Regulation of Murine Airway Eosinophilia and Th2 Cells by Antigen-Conjugated CpG Oligodeoxynucleotides as a Novel Antigen-Specific Immunomodulator

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The characteristic features of bronchial asthma reflect the orchestrated activity of Th2 cells. Oligodeoxynucleotides containing CpG motifs (CpG) have recently been highlighted as an immunomodulator that biases toward a Th1-dominant phenotype. We have previously reported that intratracheal coadministration of CpG and allergen inhibited airway eosinophilia and hyperresponsiveness in a synergistic manner. To substantiate the synergism between CpG and Ag, we introduced a covalently linked conjugate between CpG and Ag and examined the efficacy on airway eosinophilia and Th2 cytokine production. We found that the conjugated form of CpG plus Ag was 100-fold more efficient in regulating airway eosinophilia than the unconjugated mixture. The inhibitory effects lasted for at least 2 mo. The inhibition of airway eosinophilia by the conjugate was Ag specific and associated with an improvement of the airway hyperresponsiveness and the unresponsiveness of the Ag-specific Th2 cells in the regional lymph nodes. The CpG-Ag conjugate was 100-fold more effective than the unconjugated mixture for inducing in vitro Th1 differentiation in an IL-12-dependent manner. Our data show that CpG conjugated to Ag can work as a novel Ag-specific immunomodulator and imply that inhalation of allergen-CpG conjugate could be a desensitization therapy for patients with bronchial asthma. The Journal of Immunology, 2000, 164: 5575–5582.
results provide an experimental basis for a possible inhalation therapy using minute doses of the allergen-CpG conjugate in patients with bronchial asthma.

Materials and Methods

Animals and immunization

BALB/c mice were bred in our animal facility and were used at 6–10 wk of age. These animals were primed i.p. with 10 μg of chicken OVA (Sigma, St. Louis, MO) precipitated with 4 mg of aluminum hydroxide (alum) in 200 μl of PBS three times at 1-wk intervals, and then 7 days later were treated with ODN/OVA for 2 consecutive days (Fig. 1). After 6 days, they were challenged with OVA via an intratracheal route. Where indicated, keyhole limpet hemocyanin (KLH) (Calbiochem, La Jolla, CA) was used for the immunization and challenge in place of OVA, BALB/c mice transgenic (tg) for TCR specific for OVA323-339 and I-A<sup>d</sup> were established as described previously (30).

CpG and control ODN

The ODN were the same as those used in our previous report (13). The CpG ODN (1826) consisted of 20 bases containing two CpG motifs (TCAGACGTTCCTGACGTT). The control ODN (1745) was identical except that the CpG motifs were rearranged (TCCATGAGCTTCCTGACGTT). Phosphorothioate ODN were synthesized by Nihon Gene Research Laboratories (Sendai, Japan) or Takara Shuzo (Osaka, Japan). The ODN were thiolated for conjugation. LPS content of ODN was measured by minute doses of the allergen-CpG conjugate in patients with bronchial asthma.

CpG ODN were thiolated for conjugation. LPS content of ODN was measured by color development, and ODs determined using ELISA. Cytokine concentrations in the BALF or culture supernatant were determined at 450 nm were converted to concentrations (nanograms per milliliter) of control ODN:OVA was calculated to be 6.4:1. CpG was also conjugated to KLH after KLH was maleimide-activated using sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Pierce) in our laboratory. The weight ratio of CpG ODN:KLH was 1.0:1. One microgram or graded doses of CpG, where indicated, were used for the intratracheal pretreatment.

Bronchoalveolar lavage

BALB/c mice were primed, pretreated, and challenged with OVA as in Fig. 1A. Two days after the OVA challenge, the lungs were lavaged twice with PBS (0.25 and 0.20 ml each time) and ~0.4 ml of the instilled fluid was consistently recovered. The bronchoalveolar lavage fluid (BALF) was cytopsion onto microscope slides and stained with Diff-Quik (International Reagents, Kobe, Japan). Differential cell counts were performed by counting at least 300 cells per mouse. For cytokine measurement, BALF was recovered after 24 h of OVA challenge and centrifuged. The supernatants were assayed using ELISA.

