 5 weeks *(Fed)* or immunized with OVA adsorbed onto alum intraperitoneally twice with a 2-week interval *(ip/alum)*. The sera were collected and assayed for OVA-specific antibody by using ELISA. OVA-specific antibody level was expressed as optical density at 405 nm. Data are expressed as means ± SD of 4 mice. Experiments were repeated twice with similar results. *NT,* not treated; *NS,* not significant. *$P < .05$; **$P < .01$.

TABLE I. Induction of an anaphylactic reaction by intravenous injection of OVA

Mice	Weeks of feeding OVA diet	No. of mice showing anaphylactic score*			
		–	+	++	+++
OVA transgenic mice	0	9	0	0	0
	2	8	1	0	0
	5	0	2	6	1
	8	0	0	1	8
BALB/c mice	8	9	0	0	0

*Mice fed the OVA diet for various periods were challenged intravenously with OVA, and the anaphylactic reaction observed was evaluated. The extent of anaphylaxis was rated according to 4 scores, as described in the "Methods" section. Experiments were repeated twice (experiment 1 and experiment 2), and data contains results of both experiments.

in levels of specific and total IgE was observed in nontransgenic BALB/c mice, which have the same genetic background as the transgenic mice.

The serum levels of OVA-specific antibodies of other classes were examined (Fig 2). A considerable amount of OVA-specific IgG1 antibody appeared at week 2, and the level of IgG1 remained high after week 4. IgA and IgG2a responses were also induced, although those of IgM were not. The response pattern of each antibody isotype reflected a T_{H2}-type immune response predominantly, rather than a T_{H1}-type response. In the case of nontransgenic BALB/c mice, a low level of only the IgG1 subclass was detected.

Comparison of the antibody response elicited by oral administration and that elicited by intraperitoneal injection

The antibody response to orally administered OVA was compared with that elicited by intraperitoneal injection of OVA plus alum, an adjuvant well known to be effective for promotion of IgE production. As shown in Fig 3, immunization by either of these two routes elicited comparable levels of IgE and IgG1 antibodies. An IgA response was observed more prominently in the case of oral administration of OVA, whereas a stronger IgG2a response was observed in the case of intraperitoneal administration. The difference in IgM antibody levels was not significant.

Anaphylactic reaction induced by subsequent intravenous injection of OVA

OVA was administered intravenously to OVA-fed transgenic mice in an effort to induce anaphylaxis. As shown in Table I, intravenous challenge with OVA led to an anaphylactic reaction in all transgenic mice fed OVA for more than 5 weeks, and severe anaphylactic shock with labored breathing was observed in some transgenic mice fed OVA for 8 weeks. However, no anaphylactic reaction was observed in transgenic mice not fed OVA or fed OVA for 2 weeks, except for one mouse, which

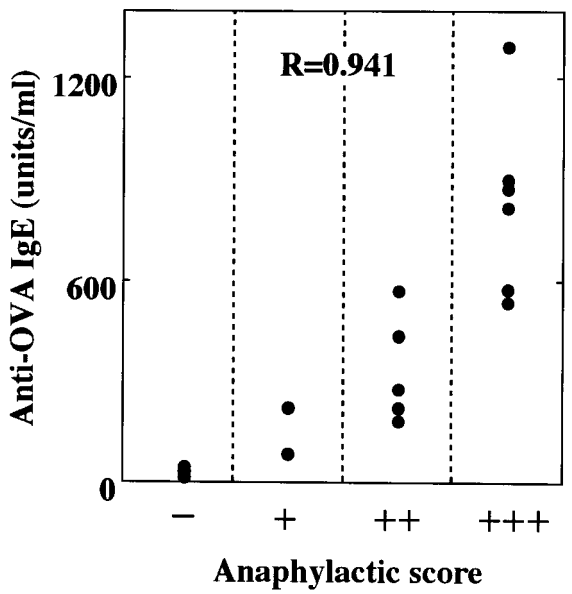

FIG 4. Correlation between anaphylactic reactions and serum OVA-specific IgE level. Anaphylactic scores and serum OVA-specific IgE levels of individual mice (n = 30) in experiment 2 of Table I are plotted. The Spearmen *R* value is shown ($P < .001$).

