

Immunostimulatory oligodeoxynucleotide induces T_{H1} immune response and inhibition of IgE antibody production to cedar pollen allergens in mice

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Background: Immunotherapy for cedar pollinosis makes use of multiple injections of allergens, but its effectiveness remains controversial. Recent studies indicate that immunization with certain protein antigens and immunostimulatory DNA sequence (ISS) oligodeoxynucleotides (ODNs) represent a potential approach to allergen-specific immunotherapy. **Objective:** We determined whether the coadministration of 2 major protein allergens, Cry j 1 and Cry j 2, of Japanese cedar pollen and ISS-ODN (5'-TGACTCTGAACGTTTCGAGATGA-3') improves the immune responses induced by protein allergens in BALB/c mice.

Methods: Mice were primed intradermally with allergens or ISS-ODN in saline solution and boosted with allergens in alum, and other mice were primed with allergens in alum and boosted with allergens/ISS-ODN. Allergen-specific IgG2a and IgG1 antibody responses were measured by means of ELISA in sera after ODN injection, and allergen-specific IgE antibody production was measured by the passive cutaneous anaphylaxis reaction. IFN- γ and IL-4 releases were also measured by ELISA in the supernatants of allergen-stimulated spleen cells. **Results:** The coadministration of allergens/ISS-ODN increased IgG2a titers and IFN- γ release in both groups of mice, whereas it decreased IgG1 titers and IL-4 release in comparison with control mice injected with allergens/mutant ODN. The coadministration additionally inhibited IgE antibody production.

Conclusion: The data demonstrate that the coadministration of cedar pollen allergens and ISS-ODNs before secondary T_{H2} and IgE responses or during ongoing primary T_{H2} and IgE responses brings about a T_{H1} -shifted immune response and inhibition of IgE antibody production, suggesting that this coadministration strategy may provide a novel type of immunotherapy for cedar pollinosis. (*J Allergy Clin Immunol* 1999;1231-8.)

Key words: Cedar pollen allergen, immunostimulatory DNA sequence, oligodeoxynucleotide, IFN- γ , IL-4, IgE, IgG2a, IgG1, T_{H1} response, T_{H2} response

Abbreviations used

CPES: Cedar pollen extract standard
ISS: Immunostimulatory DNA sequence
ODN: Oligodeoxynucleotide
PCA: Passive cutaneous anaphylaxis

Japanese cedar (*Sugi*; *Cryptomeria japonica*) pollinosis is one of the most important type I allergic diseases in Japan. The number of patients with this disease is on the increase, and more than 10% of the population in some areas is reported to be affected.¹ For cedar pollinosis, chemotherapy with antiallergic, antihistaminic, and steroid drugs, as well as hyposensitization by allergen injection, have been used. An effective cedar pollinosis treatment, however, has not been established. Allergic diseases are caused by the overproduction of T_{H2} cytokines by allergen-specific CD4⁺ T cells, followed by an IgE-induced response by mast cells.² Optimal treatment of allergic diseases requires that the T_{H2} cytokine profile of allergen-specific cells be converted into a T_{H1} cytokine profile.

Intradermal or intramuscular vaccination with naked plasmid DNA stimulates immune responses with a T_{H1} bias, with the expansion of CD4⁺ cells producing IFN- γ and cytotoxic CD8⁺ T cells, although gene vaccines stimulate antibody production inefficiently when compared with matching protein vaccines.³⁻⁷ The T_{H1} response to gene vaccination is maximal when the plasmid DNA backbone contains short (6 bp) immunostimulatory DNA sequences (ISSs) with CpG motifs.⁸ Also, the coadministration of certain protein antigens/ISS-oligodeoxynucleotide (ODN) can induce a T_{H1} immune response achieved by immunization of a plasmid DNA-encoding antigen gene with the high levels of antibody generated by protein vaccines.^{9,10} Experiments were therefore designed to determine whether this coadministration strategy can successfully improve the T_{H2} immune response induced by 2 major protein allergens, Cry j 1 and Cry j 2, of Japanese cedar pollen in mice. The data showed that the coadministration of cedar pollen allergens and ISS-ODN brings about a T_{H1} immune response and inhibition of IgE antibody production in both mice with secondary T_{H2} and IgE responses and mice with ongoing primary T_{H2} and IgE responses.

