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

Denise Serebrisky, † 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.

Antigen-induced IgE production, airway inflammation and airway hyperreactivity (AHR) 1 (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 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 Th2 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...
School of Medicine. Standard guidelines (20) for the care and use of animals were followed. Cpg-ODNs consisted of 20 bases containing 2 Cpg motifs: (TC TACGAGTTCCTGAGCT) and a control ODN, identical except for re-arrangements of the Cpg motifs (TCCATGACCTGAGGCT) 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 (DPN- albumin) were purchased from Sigma (St. Louis, MO). Abs for ELISAs were purchased from the 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 μg CA adsorbed with 2 mg alum in 0.4 ml PBS on days 0 and 7. Mice were subsequently challenged intratracheally (i.i.t.) with 100 μg CA in 0.05 ml PBS on days 14 and 21. Sensitized mice received 30 μg (low dose) or 100 μg (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 (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. acetylicholine 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 laved 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 measurement. 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-μm 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 × 10⁶/ml) were cultured in 24-well plates in the presence or absence of CA (50 μg/ml) or Con A (2.5 μg/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 μg/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 μg/ml, PharMingen) were added to the plates and incubated for an additional 1 h at room temperature. After washing, the reaction was 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 above (18). Briefly, DPN-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 μl of the mixture of RNA (5 μg)-oligo(dT) (1 μl) was incubated at 70°C for 10 min and then incubated on ice for 2 min. Reaction mixture (7 μl, 1× 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 microliner (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 μl 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 μl total volume) was conducted in 2 mM MgCl₂, 1× PCR buffer, 2.5 U AmpliTaq DNA polymerase, 2 μl 10 μM anti-sense and sense primer pairs and 2 μl 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 45 sec, annealing, 60°C for 45 sec, and 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 μl 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′-ATGCTACGGTGTCAGGAGGA-3′, 19-mer; antisense 5′-GACGTTCTCTTTCAGCTAATG-3′), B7.2 (sense 5′-GACGTTCTCTTTCAGCTAATG-3′, 20-mer, 238 bp) and B7.2 (sense 5′-CAACGTGAGCTCAAGCTCAG-3′, 20-mer; antisense 5′-TGCTTGAGCTCGAGGTCAA-3′, 238 bp) were synthesized by Life Technologies, and β-actin in the 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 μg or 100 μg/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.
PAS-stained sections of lungs from mice treated with 100 
also appeared to affect airway mucus production, we compared 
26). To determine whether postchallenge CpG-ODN treatment 
tributing to the mortality associated with acute severe asthma (25, 
mucus plugging has long been recognized as a major factor con-
asthmatic patients and in animal models of allergic asthma, and 
instances, bronchial lumens in lung from Ag-sensitized and challenged untreated mice. A, a lung 
from CpG 100-post treated mouse containing fewer PAS-positive epithelial cells (bar, 100 μm).

![Figure 1](http://www.jimmunol.org/)

**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 μg or 100 μg) 
was administered 24 h postchallenge or simultaneously with Ag sensitiza-
tion. Control ODN (30 μg) 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 ± 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 si-
multaneous treatment groups at equivalent doses, these differences 
were not statistically significant. Control ODN treatment also ap-
peared 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 num-
bers in the high dose simultaneously treated group, the total num-
bers of BALF cells were also reduced by high dose postchallenge 
CpG-ODN treated (5.3 × 10⁴) as compared with the control ODN-
treated group (8.7 × 10⁵).

