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General

CpG DNA: immunomodulation and remodelling of the asthmatic airway

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Asthma is a disorder of increasing severity and prevalence. Present understanding of the pathogenesis of asthma emphasises its inflammatory nature. Unbridled inflammation appears to induce irreversible changes, collectively known as airway remodelling. CpG oligonucleotides are a class of compounds that have been developed from studies of prokaryotic DNA. Bacterial DNA, for example, contains sequence motifs based on the dinucleotides cytosine-guanine (CpG); these motifs are suppressed in mammalian DNA and induce (as part of the innate immune system) inflammation, characterised by the induction of T helper type 1 and regulatory responses. The pattern of inflammation promoted by CpG DNA tends to suppress the cytokine and cellular responses characteristic of asthma and atopic disorders. This has led to the investigation and development of CpG DNA as a novel therapeutic approach for the treatment and prevention of these disorders. In addition to suppressing acute and persistent airway inflammation, CpG DNA also reduces the development of subepithelial collagen deposition, goblet cell hyperplasia/metaplasia, and other aspects of airway remodelling. In this paper, the effects of CpG DNA on asthma inflammation and remodelling are reviewed.

Keywords: airway remodelling, asthma, CpG oligonucleotide, inflammation, microbial products

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1. Introduction

Asthma, long considered a condition of reversible airflow obstruction, is now known to have an aspect of irreversible airway obstruction brought on by repeated episodes of acute airway inflammation. The consequence of recurrent inflammation is the development of structural changes in the airway; these processes constitute the normal reparative process (orchestrated by inflammatory mediators) gone awry, and are collectively referred to as 'remodelling'. Prominent features of these changes include subepithelial fibrosis (excess deposition of collagen in the subepithelial layer), smooth muscle hypertrophy and goblet cell (GC) hyperplasia. Until recently, the changes of remodelling were thought to be confined to the late stages of asthma; it is now known that even patients with mild asthma, as well as some asymptomatic asthmatics, can develop airway fibrosis and other chronic abnormalities. These changes have important functional consequences, including loss of distensibility of asthmatic airways, accelerated decline in airflow obstruction and airway hyper-reactivity (AHR), all of which persist even in the absence of further exposure to antigens [1,2]. Due to the impact of remodelling on lung function, the mechanisms of and potential therapeutic interventions against airway remodelling are areas of intense investigation.

Remodelling of the airway is an important cause of morbidity in persistent asthma, but therapies available at present have not been shown to reverse these changes. GC hyperplasia is a prominent feature of glucocorticoid steroid-resistant

asthma [3], and aspects of the response to glucocorticoid steroids may in fact promote epithelial damage and enhance fibroblast proliferation and collagen formation [4-6]. Recent developments in asthma therapy aimed at immunomodulation have shown promise at targeting airway remodelling. CpG DNA represents a novel class of drugs with substantial immunomodulatory properties. Preclinical studies have demonstrated the effectiveness of CpG oligodeoxynucleotides (ODNs) in the prevention and treatment of both upper and lower allergic airway disease [7-11]. Data from ongoing human trials using CpG ODNs suggest that it is a safe and well-tolerated agent that seems promising for the treatment of allergic airway inflammation [12,13]. Adjuvant activity of CpG DNA in clinical trials suggests that CpG ODNs may be uniquely well suited to immunotherapy of human disease [13]. This review summarises the effects of CpG ODNs on chronic asthma.