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METHODS

Reagents

Cedar pollen extract standard (CPES; containing 10,000 Japanese allergen units/12.5 µg/mL Cry j 1 and almost the same amount of Cry j 2 in 50% glycerin and 5% NaCl) was donated by the Japanese Society of Allergology. First, the excess glycerin and NaCl were removed and replaced with the appropriate solvent by dialysis and ultrafiltration with Amicon centrifugal filter devices (Amicon Inc), and then the CPES was filtered through a sterilized 0.2-µm filter unit and used as cedar pollen allergen in the present study. Phosphorothionate, single-stranded ODNs (ISS-ODN: 5'-TGACTGTGAACGTTCCGAGATGA-3'; mutant ODN: 5'-TGACTGTGAA GGTAGAGATGA-3')⁹ were purchased from Bex. Before injection, ODNs were precipitated in 100% ethanol, washed in 70% ethanol, and dissolved in saline solution. HRP-labeled anti-mouse IgG1 and IgG2a antibodies were purchased from Zymed, and mouse recombinant IL-4 and mouse recombinant IFN-γ were from Petro Tech EC Ltd. Biotinylated anti-mouse IL-4 mAb (BVD6-24G2) and anti-mouse IFN-γ mAb (XMG1.2) were purchased from PharMingen, and IFN-γ mAb (XMG1.2) was biotinylated in our laboratory. Ascites containing anti-IL-4 mAb (11B11)¹¹ were kindly donated by Dr N. Shinohara (Kitasato University, Sagamihara, Japan). Anti-mouse IFN-γ mAb (R46A2) was prepared in our laboratory.

Immunization protocols

Male 6- to 10-week-old BALB/c mice were purchased from Charles River Japan and subjected to immunization. CPES (containing 2 µg each of Cry j 1 and Cry j 2) dissolved in 400 µL of saline solution containing 4 mg of alum (CPES in alum) or CPES (containing 2 µg each of Cry j 1 and Cry j 2) dissolved in 200 µL of saline solution emulsified with the same volume of CFA was injected intraperitoneally 2 times at 2 weeks apart. Four weeks after the booster injection, the blood and spleens were removed. CPES (containing 2 µg each of Cry j 1 and Cry j 2) dissolved in 50 µL of saline solution containing 50 µg of ISS-ODN or mutant ODN (CPES-ODN) was injected intradermally at the base of the tail in 3 different ways: CPES-ODN in saline solution alone, CPES-ODN in saline solution 2 weeks after intraperitoneal administration of CPES in alum, and intraperitoneal administration of CPES in alum 2 weeks after CPES-ODN in saline solution. Separately, ISS-ODN (50 µg) without CPES in 50 µL of saline was injected intradermally 2 weeks before or after the immunization with CPES in alum administered intraperitoneally. The spleens were removed, and blood was collected at various times.

Determination of IgG1 and IgG2a antibody titers

The titers of CPES-specific IgG1 and IgG2a in sera were measured by means of ELISA with antibodies specific for each subclass. Briefly, ELISA plates were coated with CPES in coating buffer (2.5 µg/mL each of Cry j 1 and Cry j 2). After coating, serial samples of 2-fold diluted sera were added to the plates and incubated overnight at 4°C. After washing, HRP-labeled anti-mouse IgG1 or IgG2a was added, and the samples were incubated for 1 hour at 4°C. After additional washing, *o*-phenylenediamine substrate was added and developed, and the OD was measured at 450 nm. Antiserum pooled from CPES in alum-immunized mice was used as a standard to quantify IgG1, and the IgG1 titer of serum diluted 1:10,000 was defined as 1 U/mL. Antiserum pooled from CPES in CFA-immunized mice was used as a standard to quantify IgG2a, and the IgG2a titer of serum diluted 1:50 was defined as 1 U/mL.

Determination of IFN-γ and IL-4 release by in vitro CPES-stimulated splenocytes

Single spleen cell suspensions were cultivated in RPMI-1640 medium containing 10% FCS, 1 mmol/L sodium pyruvate, 10⁻⁵ mol/L 2-mercaptoethanol, and 100 µg/mL kanamycin at 2 × 10⁶ cells per 800 µL with CPES (containing 2 µg each of Cry j 1 and Cry j 2) for 72 hours. The quantities of IFN-γ and IL-4 in the culture supernatants were determined by means of sandwich ELISA with mAbs specific for each cytokine. The mAbs for coating the plates and the biotinylated second mAbs were as follows: anti-mouse IFN-γ (R46A2) and biotinylated anti-mouse IFN-γ (XMG1.2) for IFN-γ and anti-mouse IL-4 (11B11) and biotinylated anti-mouse IL-4 (BVD6.24G2) for IL-4. Standard curves were generated by using recombinant cytokines. The lower limit of detection was 0.4 ng/mL for IFN-γ and 78 pg/mL for IL-4.

