

CpG Oligodeoxynucleotides Can Reverse Th2-Associated Allergic Airway Responses and Alter the B7.1/B7.2 Expression in a Murine Model of Asthma¹

Denise Serebrisky,^{2*} Ariel A. Teper,^{2*} Chih-Kang Huang,* Soo-Young Lee,* Ten-Fei Zhang,* Brian H. Schofield,[†] Meyer Kattan,* Hugh A. Sampson,* and Xiu-Min Li^{3*}

CpG oligodeoxynucleotides (CpG-ODN) administered during Ag sensitization or before Ag challenge can inhibit allergic pulmonary inflammation and airway hyperreactivity in murine models of asthma. In this study, we investigated whether CpG-ODN can reverse an ongoing allergic pulmonary reaction in a mouse model of asthma. AKR mice were sensitized with conalbumin followed by two intratracheal challenges at weekly intervals. CpG-ODN was administered 24 h after the first Ag challenge. CpG-ODN administration reduced Ag-specific IgE levels, bronchoalveolar lavage fluid eosinophils, mucus production, and airway hyperreactivity. We found that postchallenge CpG-ODN treatment significantly increased IFN- γ concentrations and decreased IL-13, IL-4, and IL-5 concentrations in bronchoalveolar lavage fluids and spleen cell culture supernatants. Postchallenge CpG-ODN treatment also increased B7.1 mRNA expression and decreased B7.2 mRNA expression in lung tissues. These results suggest that CpG-ODN may have potential for treatment of allergic asthma by suppressing Th2 responses during IgE-dependent allergic airway reactions. The down-regulation of Th2 responses by CPG-ODN may be associated with regulation of the costimulatory factors B7.1 and B7.2. *The Journal of Immunology*, 2000, 165: 5906–5912.

Antigen-induced IgE production (1), airway inflammation and airway hyperreactivity (AHR)⁴ (2–4) have been well documented in patients with allergic asthma and in animal models (5, 6), and increasing evidence suggests that the Th2-type cytokines IL-4, IL-5, and IL-13, produced by activated CD4⁺ T cells play a central role in the pathogenesis of allergic asthma (6, 7). Thus, interventions that inhibit Th2 cytokine production by enhancing Th1 cytokine production, may be useful in the treatment of allergic asthma. We previously demonstrated that treatment of sensitized mice with the Th1-associated cytokines IL-12 or IFN- γ before challenge reduced IL-4 and IL-5 levels in bronchoalveolar lavage fluid (BALF) and inhibited Ag-induced eosinophilic inflammation and AHR (8, 9).

Bacterial DNA and synthetic oligodeoxynucleotides (ODN) containing CpG motifs are potent adjuvants of Th1-like responses characterized by production of IL-12, IFN- γ and IgG2a (10, 11). Consequently, immunomodulatory protocols employing CpG-ODN have recently been applied to murine models of allergic asthma. It has been reported that CpG-ODN treatment at the time of Ag sensitization or before Ag challenge have a prophylactic

effect on Ag-induced airway eosinophilia and AHR (12–15). Although CpG-ODN has also been shown to reverse an ongoing lethal Th2-driven *Leishmania major* infection in mice (16); to induce IL-12, IL-18, and IFN- γ ; and to inhibit IgE synthesis by cultured peripheral mononuclear cells from allergic patients (17), the ability of CpG-ODN to inhibit an ongoing allergic pulmonary reaction has not been reported previously.

We previously generated a mouse model of allergic asthma, which exhibits pulmonary eosinophilia, AHR, and increased Ag-specific IgE accompanied by increased IL-4 and IL-5 levels in BALF following conalbumin sensitization and challenge (18). We used this model in the present study to evaluate possible therapeutic effects of CpG-ODN on allergic pulmonary responses by administration of CpG-ODN 24 h after the first Ag challenge. Because several previous studies reported that CpG-ODN administration at the time of Ag sensitization and challenge inhibited Th-2 responses (12–15), we also used a similar protocol for comparison. We found that, in addition to its known preventive effects, CpG-ODN administered after Ag challenge also significantly reduced Ag-specific IgE production, eosinophilic inflammation, and AHR, which were associated with up-regulation of IFN- γ and down-regulation of IL-4, IL-5, and IL-13 synthesis. A previous study (19) found that CpG-ODN inhibitory effects on Th2-mediated pulmonary granulomatous inflammation were IL-12, NK cell, and B cell independent, and a role for up-regulation of B7.1 expression in Th2 response inhibition was suggested by the increased expression of B7.1, but not B7.2, by peritoneal macrophages. We also examined B7.1 and B7.2 expression by determining lung mRNA expression and found that postchallenge CpG-ODN treatment markedly decreased B7.2 mRNA and slightly increased B7.1 mRNA expression in the lung.

