

CpG oligodeoxynucleotides do not require T_{H1} cytokines to prevent eosinophilic airway inflammation in a murine model of asthma

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Background: Oligodeoxynucleotides (ODNs) containing the dinucleotide CpG in a specific sequence context (CpG-ODNs) have the ability to prevent the development of eosinophilic airway inflammation and bronchial hyperreactivity in a murine model of asthma. We have previously demonstrated that CpG-ODNs stimulate expression of the T_{H1}-inducing cytokines IFN- γ and IL-12 in a murine model of asthma and that this stimulation is associated with the protection against asthmatic inflammation.

Objective: The purpose of this study was to examine whether the protection conferred by CpG-ODNs in a schistosome egg-egg antigen murine model of asthma is dependent on the induction of IFN- γ , IL-12, or both.

Methods: C57BL/6 mice were sensitized to schistosome eggs in the presence or absence of CpG-ODNs or control ODNs and then stimulated with soluble egg antigen in the airway. The protection offered by CpG-ODNs in these mice was compared with the protection induced by CpG-ODNs in IL-12 and IFN- γ knockout mice and in mice treated with anticytokine blocking antibodies. Double-knockout mice (IL-12/IFN- γ) were also generated and used in these studies. Determinations included airway eosinophilic inflammation and bronchial hyperreactivity to inhaled methacholine.

Results: We found that CpG-ODNs confer protection against both airway eosinophilia and bronchial hyperreactivity in the absence of IFN- γ or IL-12 or in the presence of both cytokines together. However, in the absence of either IL-12 or IFN- γ , mice require 10 times as much CpG-ODNs to be protected against the induction of airway eosinophilia. The T_{H2} cytokines IL-4 and IL-5 were reduced in all of the CpG-treated mice, although less in the absence of IL-12 and IFN- γ .

Conclusion: These data indicate that CpG-ODNs prevent the generation of T_{H2}-like immune responses by multiple mechanisms, which involve, but do not require, IL-12 and IFN- γ . A direct suppressive effect of CpG-ODNs on T_{H2} responses is suggested by their reduction in IFN- γ and IL-12 knockout mice. (J Allergy Clin Immunol 1999;1258-64.)

Key words: Asthma, murine, CpG oligodeoxynucleotides, IFN- γ , IL-12, T_{H1}

Asthma is an inflammatory disease of the airways characterized by airway eosinophilic inflammation, bronchospasm, and hyperreactivity to nonspecific inhaled stimuli.¹ These attributes are mediated, to a great degree, by T_{H2}-like cytokines, which include IL-4 and IL-5 and which lead to eosinophil and mast cell chemotaxis, activation, and degranulation and B-cell production of IgE.^{2,3} Induction of T_{H2}-like cytokines and clonal proliferation of committed T_{H2} lymphocytes can be prevented and reversed by T_{H1}-like cytokines, notably IFN- γ and IL-12,⁴ which can prevent the development of T_{H2}-mediated airway inflammation.⁵ We have previously demonstrated that CpG motifs present in bacterial DNA or in synthetic oligodeoxynucleotides (ODNs) can induce release of IFN- γ and IL-12 both in vitro and in vivo.⁶⁻⁸ In addition, using a schistosome egg-schistosome egg antigen murine model of asthma, we have reported that administration of CpG-ODNs along with the sensitizing antigen can prevent the development of airway eosinophilia, release of T_{H2}-type cytokines into the airway, systemic IgE production, and bronchial hyperreactivity to inhaled methacholine.⁹ We initially hypothesized that the induction of T_{H1} cytokine expression by CpG-ODNs protects against the development of eosinophilic inflammation in asthma. In this study we specifically examined the role of IFN- γ and IL-12 in the protection against eosinophilic airway inflammation provided by CpG-ODNs by using both anticytokine antibodies and cytokine gene knockout mice.