Passive cutaneous anaphylaxis reactions

Passive cutaneous anaphylaxis (PCA) reactions were performed to measure IgE antibody production essentially according to the procedure described by Ovary et al.¹² Briefly, 0.1-mL aliquots of serially 5-fold diluted sera were injected intradermally in the back skin of adult male Sprague-Dawley rats (Japan SLC, Inc). After 24 hours, 100 µL of CPES in saline solution (containing 0.2 µg each of Cry j 1 and 2) was injected intradermally at the same point at which the serum was injected, and then 2 mL of 0.5% Evans Blue solution was injected intravenously. PCA reactions were evaluated 45 minutes after the antigen. The reciprocal of the last dilution eliciting a reaction was taken as the PCA unit of the antiserum.

Statistics

The Student *t* test was used to determine statistical differences between the CPES/ISS-ODN and CPES/mutant ODN groups.

RESULTS

IgG2a and IgG1 responses

The IgG2a and IgG1 responses to CPES in mice immunized with CPES/ODN in saline solution and CPES in alum combinations are shown in Figs 1 and 2, respectively. Mice immunized with CPES/mutant ODN produced very low titers of IgG2a and high titers of IgG1 4 and 6 weeks after injection (Figs 1, A, and 2, A). Mice immunized intradermally with CPES/ISS-ODN produced higher titers of IgG2a and lower titers of IgG1 than mutant ODN-immunized mice at 4 and 6 weeks (Figs 1, A, and 2, A).

To determine the effects of ODN on IgG subclass responses to secondary CPES immunization, CPES/ODN in intradermal saline-primed mice was injected intraperitoneally with CPES in alum 2 weeks after the primary injection. Mice primed with CPES/mutant ODN generated low titers of IgG2a and high titers of IgG1, whereas mice primed with CPES/ISS-ODN generated higher titers of IgG2a and lower titers of IgG1 than mice primed with CPES/mutant ODN at 2, 4, and 6 weeks (Figs 1, B, and 2, B). Mice primed with ISS-ODN showed no IgG2a response at 2 or 4 weeks and no IgG1 response at 2 weeks but a comparable IgG1 response to mice primed with CPES/ISS-ODN at 4 weeks (Figs 1, B, and 2, B).

To determine the effect of ODN on the ongoing primary IgG1 response, mice primed intraperitoneally with

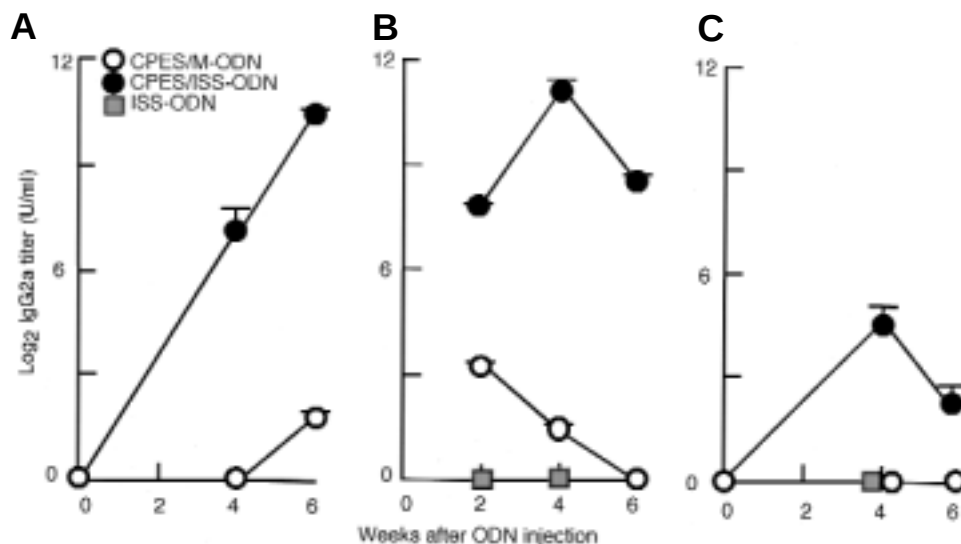


FIG 1. IgG2a responses to CPES in mice immunized with CPES/ODN and CPES combinations. The IgG2a titer was measured by means of ELISA. Primary responses of mice to CPES/ODN administered intradermally in saline solution (**A**), responses to a secondary intraperitoneal immunization with CPES in alum of mice primed intradermally with CPES/ODN or ISS-ODN in saline solution (**B**), and responses to secondary CPES/ODN or ISS-ODN in saline solution injected intradermally into mice primed intraperitoneally with CPES in alum (**C**) are shown. Data are means $\pm$ SEM of 4 mice per group. The differences in the log₂ IgG2a titers between CPES/M-ODN- and CPES/ISS-ODN-injected mice at 2, 4, and 6 weeks were statistically significant ($P < .01$).