Materials and Methods

Mice and reagents

Male AKR/J mice (6–8 wk old, purchased from The Jackson Laboratory, Bar Harbor, ME) were maintained in the animal facility at Mount Sinai

*Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029; and [†]Department of Environmental Health Sciences, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

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² D.S. and A.A.T. contributed equally to this work.

³ Address correspondence and reprint requests to Dr. Xiu-Min Li, Pediatric Allergy and Immunology, The Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574. E-mail address: Xiu-min_li@smtpplink.mssm.edu

⁴ Abbreviations used in this paper: AHR, airway hyperresponsiveness or airway hyperreactivity; CpG-ODN, CpG oligodeoxynucleotide; CA, conalbumin; i.t., intratracheally; BALF, bronchoalveolar lavage fluid; PAS, periodic acid-Schiff.

School of Medicine. Standard guidelines (20) for the care and use of animals were followed.

The CpG-ODNs consisted of 20 bases containing 2 CpG motifs: (TC CATGACGTTCTGACGTT) and a control ODN, identical except for rearrangements of the CpG motifs (TCCATGAGCTTCCTGAGTCT) as previously described (12). Both ODNs were synthesized and purified by Life Technologies (Gaithersburg, MD), and reconstituted in endotoxin-free water. Conalbumin (CA) and dinitrophenyl conjugated with albumin (DNP-albumin) were purchased from Sigma (St. Louis, MO). Abs for ELISAs were purchased from The Binding Site and PharMingen (San Diego, CA). Anti-DNP IgE, IgG1, and IgG2a were purchased from Accurate Scientific (Westbury, NY).

Ag sensitization, challenge, and CpG-ODN treatment

Mice were sensitized i.p. with 200 μ g CA adsorbed with 2 mg alum in 0.4 ml PBS on days 0 and 7. Mice were subsequently challenged intratracheally (i.t.) with 100 μ g CA in 0.05 ml PBS on days 14 and 21.

Sensitized mice received 30 μ g (low dose) or 100 μ g (high dose) of CpG-ODN i.p. 24 h after the first Ag challenge and again 1 wk later (CpG 30-post, CpG 100-post). Other mice received the same doses of CpG-ODN simultaneously with Ag sensitization and challenge (CpG 30-simul, CpG 100-simul). Control ODN-treated (ODN-30-simul), untreated Ag-sensitized and challenged mice (none), and naive mice served as additional controls.

Late phase airway response measurement, BALF cell differential counts, and lung histology

Three days after the second Ag challenge, airway responsiveness was determined by measuring airway pressure changes after i.v. acetylcholine challenge, as previously described (9, 21). The time-integrated changes in peak airway pressure, referred to as the airway pressure-time index (centimeters H₂O-s) were calculated and served as measurements of airway responsiveness. After airway response measurement, the lungs were lavaged and BALF was collected. Cytospin slides were prepared and stained, and differential BALF cell counts were determined as previously described (18, 22). In addition, BALF from 4 mice in each group were collected 24–30 h after the second challenge and used for cytokine measurements. This time point was chosen because we previously demonstrated that Th2 cytokine levels peaked at 24 h after challenge in this model (18). Lungs ($n = 4$ /group) were fixed in neutral buffered formaldehyde, and 5- μ m paraffin sections were stained with hematoxylin and eosin and periodic acid-Schiff (PAS) for evaluation of inflammatory cells and goblet cells.

Cell culture

Splenocytes were isolated and suspended in RPMI 1640 containing 10% FBS, 1% penicillin/streptomycin, and 1% glutamine. Cells (4×10^6 /ml) were cultured in 24-well plates in the presence or absence of CA (50 μ g/ml) or Con A (2.5 μ g/ml). Supernatants were collected after a 72-h culture.

Cytokine measurement

IFN- γ , IL-4, IL-5, and IL-13 concentrations in BALF and spleen cell culture supernatants were determined by ELISA according to the manufacturer's instructions (PharMingen) as previously described (18).

Ag-specific Ab measurements

Blood samples were obtained immediately after airway pressure measurements. Serum CA-specific IgE levels were measured by ELISA as described previously (18). To measure CA-specific IgG1 and IgG2a concentrations, plates were coated with CA (1 μ g/ml) and incubated overnight at 4°C and then were blocked and washed. Serum samples (1:50 dilution) were added to the plates and incubated overnight at 4°C. Plates were washed and biotinylated rat anti-mouse IgG2a, or IgG1 mAbs (0.3 μ g/ml, PharMingen) were added to the plates and incubated for an additional 1 h at room temperature. After washing, avidin-peroxidase (1:1000) was added for an additional 15 min at room temperature. After washing, the reactions were developed with 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonate) (Kirkegaard & Perry Laboratories, Gaithersburg, MD) for 30 min at room temperature and read at 405 nm.