METHODS

Animals and murine model of asthma

Six-week-old female C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, Me). All animal care and housing requirements of the National Institutes of Health Committee on Care and Use of Laboratory Animals were followed. For some studies, mice on a C57BL/6 genetic background with disrupted genes for IFN- γ (C57BL/6-*Ifng*^{tm1Ts}, Jackson Laboratory)¹⁰ or IL-12 (C57BL/6-*Il12atm1Jm*, Jackson Laboratory)¹¹ were used. The IFN- γ knockout mice produce no IFN- γ ; the IL-12 knockout mice produce no p40 and are thus unable to form active IL-12 heterodimers. In addition to the cytokine knockout mice that were commercially available, we cross-bred IFN- γ and IL-12 knockout mice on C57BL/6 backgrounds to produce double-knockout mice. Mice were genotyped by PCR of tail-derived DNA. Genes for wild-type (endogenous) IFN- γ were detected by using primers 5'-AGAAG-

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Abbreviations used

ODN: Oligodeoxynucleotide

SEA: Soluble egg antigen

TAAGTGAAGGGCCAGAG-3' and 5'-AGGGAACTGGGA-GAGGAGAAATAT-3', which amplify a 220-bp product from the endogenous *Ilg* allele; the IFN- γ disruption was detected by using primers 5'-TCAGCGCAGGGCGCCCGTTCTTT-3' and 5'-ATC-GACAAGACCGGCTTCCATCCGA-3', which amplify a 375-bp product from the targeted *Ilg* allele, as per the Jackson Laboratory protocol. Genes for wild-type (endogenous) IL-12 were detected by using primers 5'-AGTGAACCTCACCTGTGACACG-3' and 5'-TCTTTGCACCGCCATGAGC-3', which amplify a 680-bp product from the endogenous *IL12b* gene; the *neo* insert was detected by using primers 5'-CTTGGGTGGAGAGGCTATTC-3' and 5'-AGGTGA-GATGACAGGAGATC-3', which amplify a 280-bp product from the *neo* insert, as per the Jackson Laboratory protocol (Fig 1). The absence of detectable IFN- γ or IL-12 was confirmed by stimulating splenocytes from these mice with LPS (1 μ g/mL) in vitro and assaying the supernatant for IL-12 (after 24 hours in culture) and IFN- γ (after 48 hours in culture). Neither cytokine was detectable in culture supernatants. In other studies, mice lacking the type I IFN receptor were used (B&K Universal Ltd, East Yorkshire, UK).¹² These mice, on the SV/129 background, have the IFN- α receptor gene disrupted and thus are unable to respond to type I IFNs.

Mice were sensitized, on day 0, to *Schistosoma* eggs and challenged, on days 7 and 14, with soluble egg antigen (SEA, 10 μ g) as previously described.⁹ Other mice received saline solution and served as negative controls. ODNs (either CpG or control) were administered to some mice by intraperitoneal injection at the time of sensitization to the schistosome eggs. The standard dose of ODN was 30 μ g, but for some studies, a range of doses was used. Physiology studies were carried out 4 to 6 hours after the second challenge with SEA or saline, and mice were killed for whole-lung lavage 6 hours after the second challenge. For some studies, blood samples were obtained by retro-orbital puncture.

Reagents

ODNs. The CpG-ODNs that we used (#1826) consisted of 20 bases containing 2 CpG motifs (TCCATGACGTTCTGACGTT). The control ODN was identical, except that the CpG motifs were rearranged (TCCATGAGCTTCCTGAGTCT). ODNs were produced by Oligos Etc (Wilsonville, Ore) under Good Manufacturing Practice conditions and had undetectable levels of LPS.

Cytokines. Recombinant murine IL-12 was a kind gift of Dr M Gately (Hoffmann-La Roche, Nutley, NJ). Recombinant murine IFN- γ was purchased from R&D Inc (Minneapolis, Minn).

Antibodies. To block the effects of IL-12, we used the rat anti-mouse IL-12 IgG1 TOSH, which was purchased from Endogen (Woburn, Mass).¹³ To block the effects of IFN- γ , we used the rat anti-mouse IFN- γ IgG1 antibody from the hybridoma R4-6A2 (ATCC, Rockville, Md). For a control antibody, we used a rat IgG1 isotype control (Pharmingen) in equal amounts. The doses chosen were based on preliminary studies (not shown) demonstrating effective blockade of the cytokines' bioactivity.

ELISA

Murine IL-4 and IL-5 were measured by using a sandwich ELISA (R&D) according to the manufacturer's instructions.

Whole-lung lavage

After euthanasia, the trachea was cannulated, and the lungs underwent lavage with 3.0 mL of sterile normal saline (0.9% NaCl).