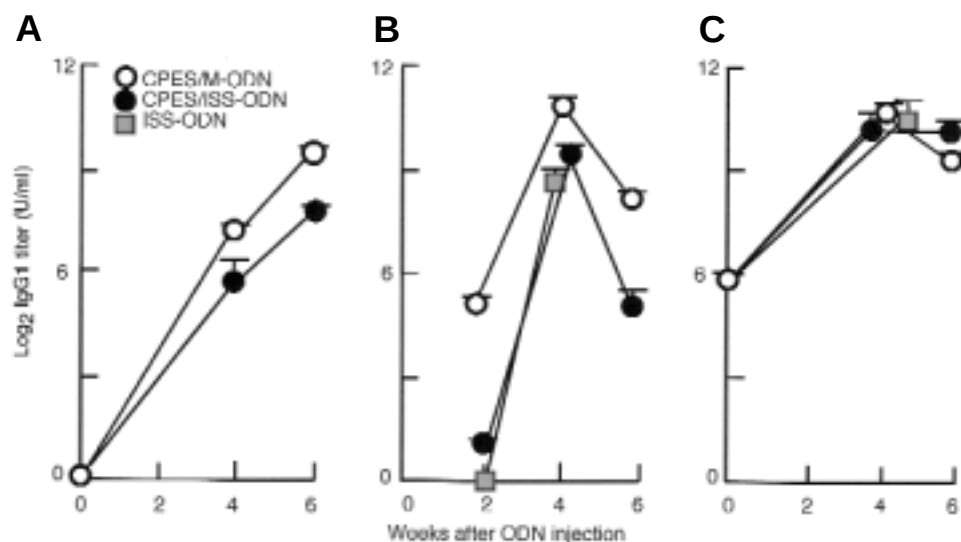


FIG 2. IgG1 responses to CPES in mice immunized with CPES/ODN and CPES combinations. The IgG1 titer was measured by means of ELISA. Primary responses of mice to CPES/ODN administered intradermally in saline solution (**A**), responses to a secondary intraperitoneal immunization with CPES in alum of mice primed intradermally with CPES/ODN or ISS-ODN in saline solution (**B**), and responses to secondary CPES/ODN or ISS-ODN in saline solution injected intradermally into mice primed intraperitoneally with CPES in alum (**C**) are shown. Data are means $\pm$ SEM of 4 mice per group. The differences in the log₂ IgG1 titers between CPES/M-ODN- and CPES/ISS-ODN-injected mice at 2, 4, and 6 weeks were statistically significant ($P < .05$), except for at 4 weeks in **C**.

CPES in alum were injected intradermally with CPES/ODN in saline 2 weeks after the primary injection. Mice injected with CPES/mutant ODN generated very small or undetectable amounts of IgG2a and high titers of IgG1. A secondary CPES/ISS-ODN injection induced an increase in IgG2a antibody production at 4

and 6 weeks, but the titers were not as high as those in CPES/ISS-ODN-primed mice (Fig 1, A, B, and C). At 4 weeks, CPES/ISS-ODN injection did not affect the IgG1 response, but at 6 weeks, the response was increased compared with CPES/mutant ODN injection (Fig 2, C). A secondary ISS-ODN injection did not

