

Oral administration of a dominant T-cell determinant peptide inhibits allergen-specific T_{H1} and T_{H2} cell responses in Cry j 2-primed mice

Kazuki Hirahara, MS,^a Saburo Saito, MD,^b Nobufusa Serizawa, PhD,^a Reiko Sasaki, MD,^c Masahiro Sakaguchi, PhD,^c Sakae Inouye, MD,^c Yoshifumi Taniguchi, ME,^d Shuichi Kaminogawa, PhD,^e and Akio Shiraishi, PhD^a *Tokyo and Okayama, Japan*

Background: Oral immunotherapy with a peptide for allergic immune responses is theoretically a promising therapy but has not been established yet.

Objective: To evaluate immune suppressive efficacy of oral administration of an immunodominant peptide, we investigated changes in T-cell proliferation, T_{H1}- and T_{H2}-cytokine production, and T_{H1}- and T_{H2}-mediated antibody production in mice after oral administration of a peptide.

Methods: Peptide p246-259, containing a dominant T-cell determinant of Cry j 2, which is the major allergen in Japanese cedar pollen, was used in this study. Groups of mice received p246-259 or PBS alone before or after they were primed intranasally with Cry j 2 and cholera toxin. In another experiment mice were primed intraperitoneally with Cry j 2 and alum. Proliferative response and cytokine production by nasal-associated lymph node cells against Cry j 2 were investigated. Amounts of systemic anti-Cry j 2 IgE and IgG antibodies were also measured.

Results: Oral administration of the peptide to mice before, or even after, the sensitization induced oral tolerance in T-cell responses against the allergen; the tolerance was associated with decreased production of T_{H1} (IFN- γ and IL-2) and T_{H2} (IL-4) cytokines. Allergen-specific T_{H1}-mediated (IgG2a and IgG2b) and T_{H2}-mediated (IgG1 and IgE) antibody responses were also inhibited.

Conclusions: Oral administration of a dominant T-cell determinant peptide induces immunologic tolerance in both T_{H1} and T_{H2} cell responses against the whole protein allergen. Our study is the first, to our knowledge, to demonstrate the potential for peptide-based oral immunotherapy in order to treat allergic immune responses. (*J Allergy Clin Immunol* 1998;102:961-7.)

Key words: Japanese cedar pollen, pollinosis, oral immunotherapy, oral tolerance, peptide, T cell epitope, T_{H1}/T_{H2}, IgE

Japanese cedar (*Cryptomeria japonica*) pollinosis is one of the most common immediate-type allergic dis-

Abbreviations used

CTX: Cholera toxin
CTB: B subunit of cholera toxin
LNC: Lymph node cell
PCA: Passive cutaneous anaphylaxis

eases in Japan, with more than 10% of the population being affected. At present, histamine receptor antagonists have mainly been used for the treatment of this disease; however, application of immunotherapy toward cedar pollinosis has not yet been established. Two major allergens were isolated from Japanese cedar pollen and identified as Cry j 1^{1,2} and Cry j 2.³⁻⁵ Patients with pollinosis exhibit both high IgE titers⁶ and T-cell reactivity⁷ against Cry j 1, Cry j 2, or both. Immunopathologic studies have revealed that T cells, especially T_{H2} cells, are involved in a wide range of allergic responses, including activation of inflammatory cells and production of allergen-specific IgE antibodies. Regulation of these T cells is potentially a fundamental therapeutic approach to cure allergic diseases.

Therapeutic administration of soluble allergenic extracts has been widely applied to allergic diseases, including pollinosis, for a number of years and is regarded as one of the therapies for modulating immune responses, although the immunologic mechanisms operating in this therapy have not been well elucidated. Recently, several new approaches for allergen immunotherapy to theoretically regulate T-cell reactivity have been examined. The principle approach is oral immunotherapy, which is based on the phenomenon of oral tolerance.^{8,9} Oral tolerance is characterized by systemic immunologic unresponsiveness against orally administered antigens, in which T-cell reactivity is profoundly suppressed by various mechanisms, such as clonal deletion,¹⁰ clonal anergy,¹¹⁻¹³ and active suppression.¹²⁻¹⁵ Clinical efficacy of oral immunotherapy with mite extract to asthmatic patients was recently reported.¹⁶ In the report the inhibition of T_{H2} cell-mediated responses was demonstrated. The second approach is a peptide-based immunotherapy,^{17,18} in which an immunodominant T-cell determinant from allergens is used. Peptides, which do not contain an IgE-binding region, have