Airway hyperresponsiveness

After 2 days of OVA challenge, airway responsiveness was assessed as a change in pulmonary resistance (R<sub>L</sub>) after injections of increasing doses of methacholine chloride (MCh) (0.1–30 mg/kg; Wako), as we described previously (13). Mice were anesthetized by i.p. injection of pentobarbital sodium (50 mg/kg; Wako) and were tracheostomized. The air flow rate at the airway opening during spontaneous breathing was monitored by a pneumotachogram connected to the tracheal tube was very small (more than 100 times) compared with the amplitude of the esophageal pressure. R<sub>L</sub> was calculated according to the subtraction method of Mead and Whittenberger (31). An average R<sub>L</sub> of three breaths at 3 min after each injection of MCh was calculated and expressed as a percentage of the baseline R<sub>L</sub> that was measured and calculated in the same way after the injection of saline used as a diluent of MCh. The provocative concentration of methacholine in milligrams per kilograms that caused a 200% increase in R<sub>L</sub>, designated PC200, was calculated by linear interpolation of the appropriate dose-response curves.

In vitro stimulation of LN cells from CpG-treated mice

BALB/c mice were primed with OVA and pretreated with CpG plus OVA or OVA alone as in Fig. 1B. Medialinal LN cells were pooled from four or five mice of each group, and 1 × 10<sup>6</sup> LN cells were cultured with 2 × 10<sup>5</sup> mitomycin C (Wako)-treated spleen cells of BALB/c mice as APCs in the presence of OVA (1000 μg/ml) or soluble anti-CD3ε mAb (0.1 μg/ml) (PharMingen, San Diego, CA). The cultures were incubated in quadruplicate for 2 days in 96-well plates, and the culture supernatants were assayed for cytokines.

In vitro stimulation of anti-OVA TCR tg T cells with CpG and OVA

Spleen cells (5 × 10<sup>6</sup>) from unimmunized anti-OVA TCR tg mice were cultured with varying doses of CpG, a mixture of OVA and CpG, or the OVA-CpG conjugate in 12-well plates for 3 days. Anti-IL-12 mAb (Genzyme, Cambridge, MA) or isotype-matched control mAb (rat IgG<sub>2a</sub>; PharMingen) was added at graded concentrations at the initiation of culture, including 0.1 μg of CpG-OVA. After an additional 3-day culture in medium, viable lymphocytes were recovered from the interface by Ficoll-Paque (Pharmacy Biotech AB, Upplands, Sweden) density gradient centrifugation and cultured with 2 × 10<sup>5</sup> APCs in the presence or absence of OVA (1000 μg/ml) in 96-well plates. Anti-CD4 (Gk-1.5) (32) or anti-CD8 (3.155) (33) mAb was added to the cultures at a 1:1000 dilution of ascites form. After 2 days, culture supernatants were assayed for IFN-γ or IL-4.

Cytokine assay

Cytokine concentrations in the BALF or culture supernatant were determined using ELISA according to the manufacturer’s recommendations. Paired anti-IL-4, anti-IL-5, and anti-IFN-γ mAbs were purchased from PharMingen. Tetramethylbenzidine reagent (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used for color development, and ODs determined at 450 nm were converted to concentrations (nanograms per milliliter) according to the standard curve. Standard recombinant mouse IL-4, IL-5, and IFN-γ were purchased from Genzyme.

Statistics

Data were expressed as the means ± SEM. For in vivo experiments, each group consisted of four to six mice. Statistical analyses were performed between different groups from the same experiment using Student’s t test. Each experiment was repeated at least twice.
Results
Efficient inhibition of airway eosinophilia by covalently linked CpG-OVA

We previously reported that CpG administered through the airway mucosa improved airway eosinophilia and hyperresponsiveness, and that concomitant administration of CpG and Ag was required for efficient improvement with few adverse effects (13). In this report, we introduced a covalently linked conjugate between OVA and CpG and compared the conjugate with the unconjugated mixture of OVA plus CpG, the regime we used in our previous experiments.

Airway eosinophilia was induced by the intratracheal challenge of OVA in the BALB/c mice sensitized with OVA/alum. One week before the challenge, these mice were pretreated with either PBS, ODN, or OVA. After 2 days of OVA challenge, the numbers of eosinophils in BALF from each mouse were counted. It is noted that the mixture of 0.1 μg of CpG plus OVA failed to inhibit eosinophilia, whereas the covalent conjugate between both revealed inhibitory activities. BALB/c mice were primed with OVA and pretreated with PBS or CpG-OVA. They were challenged with OVA at different time points after the pretreatment and examined for airway eosinophilia 2 days later. Inhibition of eosinophilia by CpG-OVA lasted for at least 2 mo. Each group consisted of four or five mice, and experiments were repeated twice with similar results. *, p < 0.05; **, p < 0.005; and ***, p < 0.002.