showed a weak anaphylactic reaction (+) with a detectable amount of OVA-specific IgE (85 U/mL; Table I and Fig 4). BALB/c mice fed OVA for 8 weeks showed no anaphylaxis. There was a correlation between the severity of the anaphylactic reaction and the serum levels of OVA-specific IgE (Fig 4). Also, there was a correlation between the serum levels of OVA-specific IgG1 and the anaphylaxis scores, with the Spearman *R* value being 0.877 (data not shown). Because anaphylaxis was not induced in OVA-fed BALB/c mice, which showed a specific IgG1 response but no specific IgE response, these data may suggest that specific IgE in transgenic mice was related to anaphylaxis. However, the possibility still remains that the stronger specific IgG1 response in those transgenic mice than in the BALB/c mice contributed to induce anaphylaxis.[17] Oral OVA challenge, instead of intravenous challenge, was not effective to induce an anaphylactic reaction (data not shown). It seems that the amount of intact OVA absorbed into the body from the intestine may be insufficient to elicit an anaphylactic reaction.

Cytokine production by splenocytes from OVA-fed transgenic mice

We examined the levels of cytokine production by spleen lymphocytes obtained from OVA-fed transgenic mice. The spleen is considered to be a key lymphoid organ involved in controlling the systemic response and the serum antibody response. As shown in Fig 5, the level of IL-4 production 1 week after the start of OVA feeding was 3 times greater than that observed in the case of splenocytes from mice not fed OVA. IL-5 was detected only in cultures of splenocytes obtained from the mice after 1 week of feeding. The enhancement of IFN-γ production at week 1 was not significant, and instead the levels decreased according to the length of the feeding period. IL-2 production rapidly decreased as a result of OVA feeding. The levels of cytokine production by purified splenic T cells were similar to those observed in the case of whole splenocytes (data not shown). These results indicate that T cells showing a high level of IL-4 production and a moderate level of IL-5 production appeared in the spleen 1 week after the start of feeding the OVA diet. In the case of BALB/c mice, no cytokines were detected in the cultures of splenocytes at any time point (data not shown).

Helper activity of splenic T cells from OVA-fed transgenic mice in promoting IgE production

It has been shown that T_{H2} cells showing high levels of IL-4 and IL-5 production are effective in helping B cells to produce IgE.[3] We examined whether T cells from the spleen of OVA-fed transgenic mice can provide help for IgE production. Splenic T cells prepared from OVA-fed transgenic mice were cultured with B cells ($B220^+$ cells) from unsensitized BALB/c mice in the presence of OVA, and IgE in the culture supernatant was assayed. The results showed that only the T cells from transgenic mice fed OVA for 1 week efficiently provided help for IgE production, whereas T cells from mice not fed OVA and those from mice fed OVA for 2 or 4 weeks did not (Fig 6).

Cytokine secretion by Peyer's patch cells from OVA-fed transgenic mice

Because an orally ingested antigen first encounters the mucosal immune system, cytokine production by Peyer's patch cells responding to OVA was investigated. The results of ELISA examining the supernatants of the cultures indicated that the levels of both IL-4 and IL-5 secretion by Peyer's patch cells were transiently elevated 1 week after the start of OVA feeding (Fig 7), which was similar to the findings obtained with splenocytes. A much larger amount of IL-5 was secreted by Peyer's patch cells (1547 ± 183 pg/mL) than by splenocytes (35 ± 6 pg/mL), whereas the level of secretion of IL-4 was much lower (70 ± 18 pg/mL for Peyer's patch cells and 469 ± 73 pg/mL for splenocytes). At this 1-week time point, the level of IFN-γ secretion also was substantially elevated in Peyer's patch cells, unlike the findings obtained with splenocytes. The level of IL-2 production was slightly elevated 1 week after the start of OVA feeding but subsequently decreased.