The lavage samples were immediately processed for total and differential (with Diff Quick Stain; Baxter Scientific, Miami, Fla) cell counts.

Physiology

Airway hyperreactivity was measured by methacholine-induced airflow obstruction by using whole-body plethysmographs (Buxco Electronics, Inc, Troy, NY) as previously described.⁹ Airway resistance was expressed as follows: $P_{enh} = ([T_e/0.3 T_r] - 1) \times [2P_{ef}/3P_{if}]$, where P_{enh} equals enhanced pause, T_e equals expiratory time (in seconds), T_r equals relaxation time (in seconds), P_{ef} equals peak expiratory flow (in milliliters per second), and P_{if} equals peak inspiratory flow (in milliliters per second). Increasing doses of methacholine were administered by nebulization (for 150 seconds), and enhanced pause was calculated over the subsequent 3 minutes.⁹

Statistics

Analysis of time-course and dose-response curves was performed by using ANOVA with post-hoc Tukey tests. Pairs of groups were compared by using the Student *t* test. *P* values for significance were set at .05. Values for all measurements are expressed as means $\pm$ SEM.

RESULTS

Effect of T_{H1} cytokines on airway eosinophilia and bronchial hyperreactivity

To ascertain whether T_{H1} cytokines were capable of preventing the development of T_{H2} -mediated airway eosinophilia in the schistosome egg-SEA murine asthma model, we first examined the effects of administering IFN- γ and IL-12 at various time points relative to sensitization and airway challenge with schistosome eggs and SEA. We found that treatment with IL-12 (1 μ g administered intraperitoneally on days 0, 1, 2, and 3), as well as with IFN- γ (10,000 U administered intraperitoneally on days 0, 1, 2, and 3), substantially reduced the subsequent development of airway eosinophilia after challenge with SEA. IL-12 therapy diminished airway eosinophils from $2.3 \pm 0.4 \times 10^6$ to $0.4 \pm 0.3 \times 10^6$, and IFN- γ therapy diminished airway eosinophils to $0.8 \pm 0.2 \times 10^6$ ($P < .05$ each).

Effect of CpG-ODNs in the absence of IFN- γ

We next explored whether IFN- γ is required for the protective effects of CpG-ODNs in the schistosome egg murine model of asthma.⁹ First, we administered anti-IFN- γ antibodies (R4-6A2) at the time of the administration of CpG-ODNs (30 μ g) and schistosome eggs. These antibodies are known to specifically block the effects of IFN- γ in vivo.¹⁴ The antibodies were administered by intraperitoneal injection 1 hour before and 3 days after the administration of schistosome eggs with or without CpG-ODNs. At the time of death, after the second airway challenge, we found that administration of the anti-IFN- γ antibody along with the CpG-ODNs and schistosome eggs only minimally decreased the protective effect of CpG-ODNs (Fig 2, A). Control antibodies had no effect. Mice who were treated with CpG-ODNs in the setting of anti-IFN- γ antibodies were also protected against the development of airway hyperreactivity, as assessed by

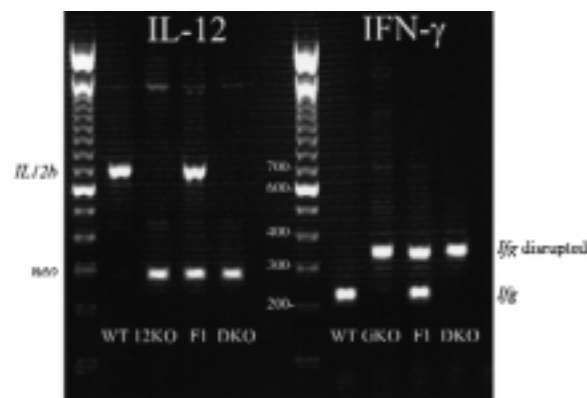


FIG 1. Genotyping of mice for IL-12 and IFN- γ . Mice on a C57BL/6 background (WT) with a disruption in the gene for IL-12 (12KO) and IFN- γ (GKO) were cross-bred, resulting in a first generation of mice heterozygous for both genes. The first-generation mice were bred to yield double-knockout (DKO) mice, with genes disrupted for both IL-12 and IFN- γ . DNA extracted from tail snips of the mice was used for PCR of the intact *Il12b* gene (680 bp), the *neo* insert in the IL-12 knockout mice (280 bp), the intact *Ifg* gene (220 bp), and the disrupted *Ifg* gene (375 bp).