From ^aSankyo Co, Ltd, Tokyo; ^bThe Jikei University School of Medicine, Tokyo; ^cNational Institute of Infectious Diseases, Tokyo; ^dHayashibara Biochemical Labs, Inc, Okayama; and ^eThe University of Tokyo, Tokyo. Received for publication Nov 10, 1997; revised May 28, 1998; accepted for publication July 17, 1998.

Reprint requests: Akio Shiraishi, PhD, Biological Research Laboratories, Sankyo Co, Ltd, 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan.

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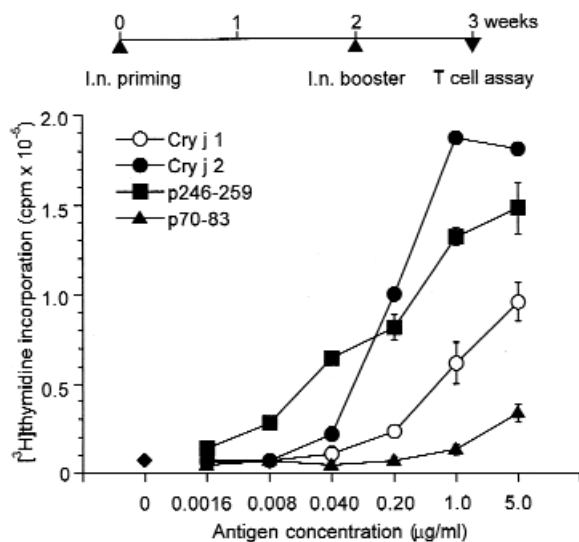


FIG 1. Proliferative responses of nasally-associated lymph node cells against Cry j 1, Cry j 2, and T-cell determinant peptides of Cry j 2. Results are indicated as mean $\pm$ SD of cpm. *I.n.*, Intranasal.

lower allergenicity compared with whole protein allergens, providing safe and stable tolerogens for the treatment. Tolerogenic behavior of a peptide on both T-cell and antibody responses against whole protein allergen was reported in animals¹⁹⁻²¹; encouraging results on its efficacy in humans are also being reported.^{22,23} The combined methods of these 2 approaches (ie, oral immunotherapy with a peptide) is thought to be more feasible for clinical application. However, the method of oral immunotherapy with a dominant T-cell determinant peptide for T_H2 -mediated allergic immune responses has not yet been examined.

We previously examined the T-cell determinants of Cry j 1 and Cry j 2 in several strains of mice and found that p246-259 of Cry j 2 is an immunodominant T-cell determinant in BALB/c mice. In this report we investigated whether oral administration of the T-cell determinant peptide would induce immunologic tolerance in allergic immune responses against native protein allergen by using pollinosis-model mice that were either intranasally primed with the allergen mixed with cholera toxin (CTX), which is known to be a mucosal adjuvant²⁴ to induce T_H2 cell responses preferentially,²⁵ or intraperitoneally primed with the allergen mixed with alum, which is known to be an adjuvant to induce strong systemic IgE response. Oral administration of the peptide to naive and allergen-sensitized mice induced oral tolerance in T-cell responses against the allergen, which was associated with decreased production of T_H1 - and T_H2 -type cytokines. In addition, feeding the peptide also inhibited allergen-specific T_H1 - and T_H2 -type antibody responses. These results indicate the potential for therapeutic efficacy of oral immunotherapy with a T-cell determinant peptide towards allergic immune responses in pollinosis.

METHODS

Mice

Six-week-old female BALB/c mice were purchased from Charles River Japan (Tokyo, Japan) and housed under conventional conditions. Experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in Sankyo.