Because there is no commercially available mouse anti-conalbumin Ab, the equivalent concentrations of Ag-specific IgE, IgG2a, and IgG1 were calculated by comparison with a reference curve generated with mouse mAbs, anti-DNP IgE, IgG2A, and IgG1 as described previously (18). Briefly, DNP-albumin was coated at the same concentration as conalbumin, and after overnight incubation at 4°C, the plates were washed and

blocked as described above. Ten serial 1:2 dilutions of murine anti-DNP IgE, IgG2a, or IgG1 Abs were added, beginning with a concentration of 1000 ng/ml. Thereafter, all steps were performed as described above. All analyses were performed in duplicate, and coefficients of variation >10% were repeated to ensure a high degree of precision.

RT-PCR

Total mRNA was isolated from lung tissues of high dose CpG postchallenge treated, simultaneously treated, sham treated, and naive mice using Trizol reagent (Life Technologies), as described by the manufacturer. The reverse transcription was performed using the Superscript Amplification System kit for cDNA synthesis (Life Technologies), as described by the manufacturer (23). Briefly, 12 μ l of the mixture of RNA (5 μ g)-oligo(dT) (1 μ l) was incubated at 70°C for 10 min and then incubated on ice for 2 min. Reaction mixture (7 μ l; 1 $\times$ PCR buffer, 5 mM MgCl₂, 0.5 mM dNTPs, 0.02 M DTT) was added to the RNA-oligo(dT) mixture and incubated at 42°C for 5 min. One microliter (200 U) Superscript II reverse transcriptase was then added, and the mixture was incubated at 42°C for 50 min. The reaction was terminated by incubating the mixture at 70°C for 15 min followed by the addition of 1 μ l RNase H for 20 min at 37°C. First strand cDNAs were either stored at -20°C or used for the PCR step.

PCR was performed as described previously (8, 24) with slight modification. Briefly, PCR (50 μ l total volume) was conducted in 2 mM MgCl₂, 1 $\times$ PCR buffer, 2.5 U AmpliTaq DNA polymerase, 2 μ l 10 μ M anti-sense and sense primer pairs and 2 μ l cDNA. PCR was conducted beginning with 95°C for 2 min followed by 25 cycles for β -actin and 35 cycles for B7.1 and B7.2 using the following temperature profile: denaturation, 94°C for 45s; primer annealing, 60°C for 45 s; and primer extension, 72°C for 90 s. This protocol was based on The manufacturer's protocol for use of Clontech Amplimer Sets (Clontech Laboratories, Palo Alto, CA) in RT-PCR, and the Superscript Kit instruction manual, as well as preliminary experiments. These cycles sufficiently amplify the β -actin and B7.1 and B7.2 expression and avoid amplification saturation. The final extension was at 72°C for 10 min. Once the PCR were complete, 10 μ l of the reaction mixture were separated by electrophoresis through a 1.5% agarose gel and visualized by ethidium bromide staining and UV irradiation. Gel images were captured using a Gel Doc Image Analysis system (Bio-Rad, Hercules, CA), and PCR product quantitation was performed by densitometry using Quantity One Software (Bio-Rad) and standardized against β -actin from the same mRNA preparation. Results were expressed as an OD ratio (B7.1 or B7.2 vs β -actin). Before analysis, the PCR product band intensities were checked to ensure that they had not reached saturation. All reactions were repeated at least 2–3 times. Oligonucleotide primers for B7.1 (sense 5'-ATGCTCACGTGTCAGAGGA-3', 19-mer; antisense 5'-GACGGTCTGTTACAGCTAATG-3', 20-mer, 238 bp) and B7.2 (sense 5'-CAACTGGA CTCTACGACTTC-3', 20-mer; antisense 5'-TGCTTAGACGTGCAGGTCAA-3', 20-mer, 209 bp) were synthesized by Life Technologies, and β -actin used in the PCR was purchased from Clontech.

Statistical analysis

Statistical analysis was performed using Student's *t* test for comparison between two groups and one-way ANOVA for comparison between more than two groups. $p < 0.05$ was considered statistically significant. All statistical analyses were performed with SigmaStat software (SPSS, Chicago, IL).

Results

Effects of CpG-ODN on AHR, inflammation, and mucus cell hyperplasia

To examine the possible therapeutic effect of CpG-ODN on allergic airway hyperreactivity, we used a posttreatment protocol in which mice were treated with CpG-ODN (30 μ g or 100 μ g/mouse) 24 h after Ag challenge. We compared the effects of postchallenge treatment to the effects produced by coadministration of CpG-ODN at the time of Ag sensitization and challenge. Postchallenge CpG-ODN treatment significantly reduced AHR and BALF eosinophil numbers when compared with untreated Ag-sensitized, challenged mice (Fig. 1). CpG-ODN administered at the time of Ag sensitization also significantly reduced BALF eosinophil numbers and AHR when compared with the untreated group. The inhibitory effect of CpG-ODN on BALF eosinophilia and AHR appeared more pronounced in the high dose treatment groups.

