

REVIEW

CpG DNA and immunotherapy of allergic airway diseases

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Summary

Atopic asthma is a highly prevalent and serious health problem for which no therapy currently offers the hope of a cure. Preindustrialized and rural populations appear relatively protected from the asthma epidemic; the hygiene hypothesis ascribes this protection to the effects of microbes and microbial products. An important immunostimulant component of microbes is DNA; bacterial DNA contains sequence motifs centred on the CpG dinucleotide, which are suppressed in mammalian DNA. Oligonucleotides containing these motifs (CpG ODN), like bacterial DNA, promote Th1 and regulatory-type immune responses. Using CpG ODN, we and others have demonstrated in murine studies that CpG ODN are effective in preventing the development of atopic airways disease. Moreover, when administered in conjunction with experimental allergen, they promote the reversal of established eosinophilic inflammation. These data suggest that CpG ODN may be a novel therapeutic tool for the treatment of atopic asthma.

Keywords asthma, atopic, bacterial DNA, cytokine, immunotherapy, T-regulatory, vaccine

Introduction

Atopy, an immune-mediated response to otherwise innocuous environmental antigens (allergens), is a major cause of airway diseases including asthma and rhinosinusitis. These disorders, which are increasing in prevalence and severity in developed but not pre-industrialized nations, account for billions of dollars of health care spending, substantial morbidity, and a mortality rate of approximately 5000 deaths annually in the USA alone. Despite considerable growth in our understanding of the molecular mechanisms of allergic inflammation, and extensive research and development expenditures by academia and the pharmaceutical industry, atopic diseases remain incurable at this time. Recent advances in therapy aimed at immunomodulation now offer the possibility of curative treatment for atopic disorders. CpG oligodeoxynucleotides (ODNs) represent a novel class of drugs with substantial immunomodulatory properties. Preclinical studies have demonstrated the effectiveness of CpG ODN in the prevention and treatment of both upper and lower allergic airway disease using murine models [1–4]. Data from ongoing human trials using CpG ODN suggest that it is a safe and well-tolerated agent that seems promising for the treatment of allergic airway inflammation.

Atopic asthma is an inflammatory disorder of the airways that is characterized by eosinophilic inflammation, bronchial hyper-responsiveness and intermittent airflow obstruction, and elevated immunoglobulin (Ig) E levels. It is now established that CD4⁺ T lymphocytes have a pivotal role in

driving the immune responses of the allergic airway. CD4⁺ T cells have been categorized into at least two populations: T helper (Th) 1 and Th2, based on their cytokine expression profiles. There also exist naturally arising regulatory T cells (T_R or Th3 cells: CD4⁺CD25⁺CD45RB^{Lo}, about 10% of peripheral T cells) that are believed to control the magnitude and character of non-regulatory T cell responses. Th1 and Th2 responses are counter-regulatory, as Th2 responses enhance asthmatic inflammation via up-regulation of interleukin (IL-) 4, IL-5, and IL-13, and the induction of Th1 responses appears to counteract these changes. Although numerous explanations have been proposed, the 'hygiene hypothesis' is one of the most discussed theories explaining the rising prevalence of atopy and asthma in developed nations. This hypothesis suggests that increased early-life exposure to microbes and their products in developing nations protects against atopic sensitization by polarizing naïve T cells towards a Th1 predominance; more recently, it has been suggested that induction of IL-10-producing regulatory T-cells may play a similar or even more important role in reducing Th2 deviation and Th2-dependent allergic disorders. While the validity of this hypothesis remains to be determined, it is now widely accepted that altered regulation of Th2 responses (and possibly the balance between Th1 and Th2 responses) is an important factor in the development of atopy.