Comparison of the conjugate with the mixture of CpG and OVA

We compared the efficiency of the conjugate with that of the unconjugated mixture of CpG plus OVA by dose-response experiments. Graded doses of the CpG-OVA conjugate or the mixture of CpG plus OVA were administered intratracheally to OVA/alum-primed BALB/c mice 7 days before the OVA challenge and the numbers of eosinophils in BALF were determined (Fig. 3). The weight ratio of OVA:CpG was 0.9 for both the conjugate and the mixture. Experiments with the conjugate (■) and the mixture ( ) were conducted independently and are depicted in the same figure, where the control responses are expressed as 100%. The covalent conjugate was calculated to be 100-fold more potent than the mixture of CpG and OVA in the inhibition of airway eosinophilia. Each group consisted of four through six mice, and experiments were repeated twice. *, p < 0.05; **, p < 0.02; and ***, p < 0.01.

Ag specificity of Ag-conjugated CpG

We examined the Ag specificity of Ag-conjugated CpG. Airway eosinophilia induced in mice primed and challenged with OVA was inhibited by OVA-CpG, whereas CpG conjugated to the irrelevant Ag KLH failed to do so (Fig. 4A). The failure was not
Inhibition of Th2 cytokines and improvement of airway hyperresponsiveness

We next examined the effects of CpG-OVA pretreatment on cytokines in BALF and airway hyperresponsiveness. BALB/c mice were primed, pretreated with PBS or CpG (0.1 μg) either conjugated or mixed with OVA, and challenged as shown in Fig. 1A. After OVA challenge, Th2 cytokines in BALF and airway hyperresponsiveness were measured. In contrast to the control PBS-pretreated group, mice pretreated with the CpG-OVA conjugate inhibited both IL-4 (Fig. 5A) and IL-5 (Fig. 5B) in BALF, which was in parallel with the eosinophilic responses. The unconjugated mixture of CpG and OVA tended to inhibit IL-4 and inhibited IL-5 significantly, in spite of the failure to inhibit eosinophilia. IFN-γ in BALF was not detectable in any of the three groups (data not shown). Airway hyperresponsiveness to methacholine was affected by the CpG-OVA conjugate in a similar manner (Fig. 5C). In comparison with the control group, the conjugated CpG-OVA improved airway hyperresponsiveness, whereas the unconjugated mixture failed to improve it significantly (p = 0.053).

Ag-specific Th2 unresponsiveness in the regional LN

To examine whether the improvement of airway eosinophilia and airway hyperresponsiveness by the CpG-OVA conjugate was associated with the inhibition of Th2 cells in the regional LN cells, OVA/alum-primed BALB/c mice were treated with OVA either alone, conjugated, or mixed with CpG (0.1 μg), and the LN cells were assessed for cytokine production in response to in vitro Ag challenge. LN cells from mice treated with OVA or a mixture of CpG plus OVA showed comparable levels of IL-4 production, whereas treatment with the CpG-OVA conjugate inhibited IL-4 production in response to OVA (Fig. 6A). Another Th2 cytokine, IL-5, produced from the LN cells was also reduced in the group treated with CpG-OVA conjugate, but not in the group treated with the mixture (Fig. 6B). IFN-γ production by OVA stimulation was not detected from any of the three groups (data not shown).

In contrast to the stimulation by the specific Ag, polyclonal stimulation of LN cells with anti-CD3 mAb revealed that T cells from the three groups produced comparable levels of IL-4 (Fig. 6C). In accord with this observation, there were no differences in IFN-γ production induced by anti-CD3 mAb between CpG-treated and CpG-nontreated groups (Fig. 6D). These results suggest that the unresponsiveness of IL-4-producing Th2 cells was induced selectively in the T cells specific for the Ag that is covalently coupled to CpG, and that CpG did not skew toward the Th1-dominant phenotype in an Ag-nonspecific manner.

circumvented by the copresence of OVA with the KLH-CpG conjugate, indicating that direct conjugation between OVA and CpG is necessary. Reciprocally, in the KLH-primed and KLH-challenged mice, OVA-CpG or the mixture of OVA-CpG plus KLH failed to inhibit eosinophilia, whereas the relevant Ag KLH-conjugated CpG, but not by OVA-CpG or OVA-CpG plus KLH. These results indicate that Ag-conjugated CpG work in an Ag-specific fashion. Each group consisted of four mice. The data shown are representative of two independent experiments. *, p < 0.02; and **, p < 0.005.