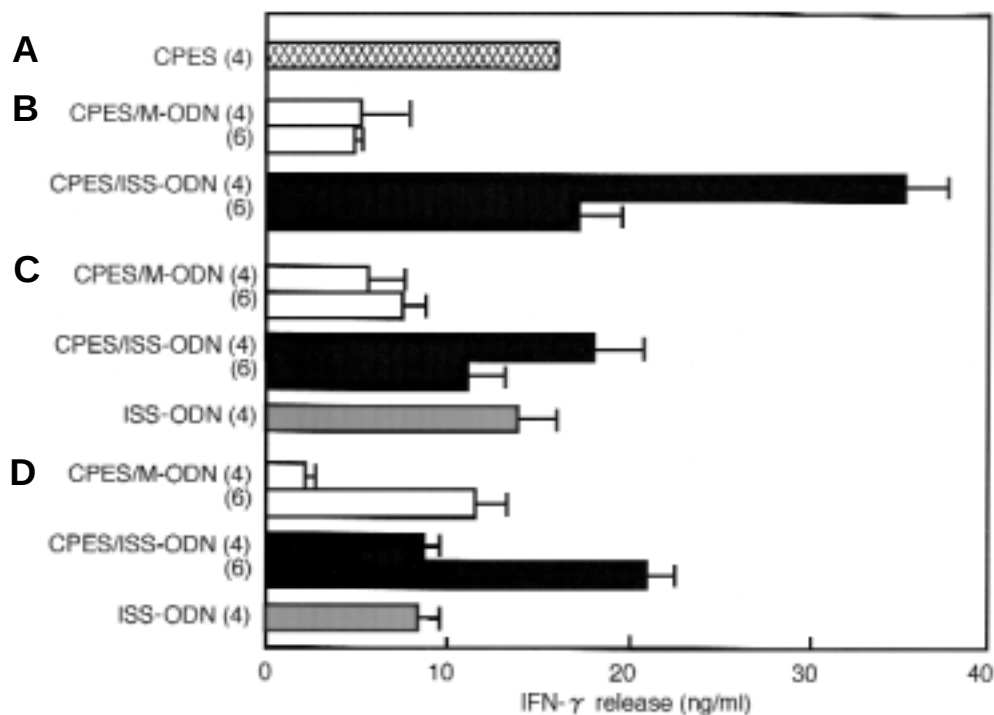


FIG 3. IFN- γ release from in vitro CPES-stimulated splenocytes from mice immunized with CPES/ODN and CPES combinations. IFN- γ release was measured by ELISA. Responses to CPES in CFA administered intraperitoneally twice at 2 weeks apart (**A**), primary responses of mice to CPES/ODN in saline solution (**B**), responses of mice primed intradermally with CPES/ODN or ISS-ODN in saline solution to a secondary intraperitoneal immunization with CPES in alum (**C**), and responses to a secondary CPES/ODN or ISS-ODN in saline solution injected intradermally into mice primed intraperitoneally with CPES in alum (**D**) are shown. The numbers in parentheses are weeks after the booster injection in **A** and after ODN injection in **B**, **C**, and **D**. Data are means $\pm$ SEM of 4 mice per group, except for a mean of 3 determinations in **A**. The differences in IFN- γ release between CPES/mutant (M) ODN- and CPES/ISS-ODN-injected mice at 4 and 6 weeks (**B**, **C**, and **D**, except for at 6 weeks in **C**) were statistically significant ($P < .05$).

affect the IgG2a response at 4 weeks and induced the same IgG1 response as CPES/ISS-ODN injection at 4 weeks (Figs 1, C, and 2, C). In many mice immunized with CPES and CPES/ODN combinations, IgG2a and IgG1 responses at 6 weeks were lower than those at 4 weeks (Figs 1, B and C, and 2, B). This might be caused by the downregulation of effector cells, such as antigen-specific T cells, on the basis of the disappearance of allergen over time, but this remains obscure.

IFN- γ and IL-4 release

Because IgG2a antibody production is typical of a T_H1 response and IgG1 antibody production results from a T_H2 immune response, these subclass profiles suggest that the coadministration of protein allergen and ISS-ODN induces different T_H -cell responses to the same antigen. Next we determined the in vitro CPES-stimulated release of IFN- γ and IL-4 by splenocytes from mice immunized with CPES/ODN in saline solution and CPES in alum combinations 4 and 6 weeks after ODN injection. Splenocytes from CPES in CFA-immunized mice released 16.1 ng/mL IFN- γ , whereas splenocytes from CPES in alum-immunized mice released 709 pg/mL IL-4 (Figs 3, A, and 4, A). Splenocytes from

CPES/mutant ODN-immunized mice released small amounts of IFN- γ and very small or undetectable amounts of IL-4 (Figs 3, B, and 4, B). Cells from mice immunized with CPES/ISS-ODN released large amounts of IFN- γ , especially at 4 weeks (Fig 3, B), but almost no IL-4 (Fig 4, B).

In splenocytes from mice primed with CPES/ODN in saline solution and boosted with CPES in alum, the CPES/ISS-ODN groups showed stronger IFN- γ responses than the CPES/mutant ODN groups, which showed weak IFN- γ responses, and the effect of CPES/ISS-ODN was notable at 4 weeks (Fig 3, C). In contrast, the CPES/ISS-ODN groups showed weaker IL-4 responses than the CPES/mutant ODN groups at 4 and 6 weeks, which showed strong IL-4 responses (Fig 4, C). The ISS-ODN group showed a weaker IFN- γ response and stronger IL-4 response than the CPES/ISS-ODN group at 4 weeks (Figs 3, C, and 4, C).