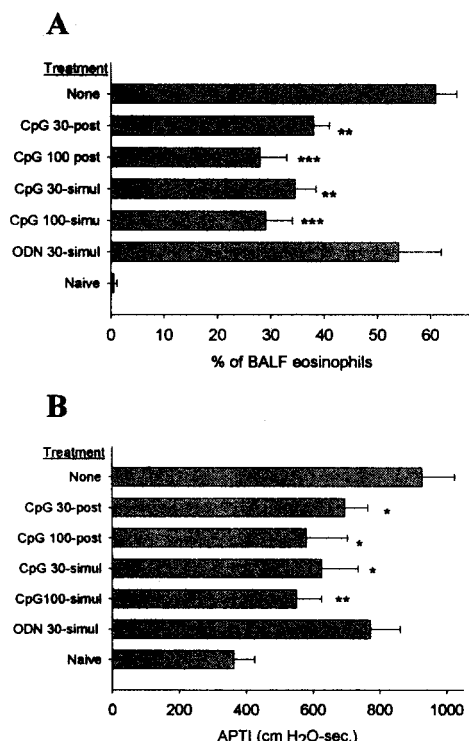


FIGURE 1. Reduction of Ag-induced eosinophilic inflammation and airway hyperreactivity by CpG-ODN. Mice ($n = 6-10$ in each group) were sensitized i.p. and challenged i.t. with CA. CpG-ODN ($30 \mu\text{g}$ or $100 \mu\text{g}$) was administered 24 h postchallenge or simultaneously with Ag sensitization. Control ODN ($30 \mu\text{g}$) was also given with Ag sensitization. The number of BALF eosinophils (A) and airway pressure-time index (APTI, B) were determined 3 days after the second challenge. Data are given as mean $\pm$ SEM of 2-3 experiments. *, $p < 0.05$ vs none; **, $p < 0.01$ vs none; ***, $p < 0.001$ vs none.

Although the number of BALF eosinophils and the degree of AHR in posttreatment groups were slightly higher than in the simultaneous treatment groups at equivalent doses, these differences were not statistically significant. Control ODN treatment also appeared to slightly reduce BALF eosinophil numbers and AHR when compared with untreated mice, but the reduction was not statistically significant. In addition, like the reduced total cell numbers in the high dose simultaneously treated group, the total numbers of BALF cells were also reduced by high dose postchallenge CpG-ODN treated (5.3×10^4) as compared with the control ODN-treated group (8.7×10^4).

Goblet cell hyperplasia is frequently observed in airways of asthmatic patients and in animal models of allergic asthma, and mucus plugging has long been recognized as a major factor contributing to the mortality associated with acute severe asthma (25, 26). To determine whether postchallenge CpG-ODN treatment also appeared to affect airway mucus production, we compared PAS-stained sections of lungs from mice treated with $100 \mu\text{g}$ CpG-ODN postchallenge to lungs from untreated mice 3 days after the second i.t. challenge. Numerous PAS-positive goblet cells were present in bronchi and bronchioles of untreated mice, and in some instances, bronchial lumens were filled with mucus (Fig. 2A). In contrast, the number of mucus-containing epithelial cells in the airways of treated mice appeared to be markedly reduced, and little or no mucus was present in the bronchial lumens (Fig. 2B). Consistent with the BALF findings, peribronchial and perivascular inflammation was also reduced by postchallenge CpG treatment

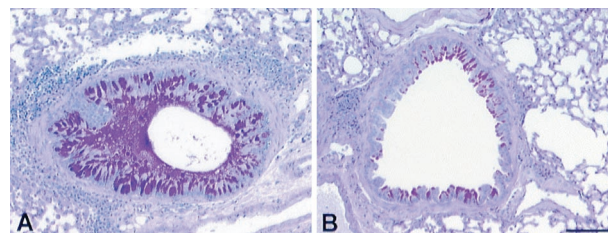


FIGURE 2. Reduction of Ag-induced bronchial mucus hypersecretion by posttreatment with CpG-ODN. Lungs were collected 3 days after the second challenge. Paraffin sections of lungs were stained with PAS. A, Many PAS-positive bronchial epithelial cells and mucus in bronchial lumen in lung from Ag-sensitized and challenged untreated mice. B, a lung from CpG 100-post treated mouse containing fewer PAS-positive epithelial cells (bar, $100 \mu\text{m}$).

(data not shown). These results demonstrate that CpG-ODN partially reversed the processes responsible for Ag-induced eosinophilic inflammation and mucus cell hyperplasia, which are associated with increased AHR in this model.

Effects of CpG-ODN on Ig synthesis

To determine the effect of postchallenge CpG-ODN treatment on humoral responses, serum CA-specific IgE, IgG1, and IgG2a Ab levels were determined by ELISA. As shown in Fig. 3, CpG postchallenge treatment as well as CpG simultaneous treatment significantly decreased IgE levels when compared with the untreated group, and this effect was more pronounced in the high dose group. Ag-specific IgG1 levels were also significantly decreased in the CpG simultaneous- and posttreated groups; however, no significant difference was observed between the high and low dose groups. IgG2a levels, in contrast, were significantly increased in both CpG treatment groups, and were higher in the high dose group. Furthermore, the decreased IgE and IgG1 concentrations and the elevated IgG2a concentrations in simultaneous- and post-treated mice receiving the same dose of CpG were not significantly different. Control ODN treatment did not significantly affect IgE, IgG1 and IgG2a levels when compared with the untreated group. These results show that CpG-ODN administered after Ag challenge can reduce IgE and IgG1 production and increase IgG2a responses.