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 con-
tributing 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 μg 
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 markedly reduced, and little 
or no mucus was present in the bronchial lumens (Fig. 2B). Con-
sistent with the BALF findings, peribronchial and perivascular in-
flammation was also reduced by postchallenge CpG treatment 
(data not shown). These results demonstrate that CpG-ODN par-
tially reversed the processes responsible for Ag-induced eosino-
philic inflammation and mucus cell hyperplasia, which are asso-
ciated 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 post-
challenge treatment as well as CpG simultaneous treatment signif-
icantly 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 signifi-
cant 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 chal-
lenge 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, 
IL4, IL-5, and IFN-γ concentrations in BALF and spleen cell cul-
turants. 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 sen-
tsitization/challenge-treated groups (Table I). These results demon-
strate that CpG-ODN reversed systemic as well as local Th2 
responses.
models of asthma. Therefore, we evaluated the relative expression
in T lymphocyte activation in human asthma or mouse
have been determined which APC or combination of APCs plays a dom-
epithelial cells, eosinophils) (28). However, it has not yet
alveolar macrophages, and dendritic cells) and nontraditional
has been suggested that many cells in murine lungs can potentially
process and/or present Ag, including “professional” APCs (B cells,
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 cy-
tokine 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-IL4 ratio was increased (14). An effect of CpG-ODN on IL-13 synthe-
sis has not been previously reported.

Discussion
Previous studies have reported that the administration of CpG-
ODN during Ag sensitization of naive mice, or before Ag chal-
lenge of sensitized mice prevents Th2-directed allergic airway in-
flammation 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 eo-
sinophilic 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 μg 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 μg 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 al-
lergen 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 aller-
gic 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, eosino-
philic inflammation, mucus production, and IgE and IgG1 produc-
tion. Interestingly, these effects were equivalent to those induced
by simultaneous CpG administration at sensitization and chal-
lenge. 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 cy-
tokine 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-IL4 ratio was increased (14). An effect of CpG-ODN on IL-13 synthe-
sis has not been previously reported.
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, IL-5, 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 Chiaramonte 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

<table>
<thead>
<tr>
<th>Group</th>
<th>IFN-γ (pg/ml)</th>
<th>IL-4 (pg/ml)</th>
<th>IL-5 (pg/ml)</th>
<th>IL-13 (pg/ml)</th>
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<tr>
<td>None</td>
<td>445 ± 2.5</td>
<td>183 ± 16</td>
<td>924 ± 4</td>
<td>7549 ± 18</td>
</tr>
<tr>
<td>CpG 30-post</td>
<td>952 ± 19***</td>
<td>70 ± 3*</td>
<td>684 ± 10,5***</td>
<td>4992 ± 248*</td>
</tr>
<tr>
<td>CpG 100-post</td>
<td>1474 ± 5.5***</td>
<td>59 ± 1*</td>
<td>491 ± 9,5***</td>
<td>3818 ± 263*</td>
</tr>
<tr>
<td>CpG 30-simul</td>
<td>1514 ± 45.5***</td>
<td>73 ± 1.5*</td>
<td>544 ± 245***</td>
<td>4383 ± 417*</td>
</tr>
<tr>
<td>CpG 100-simul</td>
<td>1797 ± 50***</td>
<td>38 ± 1*</td>
<td>372 ± 2***</td>
<td>1338 ± 142**</td>
</tr>
<tr>
<td>ODN 30-simul</td>
<td>503 ± 3</td>
<td>105 ± 1.5</td>
<td>775 ± 61</td>
<td>6912 ± 57</td>
</tr>
<tr>
<td>Naive</td>
<td>975 ± 31</td>
<td>1.9 ± 0.5</td>
<td>15 ± 2</td>
<td>95 ± 0</td>
</tr>
</tbody>
</table>

* Means ± SEM of spleens of four mice per group. *, p < 0.05 vs none; **, p < 0.01 vs none; ***, p < 0.001 vs none.
Our finding of increased B7.1 expression is similar to the finding of Chiaromonte 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 B7.1 expression by various cell types in the lung and in lungs of untreated (lane 1), CpG 100-post treated (lane 2), CpG 100-simul treated (lane 3) and naive mice (lane 4). B-Actin mRNA expression is shown for comparison. B. OD ratios of B7.1 and B7.2 vs β-actin. Results are mean ± SEM of OD ratios for B7.1 and B7.2 in each experimental group (n = 3–4/group).

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). B. β-Actin mRNA expression is shown for comparison. B. OD ratios of B7.1 and B7.2 vs β-actin. Results are mean ± SEM of OD ratios for B7.1 and B7.2 in each experimental group (n = 3–4/group).

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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References