2. Biology of CpG DNA

Although the molecular mechanisms of action of CpG DNA are not completely understood, recent advances have been made. Bacterial, but not vertebrate, DNA contains unmethylated CpG dinucleotides, which are substantially responsible for its immunomodulatory properties [14]. Antigen-presenting cells (APCs), the first cells to encounter inhaled allergens, play a critical role in the uptake, processing and presentation of antigen in the lung. APC-derived signals can then drive naive CD4⁺ T cells to a T helper (Th) type 2 response, leading to allergic airway inflammation (or a Th1 phenotype, which is counter-regulatory to the Th2 response). In the absence of APCs, allergic airway inflammation is almost completely abolished [15]. The innate immune system uses Toll-like receptor (TLR)9, one of the pathogen-associated molecular pattern recognition elements, to transduce the responses to CpG DNA [16]. T cells do not express TLR9; however, APCs, such as B cells and plasmacytoid dendritic cells, have been clearly shown to express TLR9 and can be directly stimulated by CpG ODNs [17-20]. TLR9, in concert with MyD88 (adaptor protein), plays a critical role in the signalling cascade of CpG DNA [21,22]. Once activated by CpG DNA, APCs secrete a wide array of cytokines, chemokines and other mediators that promote the activation of naive CD4⁺ cells, thereby selectively downregulating the Th2 response [11,20]. Whereas allergen-pulsed dendritic cells *in vitro* elicit marked airway inflammation, allergen-pulsed dendritic cells *in vivo* stimulated by CpG ODNs have been shown to alleviate airway inflammation [23].

Recent data suggest that the differentiation of the naive CD4⁺ T cell by the APCs is regulated by transcription factors: T-bet promotes the Th1 phenotype, and GATA3 the Th2 phenotype [24]. Mice deficient in the *T-bet* gene spontaneously develop features of acute and chronic asthma [25]. Recent *in vitro* data demonstrate that CpG ODNs induce T-bet expression in B cells (but not in naive T cells) and directly (independent of T cells) inhibit B cell production of IgE antibodies. This

is mediated via TLR9 and is associated with induction of Th1 cytokines (interferon [IFN]- γ and interleukin [IL]-12) and expression of Th1 type (IgG2a) antibodies [26]. CpG-induced T-bet expression was enhanced by IFN- γ and IL-12, but neither cytokine appears to be necessary. These findings support *in vivo* data demonstrating that CpG ODNs do not require Th1 cytokines to prevent allergic airway inflammation in a murine model of asthma [27].

3. CpG DNA and acute airway inflammation

Immunotherapy, the modulation of immune responses prominent in asthmatic inflammation, is a therapeutic option that holds promise in reversing established allergic disease [11]. Although glucocorticoid steroids provide non-specific immunosuppression, blocking any individual mediator (among the hundreds implicated in asthma) is unlikely to have a significant impact on the genesis of airway inflammation. In previous studies, the authors and others have reported the protective effects of CpG DNA on acute as well as established allergic airway inflammation in murine models of acute asthma [7,8,27-33]. In these studies, it has been reported that CpG ODNs have immunomodulatory properties that can suppress as well as reverse established airway inflammation and AHR, whether administered via systemic or mucosal routes, prior to or after antigen challenge and when used in an immunotherapy protocol with or without antigen. These effects are partially mediated through, but not dependent on, the induction of Th1 mediators, IFN- γ and IL-12; these effects are neither model- nor strain-dependent. Although CpG ODNs can influence the development of cell death, most notably seen in their effects in rescuing B cells from apoptosis [34-36], reductions in airway, bone-marrow and circulating eosinophilia are not due to promotion of eosinophil apoptosis [28].

Recently, the authors have shown that, although CpG ODNs are most effective in promoting immunomodulation when administered in conjunction with an allergen, the dose requirement for the allergen is quite low; indeed, CpG ODN administered alone, while the mice were allowed to spontaneously breathe in an environment that contained aerosolised allergen, was effective in reversing established atopic responses [8,11]. Preliminary studies were carried out using a range of concentrations of antigen and of CpG ODN; within a log or more of the concentrations used, no significant difference in immunotherapeutic effects were seen, but these effects diminished as the concentration of CpG ODNs was further reduced. In these and other studies, no adverse effects of administering CpG ODNs in doses highly effective at promoting immunomodulation have been found; when administered in significantly higher doses, neutrophilic inflammation may be induced [37]. Although the therapeutic effects of CpG DNA in protecting against acute airway inflammation are now more a paradigm than a paradox, limited data exist concerning its effects on airway remodelling.