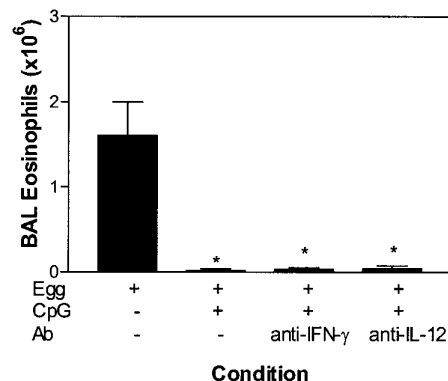
response to increasing doses of inhaled methacholine (Fig 2, B).

Because of the possibility that the antibodies, administered intraperitoneally, may not provide effective blockade against the local pulmonary effects of induced IFN- γ , we next evaluated whether CpG-ODNs would be effective in preventing the development of eosinophilic airway inflammation in the complete absence of the cytokine. For these experiments, we used IFN- γ knockout mice¹⁰ in our standard model.⁹ IFN- γ knockout mice had airway eosinophilia to a similar degree as wild-type C57BL/6 mice (Fig 3, A). Supporting the results that we obtained in the blocking antibody studies, CpG-ODNs appeared equally efficacious in IFN- γ knockout mice as in wild-type mice. In addition to blockade of airway eosinophilia, IFN- γ knockout mice treated with CpG-ODNs were also protected from the development of airway hyperreactivity (Fig 3, B).

Effect of CpG-ODNs in the absence of IL-12

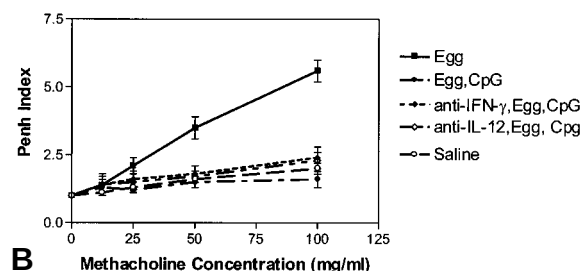
We next examined the role of IL-12 in CpG-ODN protection. These studies were conducted similarly to those described above for IFN- γ and included blocking antibody and cytokine gene knockout experiments. The antibody TOSH was administered (500 μ g administered intraperitoneally) 1 hour before and 3 days after the mice received schistosome eggs. These studies demonstrated that blockade of IL-12 only modestly reduced the protective effect of CpG-ODNs on the prevention of airway eosinophilia (Fig 2, A) and airway hyperreactivity (Fig 2, B).

We further examined the role of IL-12 in the protection offered by CpG-ODNs against the development of airway eosinophilia by using IL-12 knockout mice, which have a null mutation in the gene for IL-12 p40 and are unable to produce biologically active IL-12.¹¹ We found that IL-12 knockout mice are also protected from eosinophilic inflammation (Fig 3, A) and the develop-



A

Condition



B

Methacholine Concentration (mg/ml)

FIG 2. CpG-ODNs are effective in prevention of eosinophilic airway inflammation and bronchial hyperreactivity in the presence of antibodies to IFN- γ or IL-12. Administration of anti-IFN- γ or anti-IL-12 blocking antibodies does not significantly impair the effectiveness of CpG-ODNs in prevention of eosinophilic airway inflammation (**A**) or bronchial hyperreactivity to inhaled methacholine (**B**). There is no significant difference between the CpG groups (* P < .005 vs egg group; n = 4 for each group). BAL, bronchoalveolar lavage; *Penh*, enhanced pause.

ment of airway hyperreactivity (Fig 3, B) by administration of CpG-ODNs.

Effect of CpG-ODNs in the absence of IFN- γ and IL-12

To further evaluate whether the anti-inflammatory effects of CpG-ODNs required IFN- γ or IL-12, we next bred double-knockout mice (ie, C57BL/6 mice with disrupted genes for both IFN- γ and IL-12). When used in the schistosome egg-SEA model in the presence and absence of CpG-ODNs, airway eosinophilia (Fig 3, A) and bronchial hyperreactivity (Fig 3, B) developed in the double-knockout mice. CpG-ODNs, but not control ODNs, abrogated both of these responses.