DISCUSSION

Oral administration of OVA, a major allergen in egg,[1] to transgenic mice expressing TCR specific for a dominant T-cell determinant peptide (residues 325-334) of OVA elicited an antigen-specific serum IgE response. The anti-OVA IgE antibody level was comparable with that found in mice intraperitoneally administered OVA with alum, an adjuvant

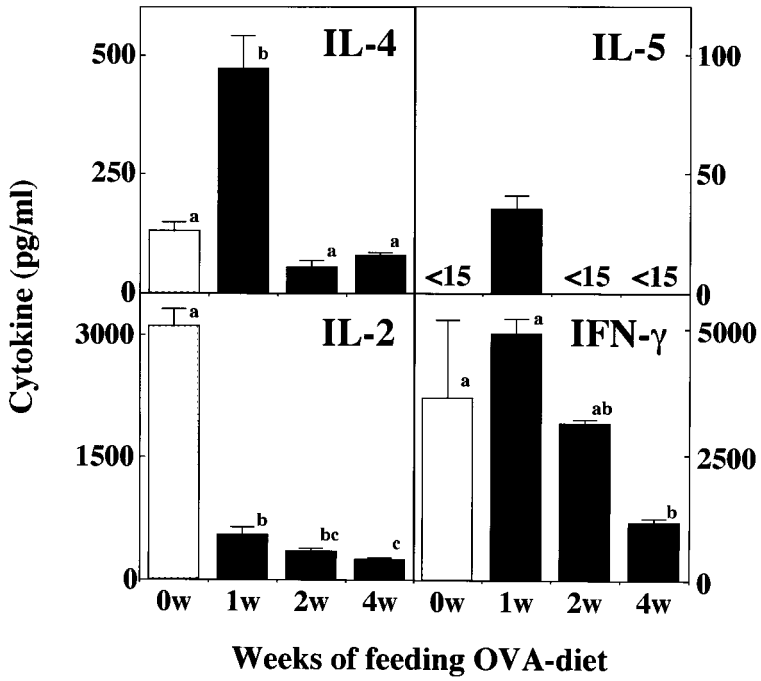

FIG 5. Cytokine production by splenocytes from transgenic mice fed the OVA diet. Splenocytes from transgenic mice fed the OVA diet for various periods were cultured with OVA for 44 hours, and the levels of cytokines in the culture supernatants were determined by using ELISA. Data are expressed as means ± SD of 3 independent spleens of transgenic mice. *Bars* not sharing a common letter are significantly different. Experiments were repeated 3 times with similar results.

known to promote IgE production. The response pattern in terms of antibody isotypes suggests that a T_{H2}-type immune response was predominantly elicited in the transgenic mice fed OVA. In addition, subsequent intravenous challenge with OVA led to severe anaphylactic reactions. Such responses were not elicited in nontransgenic BALB/c mice, which have the same genetic background as the transgenic mice. These results indicate that induction of the antigen-specific IgE response was due to the presence of a substantial population of OVA-specific T cells in these mice.

This system with TCR transgenic mice allows us to investigate the response of T cells and their contribution to the IgE production, which was different from the system of Ito et al.[13] T cells showing a high level of IL-4 production appeared in the spleen of transgenic mice fed OVA for 1 week, and these T cells were shown to have the ability to promote IgE secretion by B cells in vitro. Because nonfractionated $B220^+$ cells from unsensitized mice were used in these experiments, and because total IgE levels were measured, we cannot specify whether these T cells induced complete B-cell development and IgE secretion through cognate T cell-B cell interaction in vitro or whether the T cells enhanced IgE secretion by $B220^+$ cells that had already developed in vivo through bystander activation by cytokines in the cultures. However, at least these results suggest that splenic T cells showing a high level of IL-4 production were effective in providing in vitro helping activity for IgE secretion.