Splenocytes from CPES in alum-primed and CPES/ISS-ODN in saline-boosted mice showed stronger IFN- γ responses than those from CPES/mutant ODN-boosted mice, which showed a weak IFN- γ response (Fig 3, D). The cells from CPES in alum-primed and CPES/ISS-ODN-boosted mice showed the suppression of the IL-4

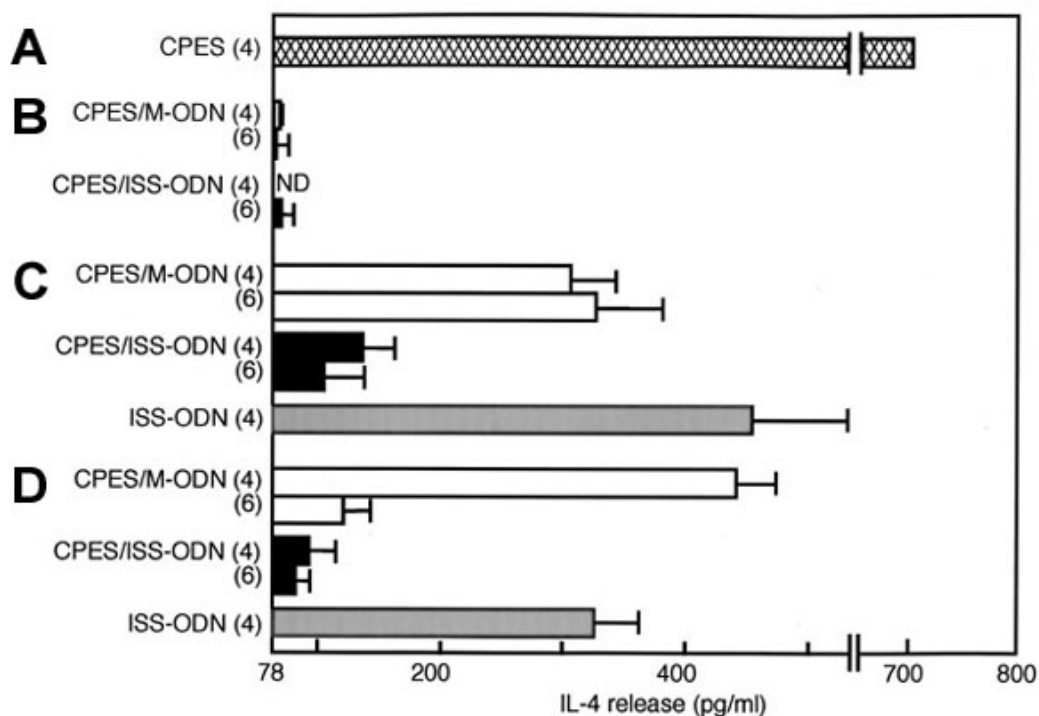


FIG 4. IL-4 release from in vitro CPES-stimulated splenocytes from mice immunized with CPES/ODN and CPES combinations. IL-4 release was measured by ELISA. Responses to CPES in alum administered intraperitoneally twice at 2 weeks apart (**A**), primary responses of mice to CPES/ODN in saline solution (**B**), responses of mice primed intradermally with CPES/ODN or ISS-ODN in saline solution to a secondary intraperitoneal immunization with CPES in alum (**C**), and responses to a secondary CPES/ODN or ISS-ODN in saline solution injected intradermally into mice primed intraperitoneally with CPES in alum (**D**) are shown. The numbers in parentheses are weeks after booster injections in **A** and after ODN injection in **B**, **C**, and **D**. Data are means $\pm$ SEM of 4 mice per group, except for a mean of 3 determinations in **A**. The differences in IL-4 release between CPES/mutant (M) ODN- and CPES/ISS-ODN-injected mice at 4 and 6 weeks (**C** and **D**, except for at 6 weeks in **D**) were statistically significant ($P < .05$). ND, Not detected.

response compared with cells from CPES in alum-primed and CPES/mutant ODN-boosted mice, which released large amounts of IL-4 at 4 weeks and small amounts at 6 weeks (Fig 4, *D*). The cells from ISS-ODN-boosted mice released almost equal amounts of IFN- γ and large amounts of IL-4 compared with cells from CPES/ISS-ODN-boosted mice at 4 weeks (Figs 3, *D*, and 4, *D*).

IgE antibody production

IgE antibody production is a consequence of an extreme T_{H2} immune response and can lead to anaphylactic reactions to protein vaccines. To evaluate whether T_{H1} stimulation by allergen/ISS-ODN coimmunization could inhibit subsequent anti-CPES IgE antibody synthesis, mice were first primed intradermally with CPES/ODN in saline solution and boosted intraperitoneally with CPES in alum or first primed with CPES in alum and boosted with CPES/ODN in saline solution. The log₅ PCA unit of mice immunized twice with CPES in alum was 5.25 (Fig 5, *A*). The mice injected with CPES/mutant ODN showed comparative amounts of IgE antibodies at 4 and 6 weeks as mice immunized with CPES in alum (Fig 5, *B* and *C*). The mice primed with CPES/ISS-ODN generated only small amounts of IgE antibodies in response to a subse-

quent boosting with CPES in alum at 4 and 6 weeks, as opposed to mice primed with CPES/mutant ODN (Fig 5, *B*). The mice primed with ISS-ODN generated larger amounts of IgE antibodies than mice primed with CPES/ISS-ODN (Fig 5, *B*). IgE antibody production in mice primed with CPES in alum and boosted with CPES/ISS-ODN was inhibited at 4 and 6 weeks compared with mice boosted with CPES/mutant ODN (Fig 5, *C*). IgE antibody production in mice boosted with ISS-ODN was larger than that found in mice boosted with CPES/ISS-ODN (Fig 5, *C*).