Because the protection conferred by CpG-ODNs on both IFN- γ knockout and IL-12 knockout mice was qualitatively similar to the protection offered to wild-type C57BL/6 mice, we next examined whether it was quantitatively identical. In the preceding studies, we used CpG-ODNs in the amount previously found to be well within the protective range (30 μ g).⁹ Therefore we proceeded to examine whether the dose-response relationship between the amount of CpG-ODNs used and the protection against eosinophilia was altered in either or both of the cytokine knockout mice. For these studies, we used our standard protocol but administered varying amounts of CpG-ODNs (0.3-30 μ g) at the time of sensitization (Fig

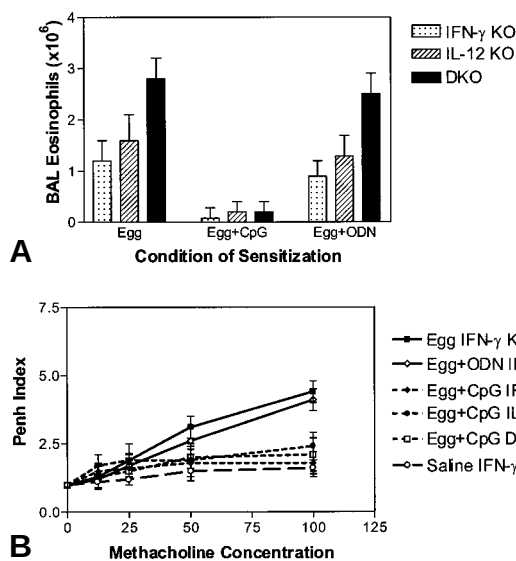


FIG 3. CpG-ODNs are effective in prevention of eosinophilic airway inflammation and bronchial hyperreactivity in IFN- γ knockout (KO), IL-12 knockout, and double-knockout (DKO) mice. Mice were sensitized to schistosome eggs in the presence or absence of CpG-ODNs or control ODNs. Knockout mice that receive CpG-ODNs, but not those who receive control ODNs at the time of sensitization, are protected against the development of airway eosinophilia (**A**) and hyperreactivity (**B**) to inhaled methacholine. Methacholine dose-response curves are shown for each strain of mice in the CpG-ODN plus schistosome egg groups, but saline control, schistosome eggs alone, and schistosome eggs plus control ODN curves are shown only for IFN- γ knockout mice. BAL, bronchoalveolar lavage; Penh, enhanced pause.

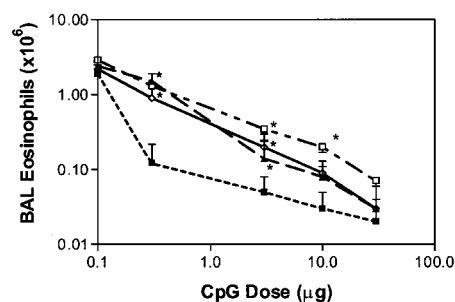


FIG 4. In the absence of IFN- γ or IL-12, mice are relatively deficient in response to CpG-ODNs. Varying amounts of CpG-ODNs (0.3, 3, 10, or 30 μ g) were administered to wild-type C57BL/6, IFN- γ knockout (KO), IL-12 knockout, and double-knockout (DKO; IFN- γ /IL-12 knockout) mice each on a C57BL/6 background at the time of sensitization to schistosome eggs. All mice who received 30 μ g and the wild-type mice who received 10 μ g of CpG-ODNs were fully protected against airway eosinophilia. There was no difference in airway eosinophilia between any of the groups that received no CpG-ODNs (not shown). A significant difference in the development of airway eosinophilia was seen between the wild-type and the knockout mouse groups, which received between 0.3 and 10 μ g of CpG-ODNs ($*P < .01$ vs wild-type C57BL/6 group; $n = 4$ mice in each group). BAL, Bronchoalveolar lavage.