Several reports have indicated that orally ingested antigens can induce a state of immunologic unresponsiveness

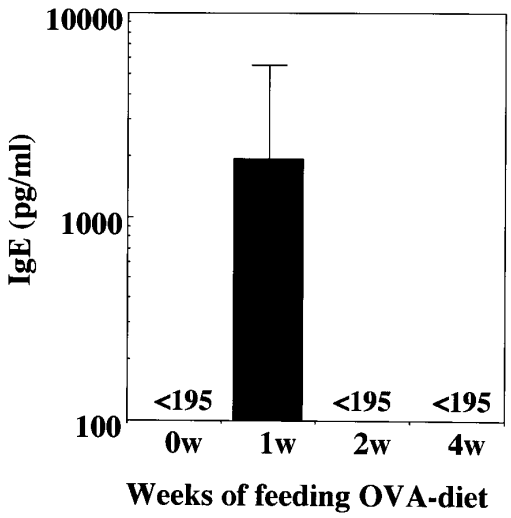

FIG 6. Helper activity of splenic T cells from OVA-fed transgenic mice in promoting IgE production. $CD4^+$ T cells were prepared from pooled splenocytes of 3 transgenic mice fed the OVA diet for various periods, and the isolated cells were cultured with naive B cells from unsensitized BALB/c mice in the presence of OVA for 7 days. The supernatants were assayed for total IgE levels by using ELISA. Data are expressed as means ± SD of 3 independent cultures. Experiments were repeated twice with similar results.

to subsequent antigen stimulation. It has been observed that in addition to downregulation of T_{H1}-driven cellular immunity, T_{H2}-driven systemic antibody responses become inhibited when higher doses of antigen are

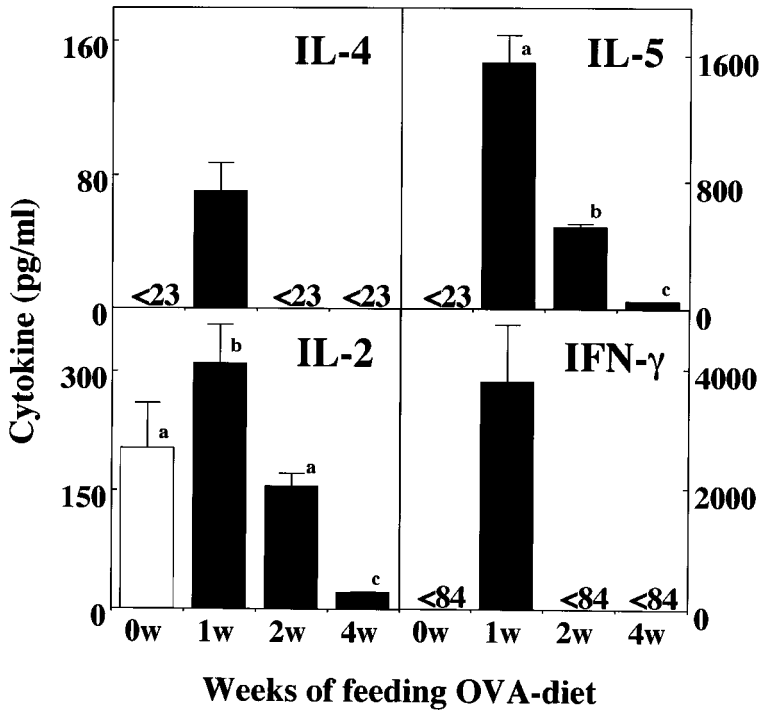

FIG 7. Cytokine production by Peyer's patch cells from transgenic mice fed the OVA diet. Peyer's patch cells were prepared from pooled Peyer's patches of 3 transgenic mice fed the OVA diet for various periods, and the isolated cells were cultured with OVA for 44 hours. The supernatants were assayed for cytokine levels by using ELISA. Data are expressed as means ± SD of 3 independent cultures. *Bars* not sharing a common letter are significantly different. Experiments were repeated 3 times with similar results.

fed.[18,19] In our system with transgenic mice, cytokine production by splenic T cells was diminished after feeding the OVA diet to the mice for more than 2 weeks. Proliferation of splenic T cells cultured in the presence of OVA was also diminished 4 weeks after OVA feeding (S. Hachimura, and S. Kaminogawa, unpublished observation). These findings suggest that T-cell tolerance ensued for both T_{H1} and T_{H2} cells after a longer period of feeding in the transgenic mice. Some or most of the T cells in an unusually large population of transgenic T cells were primed to secrete high levels of T_{H2}-type cytokines and may be responsible for development of the IgE antibody response. These T cells appear to be later tolerized by continuous feeding. Indeed, studies in human subjects, as well as those in experimental animals, have shown an enhanced T-cell response preceding the induction of tolerance.[19-21] Although further studies are necessary to clarify the relationship between the induction of T-cell tolerance and the development of a strong antibody response in these mice, our results suggest that aberrant responses in the induction phase of oral tolerance may be related to the onset of food allergy. A study examining the dependence of T cell and antibody responses on the dose of antigen fed is now in progress in our laboratory.