Therapeutic options for the treatment of asthma have been essentially unchanged, with the exception of leukotriene modifiers, for over two decades. Indeed, although the recent enhanced emphasis on anti-inflammatory therapy for persistent asthma is hoped to result in reduced morbidity mortality, and perhaps airway modelling, this strategy is aimed neither at primary prevention, nor at curative treatment, of asthma. Asthma remains a disease of complex

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and multifactorial etiology, and hundreds of mediators have been implicated in its development, including cytokines, chemokines, immunoglobulins, peptide mediators, and proteases. Although preclinical studies and clinical trials of strategies designed to eliminate the effects of many of these mediators have been carried out or are ongoing, it is perhaps reasonable to doubt that blocking any single mediator will have a profound impact on the genesis of allergic inflammation. Suppression and reversal of atopic responses using specific allergen immunotherapy (desensitization) has recently regained favour among some practitioners. Immunotherapy traditionally involves subcutaneous administration of allergen in incremental doses; its efficacy is speculated to be due to immune deviation, active regulation, deletion, or energy. Although the literature is divided regarding the use of allergen immunotherapy in asthma, recent meta-analyses demonstrate a significant beneficial response following this treatment [5, 6]. For wider acceptance, the development of reagents with both enhanced safety and improved efficacy (which regrettably often change in concert) would be ideal. One potential approach towards this may be the use of recombinant peptides (eliminating anaphylactogenic motifs) and efficacy-enhancing adjuvants.

Immune modulation of atopic responses by CpG DNA

Bacterial, but not vertebrate DNA, contains unmethylated CpG dinucleotides that are substantially responsible for its immunostimulatory properties [7]. The innate immune system uses toll-like receptor (TLR) 9, one of the pathogen-associated molecular pattern (PAMP) recognition elements, to identify and transduce the responses to CpG DNA [8]. In humans, TLR9 is expressed on antigen-presenting cells (APCs: B-lymphocytes, dendritic cells, and others) [9]; and plays a critical role in CpG-mediated activation of immune responses [10]. Once activated by CpG DNA, APCs secrete a wide array of cytokines, chemokines, and other mediators that promote the activation of naïve CD4⁺ cells, enhancing their differentiation into Th1 cells [9].

The immunology of asthmatic inflammation seems centred on the CD4⁺ T cell: Th2 cells produce cytokines and chemokines responsible for many of the manifestations of asthma, and mice depleted of CD4⁺ cells (using anti-CD4 antibody) develop neither airway eosinophilia nor airway hyper-reactivity (AHR) [11]. IL-4 and IL-5 are known to play a key role in the recruitment of eosinophils to the airway. Indeed, IL-4- and IL-5-deficient mice have limited eosinophil infiltration of their airways and reduced AHR in response to allergen sensitization and challenge [12, 13]. In contrast, IL-4 and IL-5 transgenic mice develop enhanced airway eosinophilia, although not AHR [14, 15]. Interestingly, neutralization of IL-4 has been shown to be ineffective in reversing established atopic inflammation and AHR [16, 17], and neutralization of IL-5 reverses established eosinophilia, but not AHR [18]. These data suggest that IL-4 and IL-5 may play a key role in the establishment of allergen-induced acute airway inflammation, but are only moderately important in maintaining these responses, especially with regard to AHR. This is consistent with the disappointing results of a

recently reported clinical trial using anti-IL-5 antibody, in which eosinophilia was reduced, but AHR was unchanged [19]. CpG DNA clearly inhibits IL-4 and IL-5 in murine models of acute allergic inflammation [2, 3, 20–22] and of established asthma [1], but its protective and therapeutic effects are likely to result from modulation of other mediators as well.

IL-13 has been recently shown to be critical for the development of allergen-induced AHR; this is independent of eosinophilic airway inflammation, and may result from direct effects on airway epithelial cells and promotion of goblet cell hyperplasia [23, 24]. IL-13^{-/-} mice fail to develop allergen-induced AHR, despite the presence of airway eosinophilic inflammation [25]. Moreover, adoptive transfer of antigen-specific Th2 cells generated from IL-13^{-/-} mice failed to induce AHR, in contrast to the transfer of cells generated from IL-4^{-/-} mice [25, 26]. Interestingly, glucocorticoid steroids appear to be ineffective in suppressing IL-13-induced AHR [27]. Suppression of IL-13 can reverse established AHR [28], raising the possibility that this may be a mechanism through which CpG DNA modulates established atopic airway inflammation. CpG DNA has been reported to inhibit IL-13 and goblet cell hyperplasia in a murine model of acute allergic inflammation [29], and recent studies in our laboratory using a murine model of mucosal immunotherapy suggest that CpG ODNs may reverse established AHR at least partly through suppression of IL-13 (unpublished data).