Antigens and reagents

Cry j 1 and Cry j 2 proteins were isolated from Japanese cedar pollen by affinity chromatography with mAbs specific for either Cry j 1 (065)²⁶ or Cry j 2 (N26).²⁷ Synthetic peptides p70-83 (HFT-FKVDGIIAAYQ) and p246-259 (RAEVSYPVHVNGAKF), based on the Cry j 2 sequence, were synthesized by using standard F-moc chemistry and purified by HPLC. CTX and its B subunit (CTB) were obtained from Sigma Chemical (St Louis, Mo).

Immunization

To induce a T-cell response in nasally-associated lymph node cells (LNCs), mice were injected intranasally with 1 μ g of Cry j 2 alone or combined with 1 μ g of Cry j 1. The allergen or allergens were mixed with 1 μ g of CTB and 5 ng of CTX as a mucosal adjuvant, and all antigens were dissolved in 6 μ L of PBS. Two weeks later, the mice were boosted intranasally with the same antigens. To develop antibody responses, mice were injected intranasally with Cry j 2 and the CTX adjuvant once a week for 6 weeks. One week after the last injection, the mice were bled from the tail vein. In another experiment, mice were injected intraperitoneally with 10 μ g of Cry j 2 and 2 mg of alum in 0.2 mL of PBS. Three weeks later, the mice were boosted intraperitoneally with 1 μ g of Cry j 2 and 2 mg of alum. Two weeks after the booster, the mice were bled from the tail vein. These sera were pooled as a group and kept at 4°C until antibody titers were measured.

Induction of oral tolerance

Mice were fed 200 μ g of p246-259 or p70-83 dissolved in 0.2 mL PBS by gastric intubation with a plastic animal-feeding needle. Mice were fed the peptide 4 times at intervals of 3 or 4 days, with the last feeding 3 days before immunization. To examine the inhibitory effect of feeding peptide on ongoing T-cell responses, the mice were immunized intranasally with Cry j 2 and subsequently fed with p246-259, with the first day of feeding being 7 days after the immunization. After mice were fed p246-259 4 times as described above, they were boosted intranasally with Cry j 2.

Culture medium

LNCs were cultured in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with antibiotics, namely 100 U/mL penicillin and 100 μ g/mL streptomycin (Life Technologies) and also 5×10^{-5} mol/L 2-mercaptoethanol (Life Technologies) and 1% autologous normal mouse serum.

T-cell proliferation assay

Seven days after the intranasal booster, cervical LNCs were removed, and single-cell suspensions were prepared. An erythrocyte-depleted, X-ray irradiated spleen cell suspension was prepared from normal BALB/c mice and used as antigen-presenting cells. LNCs and antigen-presenting cells were cultured at 3×10^5 and 6×10^5 cells/well, respectively, in 96-well flat bottom plates with or without antigens (1 μ g/mL unless otherwise stated) for 2 days at 37°C. Each well was then pulsed with 0.5 μ Ci of [³H]-thymidine (Amersham Japan, Tokyo, Japan), and the cells were harvested 18 hours later. The radioactivity was measured by means of the Beta-plate system (Pharmacia Biotech, Tokyo, Japan), with each sample being prepared in triplicate. The results are indicated as stimulation indices, which were calculated as follows: mean cpm in the presence of antigen divided by the mean cpm in the absence of antigen.