Although our data indicate that T cells primed by fed antigen were later tolerized, serum OVA-specific IgE levels increased during the study period until week 8. The half-life of IgE in serum is reported to be about one half of a day.[22] Thus the cells responsible for sustaining a high serum IgE level in the latter part of the study period may have been different from T cells. Basophils and mast cells can produce IL-4 and IL-5, and this is triggered by crosslinking of the Fcε receptor with IgE.[23,24] Thus once a large number of B cells has developed into plasma cells producing IgE with the aid of helper T cells, IgE production may not be easily terminated in the presence of help by lymphoid cells other than T cells. Recently, Manz et al[25] and Slifka et al[26] have shown that antigen-specific IgG-secreting plasma cells can survive and secrete antibodies for more than 3 months or 1 year. Thus plasma cells may continue to secrete antibody for a longer period than memory cells survive, although the previously recognized immunologic idea holds that plasma cells survive for a period ranging from only a few days to at most a few weeks. In addition, the long-lived plasma cells persistently secrete antibodies in the absence of continuous antigen stimulation. Considering these findings, we think that the OVA-specific IgE-secreting plasma cells that develop as a result of OVA feeding in our transgenic mouse system may be able to continue to secrete IgE without T-cell help, even after T-cell tolerance has developed.

In our experimental system the OVA fed induced an increase in the levels of total IgE in serum, and this may result from bystander activation of IgE production. A high level of total IgE was observed even at week 2, whereas only a very low level of specific IgE was detected at the same time point. B cells, which had already been sensitized by some antigens, may develop, expand, and secrete IgE in serum earlier than newly developing OVA-specific IgE-producing cells in response to the T_{H2}-type cytokines secreted by OVA-specific T cells. Indeed, the total IgE level in normal serum (at week 0) of transgenic mice (582 ± 126 ng/mL) was higher than that found in BALB/c mice (277 ± 136 ng/mL), which suggests that in the transgenic mice some B cells had developed into IgE-secreting cells even before feeding the OVA diet. In a study of patients with allergies, it has been pointed out that a high level of IgE specific for a food allergen is a risk factor for later allergic sensitization to allergens other than those in food.[27] Although the timing is different between OVA-fed transgenic mice and patients with food allergy, the mechanism of bystander activation of nonspecific IgE production may be the same in both systems. Thus our OVA-fed transgenic mouse system may be a good model for studying the mechanisms of food allergy in human subjects, collectively considering that (1) the antigen fed induced specific serum IgE, (2) T cells were tolerized after helping IgE production, and (3) the antigen fed induced bystander IgE production.

In assays of Peyer's patch cells, high levels of cytokine production were observed 1 week after the start of OVA feeding (Fig 7). However, the pattern of cytokine secretion of Peyer's patch cells differed somewhat from that of splenocytes. The ratio of IL-5/IL-4 secretion at week 1 was 22 for Peyer's patch cells, whereas it was 0.075 for splenocytes. The role of IL-5 has been discussed in terms of the development of IgA-secreting plasma cells in Peyer's patches.[28] In this transgenic mouse model, a considerable amount of OVA-specific IgA was found in the feces after feeding the OVA diet (K. Shida, S. Hachimura, and S. Kaminogawa, unpublished observation), and this may relate to the high level of IL-5 production in the Peyer's patches.

The novel IgE-responsive model shown in this article is expected to be a powerful tool for examining the molecular and cellular mechanisms of IgE production in response to oral antigen exposure. We also hope that this model will facilitate both basic studies on food allergy and the development of a novel therapy for this disease.

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