IL-12, a critical Th1 mediator that potently induces IFN- γ , has been repeatedly shown to be capable of inhibiting allergic lung inflammation; it can additionally reverse established lung inflammation and AHR has been demonstrated, through it by suppressing IL-4 and IL-13 [30]. However, an early study of inhaled IL-12 in human asthmatics was terminated because of adverse effects [31], and systemic use in mild asthmatics has been disappointing [32]. We have previously reported that while the effects of CpG DNA are partly mediated via Th1 cytokines (IL-12 and IFN- γ), these are not critical for the observed protective effects; elimination of both only minimally reduces the protective effects of CpG DNA [33]. Moreover, although CpG ODNs promote induction of Th1 cytokines, these levels are highest following stimulation with moderate concentrations of CpG ODNs, while the suppression of Th2 cytokines is maximal at higher concentrations of CpG ODNs [34]; the dose-dependent induction of IL-10 appears to be responsible for this, suggesting that IL-10 (which can suppress both Th1 and Th2 responses) may be important to CpG ODN-induced therapeutic responses.

Based on our studies and those of others, we have developed a model to explain the effects of CpG ODNs on atopic airway inflammation (Fig. 1). This model takes into account our findings that although administration of CpG alone is effective at promoting Th1-type responses, maximal protection against airway inflammation (and reduction of Th2-type responses) requires the co-administration of allergen and CpG ODNs. We suggest that the effects of CpG-ODN are mediated through altered responses of dendritic cells (DCs), which subsequently interact with CD4⁺ Th cells. We hypothesize that the presentation of antigens by DCs, in the context of the CpG-induced inflammatory milieu,

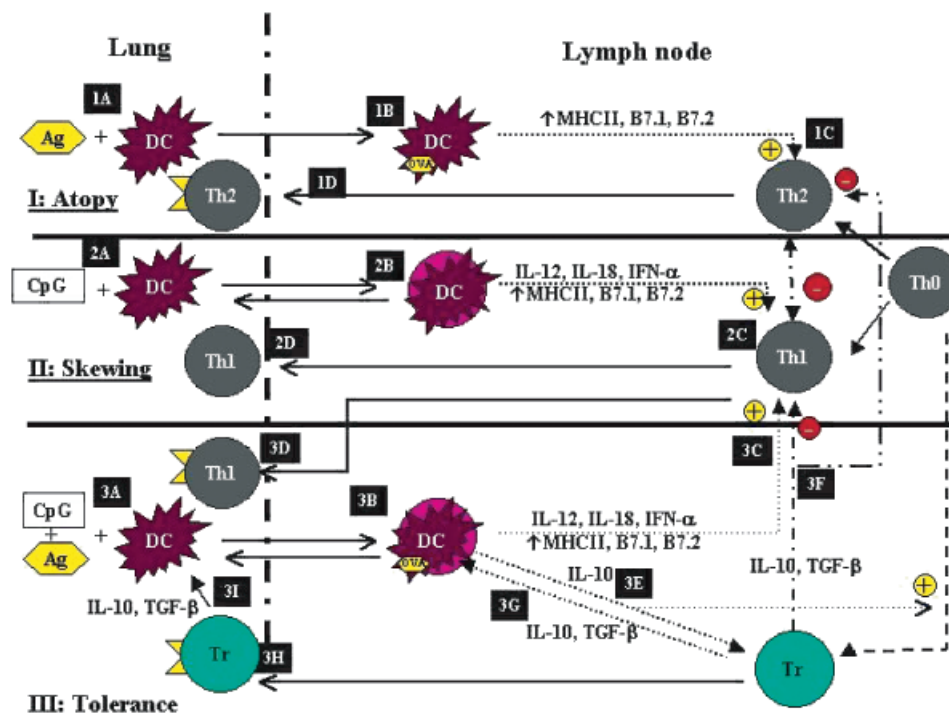


Fig. 1. (I) Atopy: Ag encountered in the airway by dendritic cells (DC) promotes Th2-type responses in sensitized mice. (II) Immune skewing: mucosal application of CpG-ODN alone promotes skewing towards Th1-type responses, in part mediated by lung DC. These responses are not Ag specific. (III) Co-administration of CpG-ODN and Ag may promote both Th1-type responses as well as (speculatively) Ag-specific regulatory T cells; these immune cells in combination may induce tolerance.