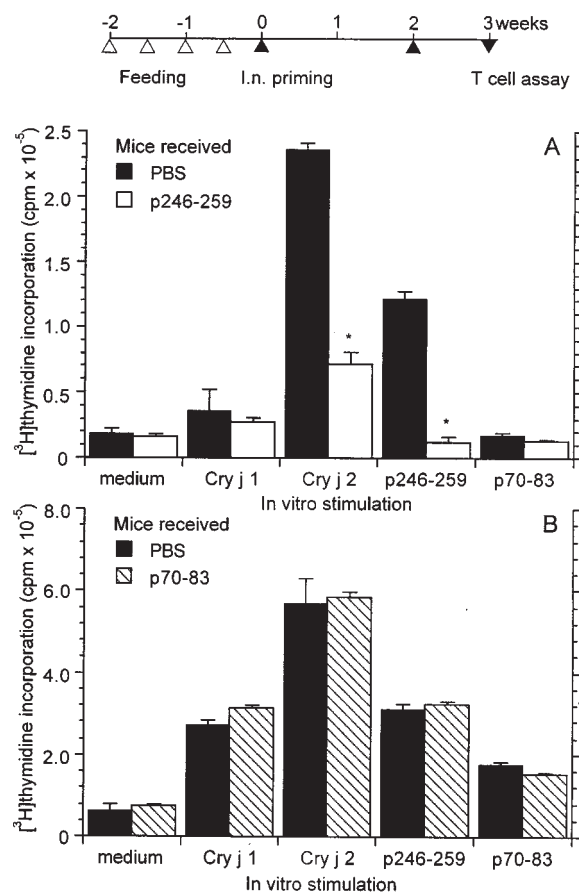


FIG 2. Proliferative responses of LNCs from mice fed with p246-259 (A) or p70-83 (B) before being primed intranasally (*i.n.*) with Cry j 1 and Cry j 2. Results are indicated as mean $\pm$ SD of cpm. * P < .01 versus control group of mice.

Cytokine assay

LNCs and antigen-presenting cells were cultured at 1.5×10^6 and 3×10^6 cells/well, respectively, in 24-well flat bottom plates with or without 1 μ g/mL of antigens for 24, 48, and 72 hours at 37°C, with 72 hours being optimal for the measurement of any cytokines. Cell-free supernatants were collected and kept at -20°C until assayed. Concentrations of IFN- γ , IL-2, and IL-4 in the samples were measured by specific ELISAs, according to the manufacturer's instructions (Endogen, Boston, Mass).

ELISA

Antigen-specific IgE antibody titers were measured by IgE capture ELISA as previously described,²⁸ with several modifications. Microtiter plates, which were coated with anti-mouse IgE (Yamasa, Chiba, Japan) and blocked with BSA, were incubated overnight with adequately diluted samples at 4°C. After washing, 0.25 μ g/mL of biotinylated Cry j 2 was added to each well for further incubation for 1 hour at room temperature. The plates were washed and treated with Europium-labeled streptavidine (Pharmacia) for 1 hour. The fluorescence intensity was measured by a microplate fluorescence reader (Fluorometer, Pharmacia). The passive cutaneous anaphylaxis (PCA) titer of anti-Cry j 2 IgE was calculated on a standard curve generated by using a reference serum with a known PCA titer.

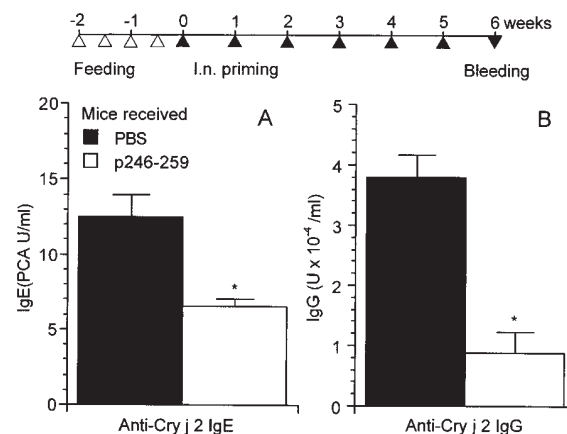


FIG 3. Inhibited anti-Cry j 2 IgE (A) and IgG (B) antibody responses in mice fed with p246-259 before being primed intranasally (*i.n.*) with Cry j 2. Data are presented as mean $\pm$ SD. * P < .01 versus control group of mice.

Antigen-specific IgG, IgG1, IgG2a, and IgG2b antibody titers were measured by an indirect ELISA. Microplates, which were coated with Cry j 2 (1 μ g/mL), were blocked with BSA, and each serum sample was added to wells and incubated for 2 hours at room temperature. After the plates were washed, alkaline phosphatase-conjugated isotype-specific antibody was added to the wells. The bound antibodies were detected by adding substrate solution, and the resulting color development was measured by absorbance at 405 nm. The relative unit of each isotype was determined by a 5-fold serial dilution of the serum.

Statistics

Statistical significance was determined by Student's *t* test and analysis of variance.