4). We found that at lower doses of CpG-ODNs (<10 μ g), both of the knockout mice were offered significantly less protection against the development of airway eosinophilia than wild-type C57BL/6 mice, and the double-knock-

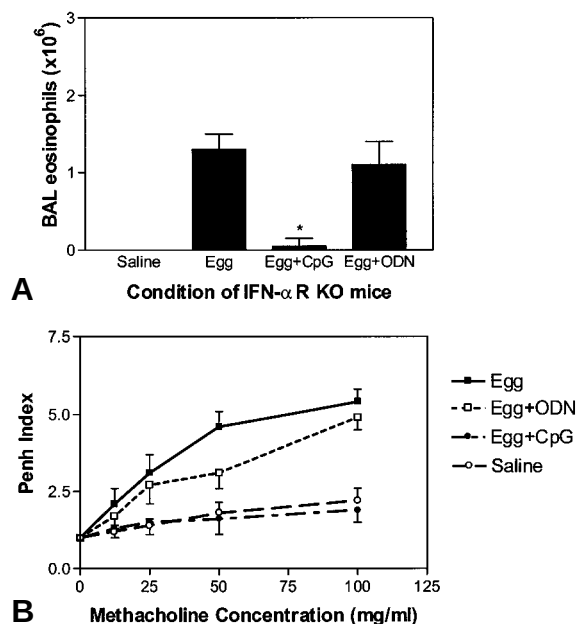


FIG 5. CpG-ODNs are effective in prevention of eosinophilic airway inflammation and airway hyperreactivity in IFN- α receptor (R) knockout (KO) mice. IFN- α receptor knockout mice were sensitized to schistosome eggs in the presence or absence of CpG-ODNs or control ODNs. CpG-ODNs, but not control ODNs, significantly reduces the development of airway eosinophilia (**A**) and methacholine-induced bronchospasm (**B**) in IFN- α receptor knockout mice ($*P < .01$ vs schistosome egg-sensitized group; $n = 4$ for each group). BAL, bronchoalveolar lavage; Penh, enhanced pause.

out mice required even greater doses of CpG-ODNs. These changes in airway eosinophilia generally paralleled the effects of CpG-ODNs on airway hyperreactivity. Wild-type C57BL/6 mice had increases in schistosome egg-induced airway hyperreactivity after doses of CpG-ODNs of less than 3 μ g, and all of the knockout mice had increased airway hyperreactivity at doses of less than 10 μ g of CpG-ODNs (data not shown).

Effect of CpG-ODNs in the absence of type I IFNs

Because we were unable to completely explain the protection offered by CpG-ODNs against eosinophilic airway inflammation and bronchial hyperreactivity through the induction of IFN- γ or IL-12, we next evaluated whether CpG-ODNs are dependent on the induction of type I IFNs for protection against eosinophilic airway inflammation. For these studies, we subjected IFN- α receptor knockout mice to the schistosome-SEA model and found that the mice had somewhat less airway eosinophilia than did the C57BL/6 mice but that CpG-ODNs provided complete protection (Fig 5, A); these mice were also protected against the development of bronchial hyperreactivity (Fig 5, B).

Effect of CpG-ODNs on T_H2 cytokines

Because we found clear evidence that CpG-ODNs provide protection in the absence of IFN- γ and IL-12, we