promotes the development of antigen-specific regulatory T lymphocytes whose interactions suppress atopic inflammation. We depict this model in Fig. 1: (I) *Atopic asthma*: DCs in the lung ingest Ag (1A), and process it en route to thoracic lymph nodes (1B), where it is presented to naïve T-cells in the context of MHC-II and costimulatory molecules (1C), promoting an Ag-specific Th2 response, a Th2 cytokine milieu in the lungs and Ag-specific Th2 cells (1D); (II) *Skewing of immune response*: DCs stimulated by CpG-ODN (2A) also mature and translocate to thoracic LN (2B); through the release of IL-12 and other soluble mediators, these cells promote Th1 responses (2C) and a Th1 milieu in the lung (2D) that is not Ag specific; (III) *Immune tolerance*: Ag encountered in the lung in the setting of CpG-ODN (3A) promotes DC maturation (3B), Ag-specific Th1 responses (3C), Ag-specific Th1 cells, and a Th1 milieu in the lung (3D) and also, perhaps by inducing the release of IL-10 and TGF-β, promotes Ag-specific regulatory T cells (derived from naïve Th0 cells) (3E) that can down-regulate both Th1 and Th2 responses (3F) and tolerize local DCs (3G); Tr cells may also return to the lung to influence the cytokine milieu (3H), and tolerize lung DCs (3I), thereby preventing or reversing Th2-mediated inflammation. In each case, DCs may be recruited to the lung. Thus, CpG-ODN-based immunotherapy can inhibit and reverse airway eosinophilic inflammation and AHR; these effects are associated with (but not dependent on) induction of Th1-type responses. The suppression of Th2-type responses appears to result from multiple influences, including perhaps regulatory-type responses characterized by IL-10 induction.

CpG DNA and acute allergic inflammation

Numerous reports now confirm the protective effects of CpG DNA on acute allergic inflammation in murine models of asthma [2, 20–22]. Our initial studies investigated whether CpG ODNs would prevent airway inflammation in a murine model of atopic asthma, in which sensitized and challenged mice develop marked airway eosinophilia, elevated serum IgE, and airway hyperreactivity (AHR) to inhaled methacholine. Mice that received CpG ODNs at the time of sensitization exhibited reductions in all these manifestations of asthma; Th2 responses in the airway (lung lavage IL-4 concentrations) were reduced, in association with an induction of Th1 cytokines (IFN-γ and IL-12) [35]. To evaluate the role of IFN-γ and IL-12 in the protection against atopic inflammation provided by CpG ODNs, we used both anti-cytokine antibodies and cytokine gene knockout mice; we found that CpG ODNs remained effective in the absence of Th1 responses, although a slight reduction in the dose-response curve can be seen [33].

Our findings have been confirmed and extended by other investigators. Broide et al. [21] used the ovalbumin (OVA) model of asthma in BALB/c mice, where CpG ODNs were administered via systemic or mucosal routes prior to antigen challenge, and found that CpG ODNs were effective in inhibiting systemic as well as airway eosinophilia; they also found that a single treatment with CpG DNA was at least as efficacious as seven consecutive daily injections of corticosteroids. Sur et al. [22] used a ragweed model of murine asthma where CpG ODNs were administered mucosally prior