RESULTS

Intranasal injection of Cry j 1 and Cry j 2 with CTX induced a proliferative response in nasally associated lymph node cells

Because of the lack of a good experimental model for cedar pollinosis, we first tried to establish a murine pollinosis model in which mice were primed intranasally with allergens from cedar pollen to induce allergic immune responses. To prime mice through their nasal mucosa, CTB with a trace amount of CTX was used as a mucosal adjuvant. Mice were injected intranasally with Cry j 1 and Cry j 2 mixed with CTB twice at an interval of 2 weeks. One week after the second injection, cervical LNCs were harvested, and the proliferative response of the cells was analyzed. LNCs were stimulated *in vitro* with various concentrations of Cry j 1, Cry j 2, p246-259, and P70-83, with the latter being dominant and subdominant T-cell determinant peptides from Cry j 2, respectively. The LNCs proliferated similarly against Cry j 2 and p246-259, with a lower response against Cry j 1 (Fig 1). The cells did not proliferate well against p70-83, which shows p246-259 is substantially a unique T-cell determinant in Cry j 2.

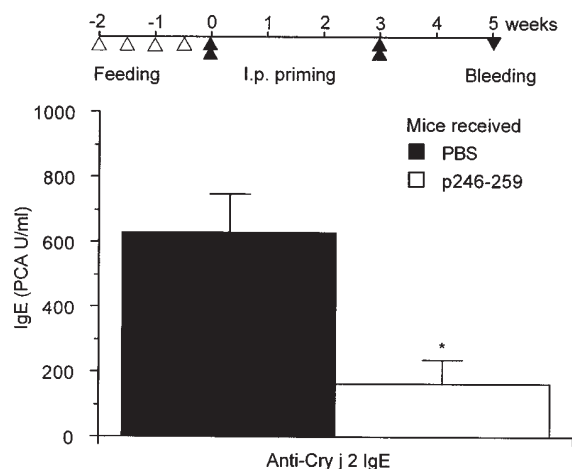


FIG 4. Inhibited serum anti-Cry j 2 IgE antibody response in mice fed with p246-259 before being primed intraperitoneally (*i.p.*) with Cry j 2 and alum. Data are presented as mean $\pm$ SD. * $P < .01$ versus control group of mice.

Thus intranasal administration of the pollen allergens was effective in inducing a proliferative response of the nasally associated LNCs, and a murine pollinosis model suitable for analysis of T-cell responses was established.

Oral administration of p246-259 induced antigen-specific oral tolerance in T-cell responses against Cry j 2

Next we investigated whether oral administration of the dominant T-cell determinant peptide would induce immunologic tolerance in T-cell responses against the whole protein allergen. Groups of mice received 200 μ g of p246-259 or PBS alone 4 times during a period of 2 weeks and were subsequently primed intranasally with Cry j 1 and Cry j 2. After boosting the mice with the same antigens, LNCs from both groups were stimulated *in vitro* with Cry j 1, Cry j 2, p246-259, or p70-83. Oral treatment with p246-259 greatly suppressed the proliferative response of LNCs (90% suppression, $P < .01$) against the peptide, and the response against Cry j 2 was also significantly inhibited by oral administration of p246-259 (70% suppression, $P < .01$) (Fig 2, A). The tolerant state was antigen-specific because response against Cry j 1 was not inhibited (Fig 2, A), which was confirmed by repeating the test. In comparison, these responses were not inhibited by feeding p70-83 (Fig 2, B). Thus oral administration of p246-259 induced the immunologic tolerance in T-cell responses against Cry j 2.