FIGURE 4. Ag specificity of Ag-conjugated CpG. BALB/c mice primed with OVA/alum (A) or KLH/alum (B) were challenged intratracheally with the relevant Ag, following the protocol in Fig. 1A. These mice were pretreated with PBS or CpG (0.1 μg) conjugated to OVA or KLH, either alone or along with the unconjugated Ag (0.1 μg). OVA-induced airway eosinophilia (A) was inhibited by OVA-conjugated CpG, but not by irrelevant Ag KLH-conjugated CpG. The copresence of unconjugated OVA with KLH-CpG failed to restore the inhibitory effects of CpG, confirming the requirement of the direct conjugation between the Ag and CpG. Conversely, KLH-induced eosinophilia (B) was inhibited by KLH-conjugated CpG, but not by OVA-CpG or OVA-CpG plus KLH. These results indicate that Ag-conjugated CpG work in an Ag-specific fashion. Each group consisted of four mice. The data shown are representative of two independent experiments. *, p < 0.02; and **, p < 0.005.

FIGURE 5. Inhibition of Th2 cytokines and improvement of airway hyperresponsiveness. BALB/c mice were primed, pretreated with PBS or 0.1 μg of CpG conjugated or mixed with 0.09 μg of OVA, and challenged with OVA. The next day, BALF was collected for IL-4 (A) and IL-5 assays (B), or after 2 days airway responsiveness to methacholine was measured (C). Inhibition of Th2 cytokines and improvement of airway hyperresponsiveness by CpG-OVA were in parallel with the amelioration of eosinophilia. Each group consisted of four or five mice, and experiments were repeated twice. *, p < 0.05; **, p < 0.005; and ***, p < 0.0002.
FIGURE 6. Ag-specific Th2 unresponsiveness in the regional LN. BALB/c mice primed, treated with OVA (0.09 μg) either alone, conjugated, or mixed with CpG (0.1 μg), and killed for mediastinal LN, as shown in Fig. 1B. A total of 1 × 10⁶ LN cells were stimulated with OVA (A and B) or anti-CD3ε mAb (C and D) in the presence of 2 × 10⁶ APCs in vitro. After 2 days, culture supernatants were assayed for IL-4 (A and C), IL-5 (B), or IFN-γ (D) by ELISA. CpG-OVA induced unresponsiveness of OVA-reactive Th2 cells in the regional LN, whereas Ag-nonspecific skewing toward a Th1 phenotype was not detected. Mediastinal LN cells were pooled from four or five mice of each group, and cultures were set up in quadruplicate. Experiments were repeated twice. *, p < 0.02; and **, p < 0.0005.

Enhanced induction of Th1 cells by the CpG-OVA conjugate

To evaluate the mechanisms underlying the in vivo Th2 unresponsiveness by CpG, we examined the in vitro effects of the CpG-OVA conjugate on T cell differentiation. Spleen cells from anti-OVA TCR tg mice were precultured in the presence of CpG or CpG-OVA, and the activated cells were restimulated with OVA for IFN-γ production. IFN-γ was not produced from the spleen cells precultured with CpG alone at any concentrations tested (Fig. 7A, △). In the presence of attendant OVA, CpG could induce IFN-γ-secreting cells at 10 μg/ml (Fig. 7A, ■), whereas a similar extent of IFN-γ was produced by as little as 0.1 μg/ml of CpG when covalently conjugated to OVA (Fig. 7A, □). Additional experiments with blocking mAbs confirmed that IFN-γ was produced by CD4+ Th1 cells, since IFN-γ production was totally inhibited by anti-CD4 mAb, whereas anti-CD8 mAb had no effects (Fig. 7B).

The Th1-skewing effects by CpG were found to be mediated in large part by IL-12; the induction of Th1 cells by the CpG-OVA conjugate was inhibited by the concomitant presence of anti-IL-12 mAb (Fig. 7C). Titration of anti-IL-12 mAb revealed that the inhibition reached a plateau at 1 μg/ml of IL-12 mAb and complete inhibition by IL-12 mAb was not attained. The control Ab had no effects even at the highest concentration (10 μg/ml). Thus, IL-12 secretion by CpG plays a major, although not exclusive, role in the Th1 development by CpG.