DISCUSSION

IgE antibody specific to Cry j 1 can be detected in up to 95% of patients with cedar pollinosis, whereas Cry j 2 is detectable in about 70% of these patients.^{13,14} More than 5 major T-cell epitopes in the Cry j 1 and Cry j 2 sequences have been identified.^{15,16} These findings suggest that the use of allergen preparations containing all of these major T-cell epitopes may be necessary for the development of immunotherapy for cedar pollinosis. Therefore we used a CPES containing Cry j 1 and Cry j 2 equally as a cedar pollen allergen for immunization and

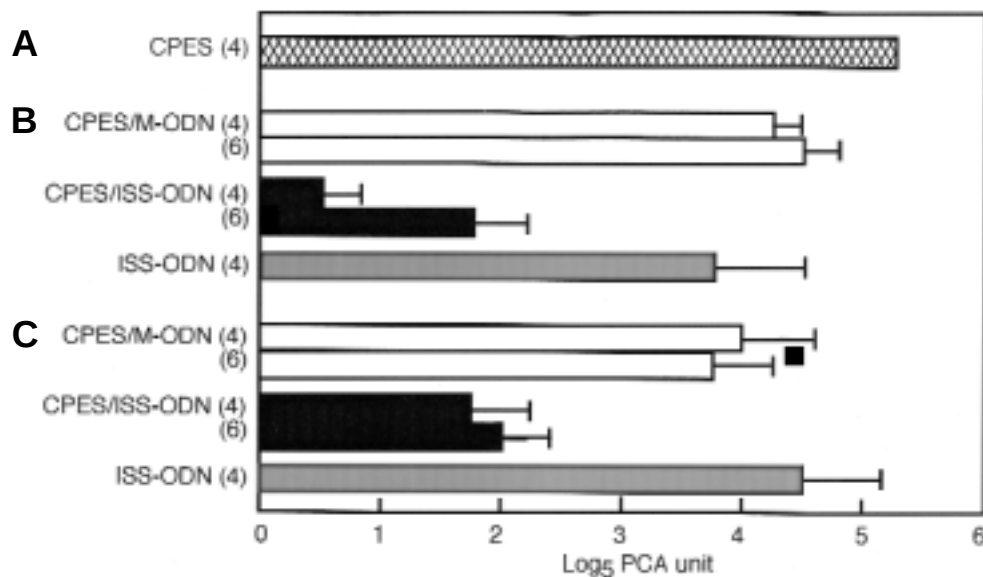


FIG 5. IgE responses to CPES in mice immunized with CPES/ODN and CPES combinations. Anti-CPES IgE antibody assays were performed by rat PCA reactions. The PCA unit represents the reciprocal of end point dilutions giving a PCA reaction. Responses to CPES in alum administered intraperitoneally twice at 2 weeks apart (**A**), responses of mice primed intradermally with CPES/ODN or ISS-ODN in saline solution to intraperitoneally administered CPES in alum (**B**), and responses of mice primed with CPES in alum to intradermally administered CPES/ODN or ISS-ODN in saline solution (**C**) are shown. The numbers in parentheses are weeks after the booster injection in **A** and after ODN injection in **B** and **C**. Data are means $\pm$ SEM of 4 mice per group, except for a mean of 3 determinations in **A**. The differences in the log₅ PCA unit between CPES/mutant (M) ODN- and CPES/ISS-ODN-injected mice at 4 and 6 weeks (**B** and **C**) were statistically significant ($P < .05$).