Oral administration of p246-259 inhibited the production of both T_{H1} - and T_{H2} -associated lymphokines

The effects of feeding peptide on cytokine production by the LNCs were examined next. After mice received

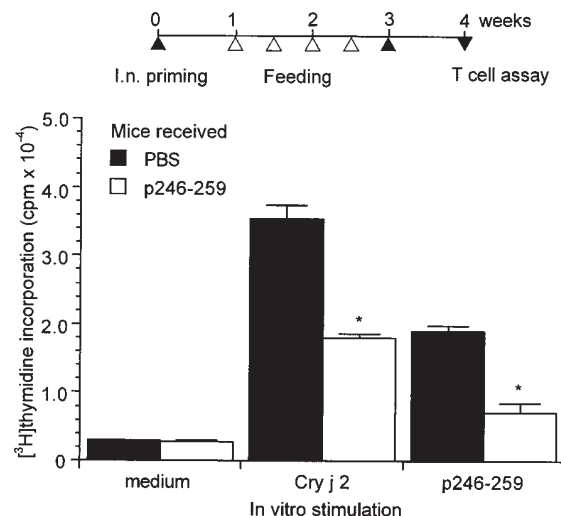


FIG 5. Inhibited proliferative response of LNCs from mice fed with p246-259 after being primed intranasally (*i.n.*) with Cry j 2. Results are indicated as mean $\pm$ SD of cpm. * $P < .01$ versus control group of mice.

p246-259 or PBS and were subsequently primed intranasally with Cry j 2, LNCs from these mice were stimulated *in vitro* with or without Cry j 2, and the amount of T_{H1} (IFN- γ and IL-2) and T_{H2} (IL-4) cell-associated cytokines in the culture supernatants was measured by specific ELISA; results are shown in Table I. LNCs from control mice produced large amounts of IFN- γ and IL-2. In contrast, LNCs from p246-259-fed mice produced significantly lower amounts of IFN- γ and IL-2, indicating that the T_{H1} -type cytokine response was rendered immunologically tolerant by feeding the peptide. In addition, the LNCs from PBS-fed mice produced a low but significant amount of IL-4; however, the cells from p246-259-fed mice did not produce detectable levels of IL-4, which indicated that T_{H2} -type responses were also rendered tolerant. Thus oral administration of p246-259 inhibited both T_{H1} - and T_{H2} -associated responses against Cry j 2.

Oral administration of p246-259 induced immunologic tolerance in anti-Cry j 2 IgE and IgG responses

Next we examined whether oral administration of the dominant T-cell determinant peptide would induce immunologic tolerance on allergen-specific IgE and IgG responses. At the time when T-cell responses were examined, IgE levels in the serum were undetectable (<0.1 PCA U/mL, data not shown). Repeated intranasal administration to mice, however, elicited significant allergen-specific IgE and IgG responses, indicating that a pollinosis model suitable for analysis of allergic antibody responses had been established. After groups of mice received p246-259 or PBS and were subsequently primed

TABLE I. Inhibited cytokine responses against Cry j 2 by oral administration of p246-259

Feeding	In vitro stimulation	Concentration of cytokines (pg/mL)		
		IL-2	IFN- γ	IL-4
PBS	Cry j 2	618 $\pm$ 71	1520 $\pm$ 300	8.7 $\pm$ 0.8
p246-259	Cry j 2	237 $\pm$ 0.6*	<50	<1.0

*P < .01 versus PBS-fed controls.

intranasally with Cry j 2 several times, the mice were bled, and anti-Cry j 2 IgE and IgG levels in the sera were measured by ELISA. Oral administration of p246-259 significantly decreased anti-Cry j 2 IgE antibody responses elicited by repeated intranasal priming (48% suppression, P < .01) (Fig 3, A). To confirm the suppressive effect of the peptide on the IgE response, mice were intraperitoneally injected with Cry j 2 and alum to develop hyper-IgE responses after they were fed with p246-259 or PBS, which revealed that feeding the peptide also significantly decreased strong anti-Cry j 2 IgE responses (74% suppression, P < .01) (Fig 4).

The anti-Cry j 2 IgG antibody response was also decreased by feeding the peptide (77% suppression, P < .01) (Fig 3, B). We then determined whether anti-Cry j 2 IgG1, IgG2a, and IgG2b antibody responses were inhibited by the oral administration of p246-259. The concentration of the specific antibody for each isotype was measured by ELISA, and the geometric mean of each relative unit is indicated in Table II. The anti-Cry j 2 antibody responses of IgG2a, IgG2b (T_{H1} -mediated), and IgG1 (T_{H2} -mediated) in p246-259-fed mice were all inhibited when compared with those in the control mice (71%, 84%, and 68% suppression, respectively; P < .05 in all isotypes). From these results, oral administration of the dominant T-cell determinant peptide induced oral tolerance in IgE and IgG responses, inhibiting both T_{H1} - and T_{H2} -mediated antibody responses.