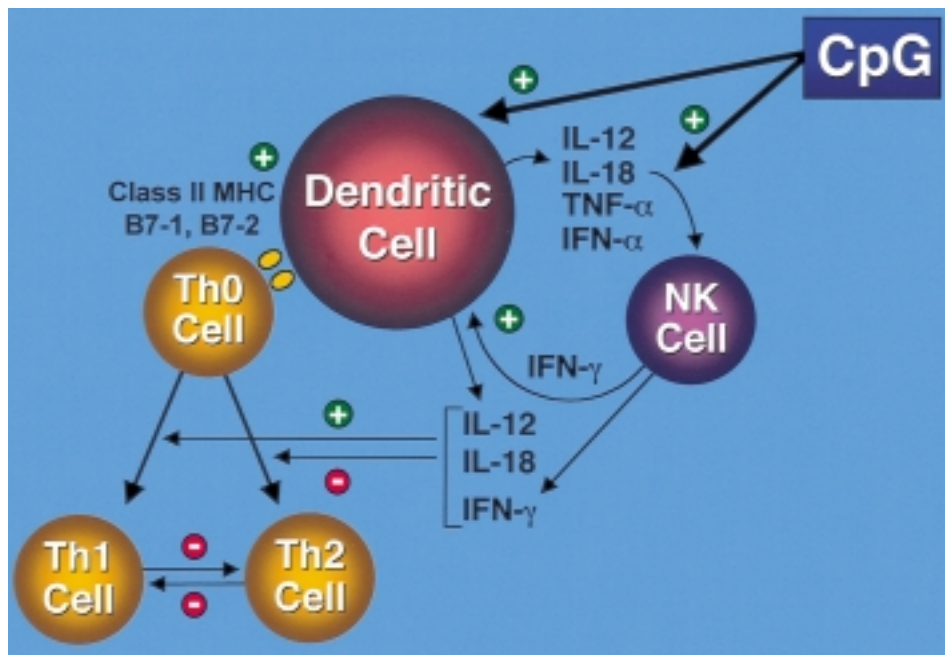


FIG 6. Proposed mechanisms through which CpG-ODNs diminish atopic inflammation. CpG-ODNs may both directly and indirectly reduce T_{H2} -mediated eosinophilic inflammatory responses. CpG-ODNs are known to induce dendritic cells (and other monocytic cells) to release mediators, such as IL-12, IL-18, and IFN- α . These cytokines induce the production of IFN- γ by natural killer (NK) cells, and this induction is augmented by CpG-ODNs. The IFN- γ further activates dendritic cells to release T_{H1} -inducing cytokines. CpG-ODNs also promote the expression of class II MHC surface markers and the costimulatory molecules B7-1 and B7-2, which interact with uncommitted T cells; these T cells, in the environment of T_{H1} cytokines from the dendritic and natural killer cells, are skewed toward T_{H1} and away from T_{H2} phenotypes.

TABLE I. Effect of CpG-ODNs on serum levels of T_{H2} cytokines in wild-type and knockout mice

	Serum IL-4			Serum IL-5		
	Control	Schistosome	CpG	Control	Schistosome	CpG
C57BL/6 mice	ND	124 $\pm$ 37 pg/mL	47 $\pm$ 41 pg/mL*	ND	83 $\pm$ 58 pg/mL	28 $\pm$ 23 pg/mL [‡]
IFN- γ knockout mice	ND	248 $\pm$ 68 pg/mL	92 $\pm$ 28 pg/mL [†]	ND	132 $\pm$ 35 pg/mL	54 $\pm$ 41 pg/mL [‡]
IL-12 knockout mice	ND	154 $\pm$ 49 pg/mL	76 $\pm$ 19 pg/mL*	ND	73 $\pm$ 49 pg/mL	68 $\pm$ 34 pg/mL
Double-knockout mice	ND	193 $\pm$ 71 pg/mL	128 $\pm$ 43 pg/mL [†]	ND	155 $\pm$ 44 pg/mL	74 $\pm$ 23 pg/mL [‡]

No significant differences were detected in any treatment group between the 4 strains of mice.

ND, Not detectable.

There were 4 mice in each group.

* $P < .05$ and $^{\dagger}P < .01$ for IL-4 levels in CpG versus schistosome mice.

[‡] $P < .05$ for IL-5 levels in CpG versus schistosome mice.

next examined whether we could demonstrate a reduction in the T_{H2} cytokines IL-4 or IL-5 in mice treated with CpG-ODNs. For these studies, we used mice (C57BL/6, IFN- γ knockout, IL-12 knockout, and double-knockout mice) that were sensitized to schistosome eggs (or received saline as controls) in the presence or absence of CpG-ODNs. Seven days after sensitization, we measured serum levels of IL-4 and IL-5 (Table I). We found that serum levels of both cytokines were undetectable in all mice who were not sensitized to the schistosome eggs and that sensitized mice of all strains demonstrated significantly greater levels of IL-4 and IL-5 than control mice. CpG-ODNs led to significant reduction of IL-4 and IL-5 in all strains of mice; there were no significant dif-

ferences in the serum levels of these cytokines in the knockout compared with the wild-type (C57BL/6) mice.