in vitro stimulation. The effect of the coadministration of CPES and ODN containing T_{H1} ISS (ISS-ODN) on the enhanced T_{H2} immune response and IgE antibody production induced in mice by CPES in alum was evaluated. The data show that the coadministration of CPES/ISS-ODN in saline increases serum IgG2a titers and decreases serum IgG1 titers (Figs 1 and 2). This combination additionally activates cell-mediated immunity, as assessed by the production of IFN- γ , whereas it decreases the release of IL-4, which induces the subsequent IgE antibody production (Figs 3 and 4). Furthermore, the treatment apparently inhibits allergen-specific IgE antibody production, as shown in the PCA reactions (Fig. 5). In some experiments the IgG1 titers were not decreased by CPES/ISS-ODN immunization, especially at 6 weeks after priming with CPES in alum followed in 2 weeks by boosting with CPES/ISS-ODN (Fig 2, C). However, these mice showed a high IFN- γ response and low IL-4 and IgE responses compared with mice boosted with CPES/mutant ODN (Figs 3, D, 4, D, and 5, C). Although IgG1 antibody production, like IgE production, is a typical T_{H2} cell response,¹⁷ IgG1 formation is much less dependent on the requirement for IL-4. The administration of anti-IL-4 antibody or anti-IL-4 receptor antibody inhibits IgE, but not IgG1, formation in mice.^{18,19} IL-4 is required for the generation of both primary polyclonal and secondary antigen-specific IgE responses.^{20,21} In contrast to IL-4, IFN- γ acts in both spontaneous and induced immune responses to suppress IgE production and also to suppress polyclonal IgG1 responses more

than antigen-specific IgG1 responses.^{20,21} Although the mechanism of the selective downregulation of IgE antibody production is not known, it is probably caused by the induction of new antigen-specific T_{H1} cells that are able to downregulate the differentiation of new T_{H2} cells, as has been shown in other systems.^{17,22} Furthermore, IgE has a very short serum half-life,²³ which is likely responsible for the rapid decrease in the IgE titer once IgE synthesis is suppressed by the secondary CPES/ISS-ODN immunization. This IgG1 response is probably not due to a small molecule of ODN (22 bp), which might be metabolized and lose its adjuvant activity long after immunization, because a secondary plasmid DNA encoding an antigen gene injected into mice primed with antigen in alum also induces an increase in IgG1 antibody production and a decrease in IgE antibody production.⁴

Intradermal immunization with a plasmid DNA encoding an antigen gene induces stronger immune responses than injection of the same plasmid DNA preparation administered intramuscularly,²⁴ suggesting that the abundance of Langerhans' cells in addition to dendritic cells, keratinocytes, and fibroblasts in the skin may facilitate the initiation of immune responses, particularly at very low antigen concentrations derived from plasmid DNA.^{24,25} In contrast, the experiment with protein antigen showed that the density and state of activation of antigen-presenting macrophages and dendritic cells at the injection site have a strong influence on subsequent immune responses.^{25,26} In the present study mice immu-

nized intradermally with protein allergen/ISS-ODN showed almost the same response as mice immunized intramuscularly (data not shown).

The mechanism by which protein allergen/ISS-ODN vaccination induces a T_{H1} response is thought to be as follows.^{2,9} The response is the consequence of the activation of the innate immune response by the ISS, and the activation products of the ISS, IFN- γ together with IL-12 and IL-18 (not determined in the present study), are established inducers of IFN- γ synthesis and promote the differentiation of naive T_H cells to T_{H1} lymphocytes. In the presence of a protein allergen, this response promotes the differentiation of naive $CD4^+$ T cell toward T_{H1} cells, leading to a subsequent production of IFN- γ . In fact, the injection of ISS-ODN without allergen induced neither the T_{H1} -shifted immune response nor the inhibition of IgE antibody production compared with the coinjection of allergen and ISS-ODN (Figs 1-5). Thus this mechanism by ISS activation works to induce a T_{H1} response to a coadministered protein allergen, but further proof is required. In the present study the results represent the total immune responses to 2 allergens, Cry j 1 and Cry j 2, and each of the 2 independent immune responses is presumed to correlate with ISS activation.

Recently, several new approaches to immunotherapy for cedar pollinosis have been developed. Modifications of cedar pollen allergen T-cell epitopes, such as the conjugation of Cry j 1 with pullulan²⁷ and single amino acid substitutions on Cry j 1-derived peptides,²⁸ enhance T_{H1} cytokine production and inhibit IgE antibody production. Previously, we reported that the oral administration of cedar pollen extracts causes unresponsiveness of allergen-specific T cells and the suppression of IgG, IgG1, IgG2a, and IgE antibody production in mice. In addition, lymphocytes in the intestine and liver of mice fed allergens would be involved in the mechanism.^{29,30} Also, oral administration of a dominant T cell-determinant peptide inhibits T_{H1} and T_{H2} cell responses in Cry j 2-primed mice.³¹ The mechanism by which the oral administration of allergens induces the inhibition of IgE antibody production is likely to differ from that induced by the coadministration of allergens/ISS-ODN.

The present results demonstrate that the coadministration of cedar pollen allergen and ISS-ODN brings about a T_{H1} immune response and inhibition of IgE antibody production in both mice with secondary T_{H2} and IgE responses and mice with ongoing primary T_{H2} and IgE responses, suggesting that this coadministration strategy may provide a novel type of immunotherapy for cedar pollinosis.

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