Oral administration of p246-259 to sensitized mice inhibited ongoing T-cell responses against Cry j 2

It is quite important that feeding a tolerogenic peptide has suppressive efficacy on ongoing immune responses in the case of clinical application. We assessed whether oral administration of p246-259 to mice after they were sensitized with Cry j 2 would induce oral tolerance. Animals were primed intranasally with Cry j 2. Seven days later, when significant proliferative responses of LNCs were already developed (data not shown), the mice received p246-259 or PBS by gastric intubation. After they were boosted intranasally with the antigen, LNCs were stimulated in vitro with p246-259 or Cry j 2. The proliferative responses of LNCs against Cry j 2 and p246-259 were inhibited by the oral administration of the peptide (49% and 62% suppression, respectively; P < .01 in both) (Fig

TABLE II. Inhibited all anti-Cry j 2 antibody isotype productions by oral administration of p246-259

Feeding	Intranasal priming	Mean concentration of anti-Cry j 2 antibody (U/mL)		
		IgG1	IgG2a	IgG2b
PBS	Cry j 2	7500	1200	330
p246-259	Cry j 2	2200*	190*	105*

*P < .05 versus PBS-fed controls.

5). The results indicated the potential benefit of oral immunotherapy with a T-cell determinant peptide for allergic immune responses in already sensitized patients with pollinosis.

DISCUSSION

Oral immunotherapy with a peptide is theoretically a promising method to cure the fundamental cause of allergic responses by modulating pathogenic T-cell responses because oral tolerance is well known to induce profound antigen-specific unresponsiveness in pathogenic T cells and because the T cell-determinant peptide, which lacks an IgE-binding region, has lower allergenicity than the whole protein allergen. In addition, oral administration is safer and more practical than the standard subcutaneous administration, which sometimes evokes an adverse reaction and is accompanied by pain. In this study we demonstrated the efficacy of oral treatment with an immunodominant peptide on allergic immune responses by using the pollinosis mouse model.

The lack of a suitable animal model for allergic diseases is one reason for our uncertainty as to the efficacy of immunotherapy for allergic diseases and for the lack of information on mechanisms operating in allergen immunotherapy. We have shown here that intranasal administration of a pollen allergen with a mucosal adjuvant induced T-cell activation in nasally associated lymph node cells and that repeated priming elicited a systemic IgE antibody response, which reflects the route of sensitization and, in some part, the allergic immune responses in humans. Histologic analysis of the nasal mucosa revealed that weak acute inflammation was developed after 2 injections of the allergens and CTX adjuvant (data not shown). However, the inflammation was not developed by injection of allergen alone, which indicated the inflammation was not an allergen-specific but rather an adjuvant-mediated reaction. Thus the histologic analysis was not useful for the detection of immune suppression in this case.

We investigated the efficacy of peptide-based oral immunotherapy, focusing on allergen-specific immune responses. We showed that the oral administration of p246-259 to mice before they were sensitized with Cry j 1 and Cry j 2 induced diminished proliferative responses of T cells against Cry j 2 but not against Cry j 1. This antigen-specific T-cell unresponsiveness was not induced by p70-83, a weak T-cell determinant peptide. These results

indicate that the pathogenic peptide itself can be a tolerogenic peptide and support the theory that T-cell dominance is correlated with the ability to induce immunologic tolerance. Our data suggested that anergy was induced in p246-259-specific T cells. Although precise mechanisms of oral tolerance have not been elucidated yet, we showed the effective induction of tolerance by using a single dominant T-cell determinant peptide. We also showed that feeding p246-259 significantly decreased the proliferation of T cells against Cry j 2 even after the mice were sensitized with the allergen, which is encouraging for the clinical application of peptide-based oral immunotherapy.