DISCUSSION

Immunostimulatory CpG-ODNs have a wide range of effects on inflammation and immune responses.^{8,16-20} Recently, we have shown that CpG-ODNs are also effective in prevention of eosinophilic airway inflammation in a murine model of asthma.⁹ Most of these actions of CpG-ODNs have been associated with, or attributed to, the induction of a T_{H1} -like immune response. This response is characterized by production of IFN- γ and IL-12. To evaluate whether the association between the T_{H1}

cytokine expression and the protection offered by CpG-ODNs against the development of eosinophilic airway inflammation was causal, we initiated the studies described in this article.

Our initial hypothesis, that protection against T_{H2} -mediated airway eosinophilia in the schistosome-SEA murine model of asthma by CpG-ODNs is mediated through induction of IFN- γ , IL-12, or both, was supported by our finding that either cytokine, in sufficient quantity, could prevent the development of airway eosinophilia. Subsequently, however, we were surprised to find that neither cytokine alone, nor a combination of both, was absolutely required for protection. Nevertheless, the altered dose-response relationship between CpG-ODNs and the prevention of airway eosinophilia, which we found in both IFN- γ and IL-12 knockout mice does support the hypothesis that these cytokines can mediate or contribute to the response to CpG-ODNs. Therefore we remain convinced that induction of the T_{H1} cytokines IFN- γ and IL-12 are important in mediating the protective effects of CpG-ODNs.

Redundancy in the regulation of immune responses is more the norm than the exception. In the present system it seems likely that induction of both IFN- γ and IL-12 contribute to the ability of CpG-ODNs to prevent the development of airway eosinophilia. Previous studies have demonstrated the efficacy of both IFN- γ ²¹ and IL-12^{5,22} in diminishing or preventing eosinophilic inflammation in murine models of asthma. Interestingly, Wynn et al²³ have demonstrated that, in the absence of IFN- γ , administration of IL-12 may actually increase the development of eosinophilic granuloma formation in a murine model of schistosome egg-induced pulmonary granulomatous disease. In this same study IL-12 administration did promote IL-2 production, suggesting that IL-12 inhibits T_{H2} responses primarily through the induction of IFN- γ while promoting (to a limited degree) T_{H1} responses through direct effects that are not dependent on the production of IFN- γ .

Although our studies demonstrate that both IFN- γ and IL-12 can be involved in the protection against the development of eosinophilic airway inflammation offered by CpG-ODNs, they suggest that other mechanisms may also be important in inducing this protection. One group of candidates for mediators of CpG-ODN effects is type I (α or β) IFNs. Each of these cytokines has the potential to induce T_{H1} -type responses and thus downregulate a T_{H2} -mediated immune response. Synergistically with IL-12, IFN- α can increase production of IFN- γ by natural killer cells²⁴ and induce T_{H1} clonal expansion.¹³ In addition, it may prevent IL-4-mediated suppression of T_{H1} cells,²⁵ IFN- β stimulates T_{H1} clonal proliferation²⁶ and induces IFN- γ and IL-12 from peripheral lymphocytes.²⁷ IFN- β also can downregulate T_{H1} responses in some settings by inhibiting IL-12 secretion²⁸ and T_{H1} cell development.²⁹ Nevertheless, the type I receptor knockout mice do not appear to be more T_{H2} deviated in our asthma model, and our data demonstrating that CpG-ODNs are effective in these

mice suggest that type I IFN responses are not central to the effects of CpG-ODNs.

Finally, there is a suggestion that CpG-ODNs may be able to directly downregulate T_{H2} responses. We found suppression of the T_{H2} cytokines IL-4 and IL-5 in mice who were sensitized to schistosome eggs in the presence of CpG-ODNs, even in the absence of IL-12 and IFN- γ . These data are also, however, consistent with the hypothesis that alternative T_{H1} -like signals are induced by CpG-ODNs.

Thus we have demonstrated that the CpG-ODNs can effectively prevent the development of eosinophilic airway inflammation and bronchial hyperreactivity in the absence of IFN- γ , IL-12, or both. However, in the absence of either or both cytokines, significantly greater amounts of CpG-ODNs are required to protect against eosinophilic airway inflammation. Furthermore, type I IFNs do not appear to be central in mediation of this protective effect. CpG-ODNs may work through currently unidentified mediators of inflammation, may directly suppress T_{H2} responses, or may take advantage of the great redundancy in the inflammatory pathways that modulate T_{H1} and T_{H2} activity (Fig 6).

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