Effects of CpG-ODN on IL-13, IL-4, IL-5, and IFN- γ synthesis

To assess the effects of CpG-ODN treatment on Th2 cytokines associated with allergic airway responses, we measured IL-13, IL-4, IL-5, and IFN- γ concentrations in BALF and spleen cell culture supernatants. Consistent with our previous findings in this model (9) (18), IFN- γ concentrations were markedly lower, and IL-13, IL-4, and IL-5 concentrations were markedly higher in BALF from untreated mice after Ag sensitization and challenge than in naive mice (Fig. 4), demonstrating predominantly a Th2 response. In contrast, IFN- γ levels were significantly increased and IL-13, IL-4, and IL-5 concentrations were markedly decreased in BALF from both CpG posttreatment, and CpG simultaneous treatment groups. Differences in IFN- γ , IL-13, IL-4, and IL-5 concentrations between control ODN-treated and untreated groups did not reach statistical significance.

Furthermore, increased IFN- γ and decreased IL-13, IL-4, and IL-5 levels were also observed in splenocyte culture supernatants from CpG-ODN postchallenge treated as well as simultaneous sensitization/challenge-treated groups (Table I). These results demonstrate that CpG-ODN reversed systemic as well as local Th2 responses.

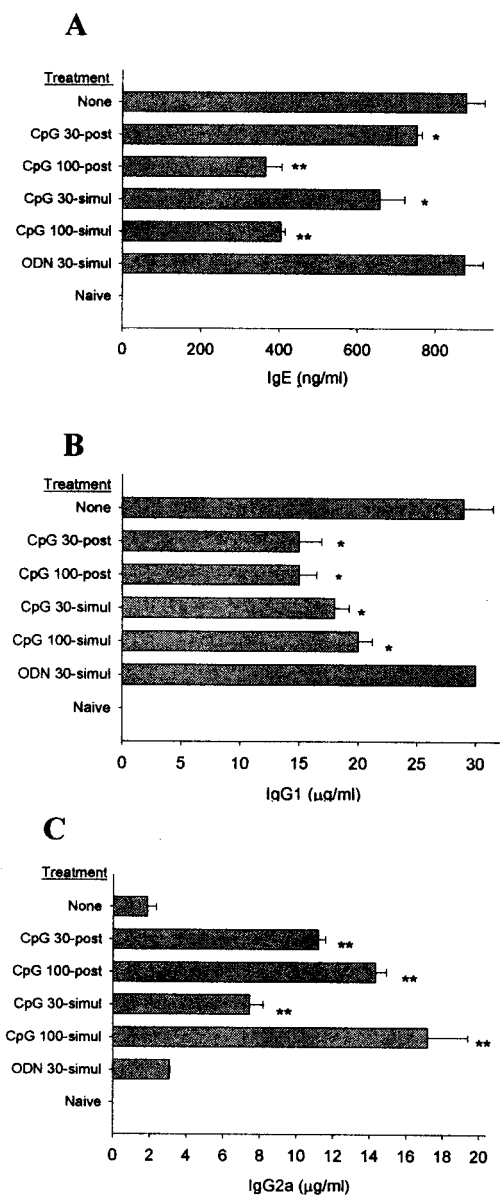


FIGURE 3. Reduction of IgE and IgG1, and increase of IgG2a levels by CpG-ODN. Sera from different groups of mice ($n = 6-10$) were obtained and CA-specific IgE, IgG1, and IgG2a concentrations were determined by ELISA. Data are given as mean $\pm$ SEM of two to three experiments. *, $p < 0.05$ vs none; **, $p < 0.01$ vs none.

Effects of CpG-ODN on B7.1 and B7.2 mRNA expression

Selective expression of B7.1 vs B7.2 has been shown in many models to preferentially influence Th1 and Th2 responses, respectively (27). It has also been reported that up-regulation of B7.1 by CpG-ODN appeared to be involved in preventing a Th2 response (19). Therefore, we determined whether postchallenge CpG-ODN treatment also influenced B7.1 and B7.2 expression in the lung. It has been suggested that many cells in murine lungs can potentially process and/or present Ag, including “professional” APCs (B cells, alveolar macrophages, and dendritic cells) and nontraditional APCs (epithelial cells, eosinophils) (28). However, it has not yet been determined which APC or combination of APCs plays a dominant role in T lymphocyte activation in human asthma or mouse models of asthma. Therefore, we evaluated the relative expression of B7.1 and B7.2 mRNA in whole lung tissue by semiquantitative

RT-PCR. As shown in Fig. 5, B7.2 expression was markedly increased, whereas B7.1 expression was decreased in the Ag-sensitized/challenged/untreated group as compared with naive mice. CpG posttreatment reduced B7.2 expression by 60% and increased B7.1 expression by 28% compared with the untreated groups. Simultaneous CpG treatment reduced B7.2 expression by 43% and increased B7.1 expression by 50%. These results show that CpG-ODN has an immunoregulatory effect on lung B7.1 and B7.2 expression and that postchallenge CpG-ODN treatment had a greater effect on suppression of B7.2 expression whereas simultaneous CpG-ODN treatment appeared more effective in increasing B7.1 expression.