A notable finding of our study is that the mucosal administration of the antigen inhibited immune responses induced by mucosal challenge with the same antigen. It has been well documented that oral administration of antigen induces immunologic unresponsiveness subsequent to the parenterally and nonmucosally induced immune responses, such as responses against the autoantigens. However, before our study, it was not evident whether oral tolerance was effective on mucosally induced immune responses, which were common in allergy to inhalant allergens. We showed that feeding peptide inhibited the immune responses developed by the nasal route. The results support the potential of oral immunotherapy toward mucosally induced allergic diseases like pollinosis.

To elucidate the mechanism operating in T-cell unresponsiveness, cytokine productions by nasally-associated LNCs were determined. The data indicated that both T_{H1} (IFN- γ and IL-2) and T_{H2} (IL-4) cytokine responses against Cry j 2 decreased after oral treatment with the peptide. Marked reduction of proliferative and IL-2 responses of LNCs suggested that clonal anergy was induced in T_{H1} cells, which is supported by previous reports demonstrating that T_{H1} cells are selectively anergized in states of oral tolerance¹¹ with intact or enhanced responses of T_{H2} cells.²⁹ The inhibited IgG2a and IgG2b antibody responses against Cry j 2 also indicated that T_{H1} cells are induced into a state of tolerance.

On the other hand, it is of note that the T_{H2} response was also decreased by feeding the peptide. Although the concentration of IL-4 was relatively low when T-cell responses were examined, the inhibitory effect of the peptide on both anti-Cry j 2 IgG1 and IgE antibody responses indicates that T_{H2} cells were rendered tolerant by administering the dominant T-cell determinant peptide. It was difficult to tolerize against T_{H2} -type responses systemically, although this was achieved by feeding large doses of antigen.^{29,30} Melamed et al²⁹ demonstrated that a large amount of antigen, 4 mg of ovalbumin per day for at least 20 days, had to be fed to mice to induce tolerance in T_{H2} cells. Our results seem to argue against their finding because in our study feeding mice with 0.2 mg of peptide per day 4 times during a 2-week period induced tolerance in T_{H2} cells. Feeding mice with 0.04 mg of peptide for 4 days also induced tolerance, but feeding 0.008 mg of peptide for 4 days did not (data not shown). The difference in dose required to induce T_{H2}

cell tolerance may be related to the affinity of antigen to the major histocompatibility complex molecule because optimal concentration of p246-259 to stimulate T cells in vitro was 1 μ g/mL (Fig 1), whereas that for ovalbumin was 100 μ g/mL.²⁹ We previously reported that an intradermal administration of a dominant T-cell determinant peptide from milk allergen suppressed both T_{H1} - and T_{H2} -type antibody responses.¹⁹ This, taken together with the above information, led us to believe that tolerance in T_{H2} cells may depend on the experimental system and on the nature of the tolerogen.

Results obtained from cytokine and antibody isotype analysis indicated that both T_{H1} - and T_{H2} -type responses were inhibited in the state of oral tolerance. Furthermore, inhibition of ongoing T-cell response was also demonstrated. The greatly inhibited immune responses suggest that some mechanisms actively suppressing the function of pathogenic T cells are involved in oral tolerance. We are now investigating the contribution of mucosal immunity to the immune suppression because a number of new types of cells, such as $\gamma\delta$ T cells³¹⁻³⁴ and T_{H3} cells,^{9,15} generated in the mucosal immune system have been found to mediate immune suppression in oral tolerance. Although precise mechanisms remain to be elucidated, our results demonstrated the profound antigen-specific immunosuppressive feature of peptides administered by the oral route.

In conclusion, oral administration of an allergen-derived dominant T-cell determinant peptide induced immunologic tolerance in both T_{H1} and T_{H2} cell responses against the whole protein allergen and resulted in an inhibited allergen-specific IgE and IgG antibody response. These results indicate the potential and notable efficacy of allergen-derived T-cell determinant peptide for peptide-based immunotherapy in T_{H1} - and T_{H2} -mediated allergic immune responses. Because T-cell determinants of Cry j 1³⁵ and Cry j 2³⁶ in patients with pollinosis were recently reported, our results open the way for the clinical application of oral immunotherapy with those peptides to patients with pollinosis.

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