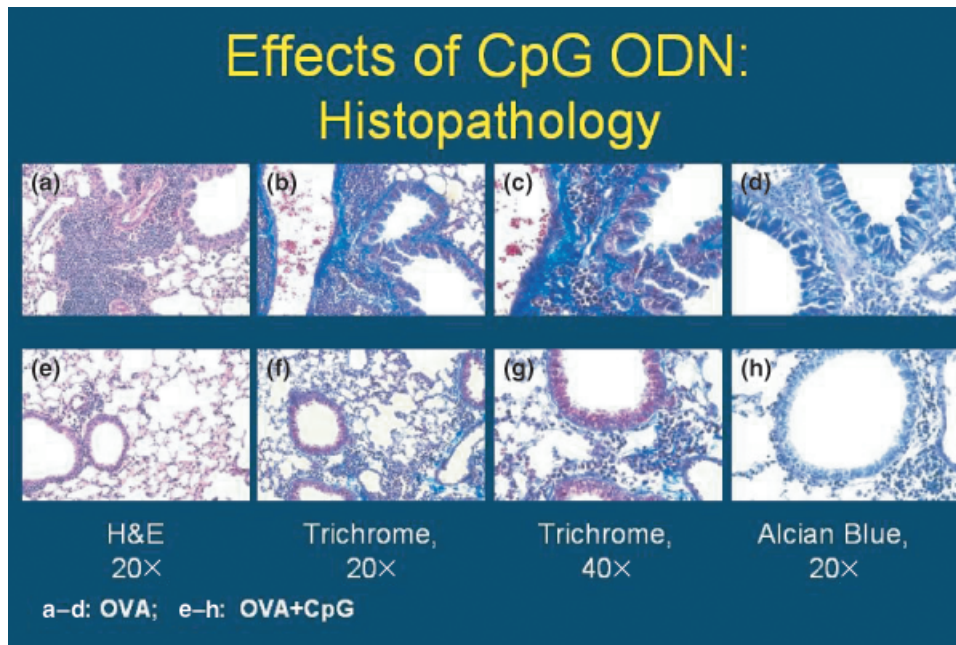


Fig. 2. Effects of CpG-ODNs on preventing airway remodelling in chronic asthma. C57BL/6 mice were injected (days 0 and 14) with OVA alone (a–d) or with CpG-ODN (e–h), then exposed to aerosolized OVA three times weekly for 6 weeks. Those mice that received CpG-ODN were significantly protected against the development of peribronchial eosinophilic inflammation (a and e), subepithelial collagen deposition (b, c, f, and g), and goblet cell hyperplasia (d and h). Adapted from Jain et al. [4].

to antigen challenge; the protective effects lasted at least 6 weeks after administration of CpG ODNs. Shirota et al. [36] similarly demonstrated that mucosal administration of CpG ODNs administered with antigen, either independently or as a conjugated compound [37], prevented airway eosinophilia and AHR.

These studies show that CpG DNA, administered at the time of allergen sensitization, has immunomodulatory properties that can suppress airway inflammation and AHR (while inducing Th1 cytokines and suppressing Th2 cytokines). These effects are partially mediated through induction of IFN- γ and IL-12, and are neither model- nor strain-dependent. Although primary prevention of atopic disease has not previously been a goal of pharmacotherapeutic agents, these results suggest the possibility of immunomodulatory strategies for individuals at high risk (due to family history or other factors) of developing atopic disease.

CpG DNA and established asthma

Reversal of established eosinophilic airway inflammation is more challenging than preventing its development. Sensitization to various allergens in atopic asthma most often develops in early life; certainly there is no doubt that our patients have been sensitized and/or Th2 committed by the time they seek treatment for their atopic disorders [38]. Recently, we have demonstrated that systemic administration of CpG DNA and allergen is effective at reversing established airway eosinophilic inflammation [1]. In these studies, mice were sensitized to, then challenged by inhalation of OVA; using this model, significant airway eosinophilia develops within 48 hours of the first airway challenge, and increases with additional challenges [39, 40]. Immunotherapy, consisting of four biweekly subcuta-

neous injections of CpG (or control) ODNs and/or OVA, was begun after 2 weeks of inhaling the experimental allergen, at which time eosinophilic airway inflammation was well established. Mice that had been treated with the combination of CpG ODNs and OVA (but not those treated with either agent alone) had reduced OVA-specific IgE levels and were significantly protected against Ag-induced eosinophilic airway inflammation and AHR, seen in untreated mice following OVA inhalation. Successful immunotherapy was associated with induction of IFN- γ and IFN- γ inducible protein-10 (IP-10) and suppression of IL-5 and eotaxin.