Discussion

Previous studies have reported that the administration of CpG-ODN during Ag sensitization of naive mice, or before Ag challenge of sensitized mice prevents Th2-directed allergic airway inflammation and AHR (12–15). Although several studies investigated the effects of CpG given at the time of Ag challenge, the reported efficacy of CpG-ODN in preventing Ag-induced eosinophilic inflammation and AHR varies widely between different reports. Sur et al. (14) found that i.t. administration of CpG with Ag challenge had no effect on eosinophilic inflammation. Shirota et al. (29) found that CpG was effective when coadministered into the lung with Ag, but not alone. We found that i.p. administration of 30 μ g CpG into AKR/J mice at the time of challenge was more effective than i.t. administration of the same dose in suppressing AHR, but administration of 100 μ g CpG equally suppressed AHR whether given i.p. or i.t. (30). The varying results in these studies are most likely a consequence of the CpG dose or murine strain used. We previously showed that AKR mice produced stronger IgG2a than BALB/c mice after plasmid DNA-encoding peanut allergen immunization (31).

Recently, CpG-ODN has also been shown to have therapeutic potential by inhibiting IgE synthesis, and inducing IL-12, IL-18, and IFN- γ synthesis by cultured peripheral mononuclear cells from allergic patients (17), and by reversing an ongoing lethal Th2-driven *L. major* infection (16). These findings suggest that CpG-ODN may have therapeutic potential for ameliorating allergic airway inflammation and AHR. However, until now there has been no direct experimental evidence to support this hypothesis. In this study, we report for the first time that administration of CpG-ODN after Ag challenge can significantly reduce AHR, eosinophilic inflammation, mucus production, and IgE and IgG1 production. Interestingly, these effects were equivalent to those induced by simultaneous CpG administration at sensitization and challenge. Although the reversal was not total, these findings support further research into the possible use of CpG-ODN therapy for treatment of allergic AHR.

As recently reviewed by Romagnani et al. (7), Th2 cytokines play a central role in the pathogenesis of asthma. IL-4/IL-13 promotes B cell switching to IgE production and mucus hypersecretion. IL-5 has been shown to be the primary determinant of eosinophil priming, activation, recruitment, and survival. Although up-regulation of Th1 cytokines by CpG-ODN administered before Ag challenge has been well documented, the effects of CpG-ODN on Th2 cytokine production have not been comprehensively characterized. It has been reported that CpG-ODN pretreatment reduced IL-4 and/or IL-5 (12, 13, 29). However, it also has been reported that CpG-ODN treatment did not decrease IL-4 synthesis by spleen or lung cells, but due to enhanced IFN- γ production, the IFN- γ /IL-4 ratio was increased (14). An effect of CpG-ODN on IL-13 synthesis has not been previously reported.