Discussion

CpG found in bacterial but not vertebrate DNA are sensed by immune cells as a sign of the presence of pathogens and evoke host defense mechanisms such as the activation of B and NK cells and the induction of Th1 cell differentiation (23, 26, 34–36). CpG, with features that counteract Th2 responses by inducing Th1 cells, have been applied for the regulation of bronchial asthma in murine experimental models (27, 28, 37). We previously reported that CpG improved airway eosinophilia and hyperresponsiveness when both CpG and Ag, but not CpG alone, were administered via the airway mucosa (13). In the present study, we introduced the covalently linked conjugate between CpG and OVA to further demonstrate the synergism between CpG and Ag. We found that the direct conjugate was 100-fold more effective than the unconjugated mixture in the inhibition of airway eosinophilia (Fig. 3) and in vitro Th1 differentiation (Fig. 7A). Airway hyperresponsiveness and Th2 cytokine levels in BALF were also inhibited (Fig. 5), and these phenomena were associated with the unresponsiveness of Ag-specific Th2 cells in the regional LNs (Fig. 6, A and B).

Bronchial asthma is essentially under the control of unfavorable Th2 cells from which various inflammatory responses stem. We
have been investigating regulatory subsets of CD4+ T cells in regional LNs where activation by Ag and commitment for differentiation of T cells take place. We reported that TGF-β-producing CD4+ T cells induced by oral or tracheal tolerance or Th1 cells induced upon exposure to Mycobacterium tuberculosis neutralized Th2 cells in the regional LNs, along with the inhibition of downstream airway inflammation (10–12). As in these previous experiments, the present study also demonstrated the unresponsiveness of Th2 cells in the mediastinal LNs (Fig. 6, A and B), namely, Th2 tolerance.

Then, what are the underlying mechanisms for the Th2 tolerance in the present experiments? Dominance of Th1 over Th2 cells? We could not observe an increase in IFN-γ levels in BALF after the pretreatment with 0.1 μg of CpG conjugated to OVA (data not shown). In accord with this was a failure to demonstrate IFN-γ production in response to Ag stimulation by the LN CD4+ T cells from the mice receiving CpG-OVA instillation (data not shown). Nor could we observe an Ag-nonspecific deviation of the immune responses toward a Th1-dominant phenotype (Fig. 6, C and D). Thus, no apparent evidence for an in vivo Th1 induction was obtained. In contrast to the in vivo results, however, the in vitro experiments showed that the CpG-OVA conjugate was a 100-fold more potent inducer of Th1 cells than the unconjugated mixture (Fig. 7A). How can we reconcile these differences? We used only 0.1 μg of CpG in this study, which was 1000-fold lower than that in the study by Broide et al. (28) in which 100 μg of CpG was transmucosally administered for the regulation of asthma and the induction of IFN-γ-secreting CD4+ splenocytes. Since the amount of CpG we used was so minute, the induced Th1 cells might have been below detection. Based on the notion that a balance between Th1 and Th2 cells could determine the outcome of immune responses, these Th1 cells could manage to partially counteract the Th2 activity and inhibit the downstream inflammation, whereas the Th1 cells may not have been potent enough to demonstrate their presence as IFN-γ producers. The precise mechanisms remain to be elucidated.

CpG have been used to control asthma in animal models (28, 29, 37). Our experiments are different from others in two important points. First, we showed the need for Ag coadministration with CpG. In accord with us was the report by Kline et al. (37), who reported that i.p. coadministration of CpG with Ag prevented airway inflammation. However, Broide et al. (28) reported that intratracheal instillation of CpG without the accompanying Ag inhibited airway eosinophilia and hyperresponsiveness. In addition, Sur et al. (29) reported that intratracheal administration of CpG prevented allergic lung inflammation, whereas coadministration of CpG with Ag did not suppress lung inflammation. This absence of a need for accompanying Ag was in sharp contrast to our contention. CpG in the absence of attendant Ag would skew immune responses toward a Th1-dominant phenotype in an Ag-nonspecific manner, because CpG would not know a priori which Ag-specific immune systems they affect. It was also reported that CpG acted as powerful adjuvants only when coinjected with Ag (22).

Another point is the doses of CpG. We instilled 0.1 μg of CpG in a form of a CpG-OVA conjugate, whereas 30–100 μg of CpG was used in other studies (28, 29, 37). In light of the pathogenic roles of Th1 cells in various types of autoimmunity (38), the effects of CpG on the activation of autoreactive Th1 cells and autoimmunity are unpredictable and controversial (39–42). The toxicity of CpG would be another problem. Bacterial DNA was reported to increase the toxicity of LPS (24). CpG also increased serum TNF-α levels and mortality in CpG-treated mice (25–27). Exacerbation of local inflammation by CpG was also reported (43, 44). To avert possible adverse effects, it would be desirable to minimize the therapeutic doses of CpG for allergic diseases. In this report, we introduced a covalently linked conjugate between CpG and OVA and could reduce the doses of CpG owing to the effective synergism.