Our findings are in accord with those of Serebrisky et al. [29]; although this study administered CpG ODNs only 24 h after each of two allergen challenges, they also noted that CpG ODNs can reverse Th2 responses following sensitization. Santeliz et al. [41] used a conjugated compound (CpG ODNs chemically conjugated with the ragweed allergen, Amb a 1) and demonstrated that this approach is also effective in reversing established airway disease. CpG ODNs were administered after the first antigen challenge, and responses were assessed after the second challenge in their model. They also assessed IFN- γ and IL-5 production *in vitro*, and found that the effects of CpG DNA on established AHR were associated with induction of IFN- γ , but surprisingly were independent of IL-5 suppression.

CpG DNA and airway remodelling

Although acute inflammatory responses in atopic asthma appear fully reversible, repeated episodes induce a chronic state of inflammation; this may lead to the development of irreversible responses, collectively referred to as airway 'remodelling'. Prominent features of remodelling include

subepithelial fibrosis (excess deposition of collagen in the subepithelial layer), smooth muscle hypertrophy, and goblet cell hyperplasia. These changes have important functional consequences, including loss of distensibility of asthmatic airways, permanent airflow obstruction, and bronchial hyper-reactivity, all of which persist even in the absence of further exposure to antigens [42, 43]. Goblet cell hyperplasia has been shown to be a feature of glucocorticoid steroid-resistant asthma [27], and aspects of the response to glucocorticoid steroids may in fact enhance collagen formation [44]. Remodelling of the airway may be an important cause of morbidity in persistent asthma, and currently available therapies are not capable of reversing these changes.

Based on these concerns, we have recently examined the effects of CpG ODNs on airway remodelling [4]. For these studies, we developed a murine model of chronic asthma in which OVA-sensitized mice were subjected to repeated inhalational exposure to OVA aerosol three times weekly for 6 weeks. These mice developed significant subepithelial fibrosis and goblet cell hyperplasia/metaplasia in association with chronic AHR. In contrast, mice that were treated with CpG DNA at the time of sensitization had significantly fewer features of airway remodelling (Fig. 2), including reduced thickness of the subepithelial collagen layer, lower total lung collagen content, and less goblet cell hyperplasia. These results suggest that CpG ODNs may prevent chronic inflammatory structural changes in the airways seen in chronic asthma.

Although the immune stimuli for airway remodelling remain unclear, considerable evidence implicates IL-13 in the regulation of many aspects, including subepithelial fibrosis and goblet cell hyperplasia [23, 45]. The suppression of IL-13 in chronic asthma by CpG DNA may be accomplished through release of regulatory cytokines, such as IL-10 and TGF- β . These cytokines are products of regulatory T cells (CD4⁺CD25⁺CD45RB^{Lo}) that can modulate allergic Th2 responses [46, 47]. We have recently demonstrated elevated levels of BAL TGF- β in association with inhibitory effects of CpG DNA on airway remodelling [4]; we have also shown that CpG ODNs may inhibit established Th2 responses through induction of IL-10 [34]. It remains an intriguing possibility that some of the effects of CpG DNA in chronic asthma may be mediated via immunosuppressive regulatory T cells.

Conclusion

Recent data from human trials using CpG DNA suggest that it is well tolerated and a potent immunomodulatory agent [48]. To date, most of our understanding of the effects of CpG DNA on asthma and atopic inflammation is derived from animal models; these have clear limitations that prevent direct extrapolation of findings to the treatment of clinical asthma. Nevertheless, while its precise mechanisms continue to be fully delineated, CpG DNA offers considerable promise as a novel treatment for atopic inflammation. It may prove to be an important disease-modifying therapy, or even curative therapeutic agent for asthma.

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