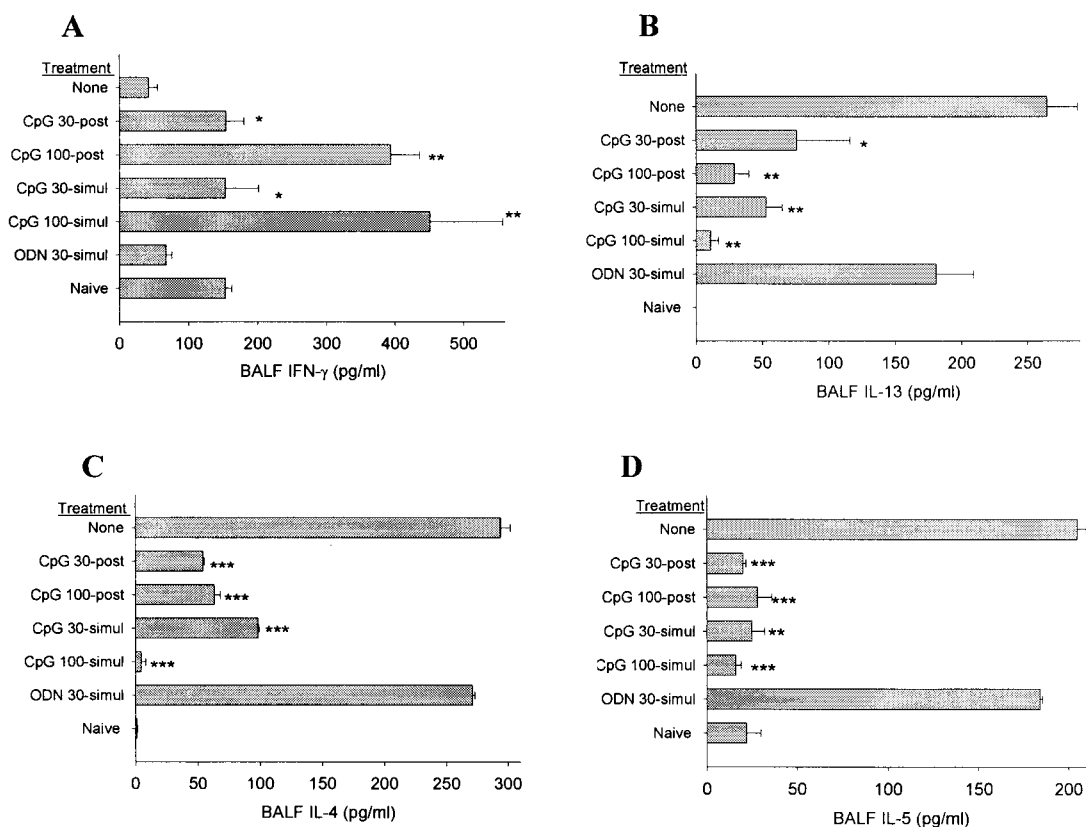


FIGURE 4. BALF cytokine concentrations. BALF were collected 24–30 h after the second Ag challenge from each group ($n = 4/\text{group}$). Cytokines IFN- γ , IL-13, IL-4, and IL-5 were determined by ELISA. Data are given as mean $\pm$ SEM, *, $p < 0.05$ vs none; **, $p < 0.01$ vs none; ***, $p < 0.001$ vs none.

In this study, we found that CpG-ODN administered 24 h after i.t. Ag challenge, the time of peak Th2 cytokine expression in this model (18), suppressed IL-4, IL-5, and IL-13 synthesis and increased IFN- γ synthesis. These results suggest that the therapeutic effect of CpG on eosinophilic inflammation, IgE levels, and AHR in this model may be a result of down-regulation of TH2 cytokine levels. Previous studies showed that anti-IL-4 or anti-IL-13 receptor Abs suppressed Ag-induced AHR, but not eosinophilic inflammation (32, 33), and that anti-IL-5 Ab administered after Ag challenge suppressed eosinophilic inflammation but had little effect on AHR (34). Because natural allergic inflammatory reactions are mediated by a combination of Th2 cytokines, CpG-ODN administration may offer some advantage over therapeutic administration of single Abs against IL-4, IL5, or IL-13, or their receptors.

It has been suggested that the prophylactic effect of CpG-ODN on Th2-driven allergic airway responses is associated with the induction of IL-12 (12) and IFN- γ (14). However, it has also been reported that blocking IL-12 or IFN- γ by specific Abs in vitro only

partially reduced CpG-ODN inhibition of IL-5, IL-3, and GM-CSF production (13). These findings suggest that suppression of Th2 responses by CpG-ODN is only partially attributable to induction of IL-12 or IFN- γ . A more recent study by Chiamonte et al. (19) showed that the preventive effect of CpG-ODN on Th2-mediated schistosome egg-induced pulmonary inflammation was not blocked in IL-12-deficient mice and was only partially decreased in IFN- γ and IL-10/IL-12 double knockout mice, demonstrating that CpG-ODN-induced suppression of Th2-mediated inflammation is not IL-12 dependent. This study also found that CpG-ODN increased B7.1, but not B7.2 expression by activated macrophages from IL-12 and IL-10/IL-12 double knockout mice as well as wild-type mice. These findings suggest that up-regulation of B7.1 may play an important role in CpG-ODN suppression of Th2 responses.

In the present study, we found that CpG-ODN treatment altered B7.1 and B7.2 mRNA expression in the lung with a greater increase in B7.1 mRNA in the simultaneously treated groups and a greater decrease in B7.2 mRNA in the postchallenge treated group.

Table I. Cytokine levels in Ag-stimulated spleen cell culture supernatants^a

Group	IFN- γ (pg/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	IL-13 (pg/ml)
None	445 $\pm$ 2.5	183 $\pm$ 16	924 $\pm$ 4	7549 $\pm$ 18
CpG 30-post	952 $\pm$ 19***	70 $\pm$ 3*	684 $\pm$ 10.5**	4992 $\pm$ 248*
CpG 100-post	1474 $\pm$ 5.5***	59 $\pm$ 1*	491 $\pm$ 9.5***	3818 $\pm$ 263*
CpG 30-simul	1514 $\pm$ 45.5***	73 $\pm$ 1.5*	544 $\pm$ 245**	4383 $\pm$ 417*
CpG 100-simul	1797 $\pm$ 50***	38 $\pm$ 1*	372 $\pm$ 2***	1338 $\pm$ 142**
ODN 30-simul	503 $\pm$ 3	105 $\pm$ 1.5	775 $\pm$ 61	6912 $\pm$ 57
Naive	975 $\pm$ 31	1.9 $\pm$ 0.5	15 $\pm$ 2	95 $\pm$ 0

^a Means $\pm$ SEM of spleens of four mice per group. *, $p < 0.05$ vs none; **, $p < 0.01$ vs none; ***, $p < 0.001$ vs none.