Then, what are the mechanisms underlying the synergism of OVA-CpG conjugate? OVA is picked up, processed, and presented to Th cells by APCs, whereas CpG promote elaboration from APCs of IL-12, which destine T cell development toward a Th1 phenotype. A. APCs engulfing CpG-OVA would present antigenic peptide along with the secretion of IL-12. Since anti-OVA Th cells recognize and are in close proximity to the antigenic peptides presented on the IL-12-secreting APCs, IL-12 could be effectively targeted to the Th cells binding to the IL-12-secreting APCs. This key step would allow CpG, which is Ag nonspecific in nature, to behave in an Ag-specific manner, because the effects of CpG are concentrated on the T cells specific for the Ag that is conjugated to CpG. Such Th1 cells would counteract Th2 cells and inhibit downstream inflammation at the time of antigenic challenge. B. When small amounts of OVA and CpG are administered in the mixed form, the chances for CpG-laden APCs to simultaneously pick up OVA are less likely than when using the CpG-OVA conjugate. Although CpG-laden APC would secrete IL-12, the secreted IL-12 would not reach to the Ag-specific Th cells because the IL-12-secreting APCs would not have presented the Ag. Another APC would phagocytose the Ag without CpG and be likely to induce and activate Th2 cells. Upon intratracheal challenge with Ag, there would be few Th1 cells that counteract Th2 cells, resulting in Th2 dominance. 

FIGURE 8. Hypothetical mechanisms for the synergism of covalent conjugation between CpG and OVA. OVA is picked up, processed, and presented to Th cells by APCs, whereas CpG promote elaboration from APCs of IL-12, which destine T cell development toward a Th1 phenotype (14, 15). APCs engulfing CpG-OVA would present antigenic peptide along with the secretion of IL-12 (Fig. 8A). Since anti-OVA Th cells recognize and are in close proximity with the antigenic peptides presented on the IL-12-secreting APCs, IL-12 could be effectively targeted to the Th cells binding to the IL-12-secreting APCs. This key step would allow CpG, which is Ag nonspecific in nature, to behave in an Ag-specific manner because the effects of CpG are concentrated on the T cells specific for the Ag that is conjugated to CpG. Such Th1 cells would counteract Th2 cells and inhibit downstream inflammation upon antigenic challenge (11). When OVA and CpG are administered in the mixed form, the chances for CpG-laden APCs to simultaneously pick up OVA are less likely than when using the CpG-OVA conjugate (Fig. 8B). Although CpG-laden APCs would secrete IL-12, the secreted IL-12 would not reach the Ag-specific Th cells because the IL-12-secreting APCs would not have presented the Ag. Another APC would phagocytose the Ag without CpG and be
likely to induce and activate Th2 cells. Upon intraotracheal challenge with Ag, there would be few Th1 cells that counteract Th2 cells, resulting in Th2 dominance.

We previously observed a similar synergism between Ag recognized by T cells and an inducer of IL-12 from APCs (11). We reported that both the Ag and M. tuberculosis bacilli, but not either one alone, were essential and adequate for the efficient induction of Ag-specific Th1 cells and suggested that the copresentation of the Ag and M. tuberculosis bacilli would occur at the level of APCs secreting IL-12 (11). Therefore, the link of Ag and a modulator of IL-12 secretion would be a useful tool for Th1 development.

Direct conjugation of Ag to ODN might unveil unexpected immunomunogen aspects of DNA; it has been reported that DNA complexed to proteins or peptides induces the production of lupus-like anti-dsDNA Abs (45–47), whereas immunization with the DNA-protein complex was reported to attenuate murine lupus despite the induction of pathogenic Abs (48). As discussed above, the reasons for the possible deteriorating effects of autoimmune diseases by Cpg-protein conjugates need to be clarified.

In summary, we described a novel approach that controls bronchial asthma in a murine model. Since the effects of the transmucosally administered Cpg-allergen conjugate lasted for >2 mo, periodical inhalation of allergen-Cpg conjugate could be a clinically useful therapy for bronchial asthma. Efficient induction of Ag-specific Th1 cells would also be beneficial for infection and cancer therapies.

Acknowledgments

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References