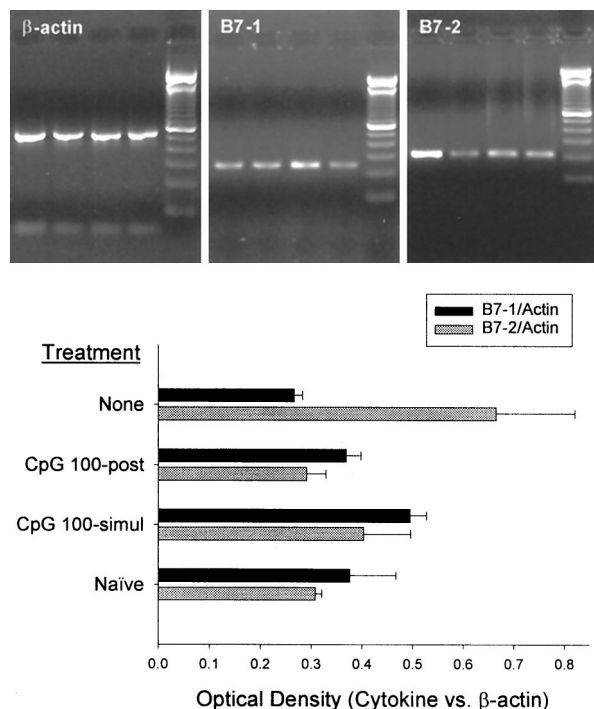


FIGURE 5. Semiquantitative analysis of B7.1 and B7.2 mRNA expression in the lung by RT-PCR. *A*, Gel illustration of density of B7.1 and B7.2 mRNA in lungs of untreated (lane 1), CpG 100-post treated (lane 2), CpG 100-simul treated (lane 3) and naive mice (lane 4). β -Actin mRNA expression is shown for comparison. *B*, OD ratios of B7.1 and B7.2 vs β -actin. Results are mean $\pm$ SEM of OD ratios for B7.1 and B7.2 in each experimental group ($n = 3$ –4/group).

Our finding of increased B7.1 expression is similar to the finding of Chiaramonte et al. (19). Although CpG depression of B7.2 expression has not been previously reported, our finding that suppression of B7.2 by CpG-ODN may be involved in the reduction of Th2 responses is compatible with findings that B7.2, but not B7.1, preferentially costimulates the initial production of IL-4 (35) and that anti-CD86 (B7.2), but not anti-CD80 (B7.1) treatment of mice significantly inhibited Ag-induced AHR, eosinophilia, and Ag-specific IgE, which was associated with the reduction of IL-4 and IL-5 (36, 37). Taken together, the above findings suggest that the suppressive effects of CpG-ODN on Th2 responses involve at least two immunoregulatory pathways. The first is induction of Th1 responses via activation of B7.1 expression. This pathway most likely explains the prophylactic effect of CpG-ODN on Th2 responses, described by Chiaramonte et al. (19). The second pathway, decreasing B7.2 expression, may more likely explain the therapeutic effect of CpG-ODN on ongoing Th2 responses. Several previous studies, mainly using cultured dendritic cells, found that CpG-ODN increased B7.2 expression (38–40). However, expression of B7.1 or B7.2 by cultured DC is dependent on the maturation state of DC and culture conditions. Thus, these previous studies are not necessarily contradictory to our finding of decreased B7.2 expression after CpG exposure of a mixed population of inflammatory and noninflammatory cells such as bronchial epithelial cells, in an allergic inflammatory milieu in vivo. Nevertheless, as in the case of CpG prophylactic effects on airway responses by CpG, the exact mechanisms responsible for the therapeutic effects of CpG on Th2-mediated inflammation and AHR in our study are largely unknown. Further research is necessary to assess the mechanisms of actions of CpG underlying these effects, including elu-

cidation of B7.1 and B7.2 expression by various cell types in the lungs of CpG-treated lungs.

In summary, we have demonstrated for the first time that the systemic administration of CpG-ODN can partially reverse Ag-induced airway inflammation and AHR, suggesting a potential approach for the treatment of allergic asthma. Although the mechanisms underlying these effects are not fully understood, down-regulation of Th2 cytokines likely contributes to the reduction of allergic airway responses. Furthermore, the down-regulation of Th2 responses by CpG-ODN may be associated with regulation of the costimulatory factors B7.1 and B7.2.

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