4. CpG DNA and subepithelial fibrosis

Subepithelial fibrosis, defined as deposition of collagen in the subepithelial and submucosal compartments of the airway, correlates with AHR and is the main determinant of the irreversible (or persistent) component of airflow obstruction [38,39]. The major site of functionally relevant subepithelial fibrosis remains controversial, leading to disparate conclusions regarding the correlation between subepithelial fibrosis and asthma severity [39]. Chu *et al.* reported that the amount of collagen deposition at the large airway level in human asthmatics might not predict the clinical severity of disease [40]. However, studies assessing airway wall thickness using high resolution computed tomography have reported an increase in thickness in the small airways, which is not always observed in the large airways [41,42]. In addition, enhanced airway wall thickness is predominantly seen in the small airways in non-fatal asthma [43]. The authors have reported the effects of CpG DNA on subepithelial fibrosis at the level of intrapulmonary conducting (medium and small size) airways in a murine model of chronic asthma induced by repeated allergen exposures, where mice treated with CpG ODNs had a 40% decrease in the subepithelial collagen thickness associated with significant reduction in the total lung collagen content [10]. More recently, Cho *et al.* confirmed these findings by demonstrating that CpG ODNs inhibit subepithelial collagen deposition, with protection lasting up to 6 months [44].

Transforming growth factor (TGF)- β 1, a multifunctional cytokine, is believed to play a critical role in epithelial homeostasis and modelling of the asthmatic airway [45]. It may either stimulate or inhibit fibroblasts depending on the local cytokine milieu. TGF- β is also known to be a product of regulatory T cells (CD4⁺CD25⁺CD45RB^{Lo}) that can modulate allergic Th2 responses [46,47]. Recent studies have indicated a protective role for TGF- β in asthma. Hansen *et al.* demonstrated that ovalbumin (OVA)-specific CD4⁺ T cells engineered *in vitro* to produce TGF- β reversed allergen-induced AHR and airway inflammation; these effects were impaired by neutralisation of TGF- β [48]. Haneda *et al.* demonstrated that TGF- β -producing CD4⁺ T cells generated by oral or tracheal tolerance to OVA abolished antigen (OVA)-specific eosinophilic airway inflammation [49,50]. They also reported that adoptive transfer of CD4⁺ lymph node T cells from OVA-tolerant mice inhibited eosinophilic airway inflammation in OVA-sensitised mice, a response that required TGF- β activity. Blockade of TGF- β -mediated signalling via overexpression of Smad7 (intracellular antagonist of TGF- β signalling) has been shown to enhance allergic airway inflammation and AHR [51]. These studies, however, did not address chronic airway inflammation. Based on these studies, it has been speculated that TGF- β may play a role in the inhibition of subepithelial fibrosis in the authors' murine model of chronic asthma. It was found that treatment with CpG ODN-based immunotherapy increased TGF- β levels in bronchoalveolar

lavage (BAL) fluid; this was associated with significant inhibition of subepithelial fibrosis [10]. The TGF- β may be a product of immunosuppressive regulatory T cells. This is in contrast with the study by Cho *et al.* [44], who found that the CpG DNA-mediated inhibition of subepithelial fibrosis was associated with reduced BAL TGF- β .

In asthma patients, TGF- β levels in the airways are higher than those in normal subjects [52]. Epithelial damage from repeated bouts of inflammation may lead to TGF- β induction as a marker of ongoing epithelial repair [53]. It is conceivable that TGF- β may lead to appropriate repair and inhibition of airway scarring in some cases, but remodelling and increased subepithelial fibrosis in others. Although the role of TGF- β remains uncertain and requires further study, studies by Cho *et al.* and the authors of this paper are in agreement that CpG DNA may inhibit subepithelial fibrosis in chronic asthma.

IL-13 is a central mediator of asthma and may be critical to the development of airway remodelling; subepithelial collagen deposition does not occur in IL-13^{-/-} mice exposed to repeated allergen exposures [54]. In addition, mice genetically engineered to overexpress IL-13 demonstrate enhanced subepithelial fibrosis [55]. These studies suggest a critical role for IL-13 in the regulation of subepithelial fibrosis, and that inhibition of IL-13 might be a target for antiremodelling therapy. CpG ODNs have inhibitory effects on IL-13 in acute asthmatic inflammation [32]. Recently, the authors have shown that CpG DNA also reduces BAL IL-13 levels in mice with chronic allergen-induced asthma [11]. Although IL-13 has been shown to act in concert with IL-4 and -5 in modulation of acute airway inflammation and AHR [56], subepithelial fibrosis following repeated allergen challenge occurs independent of IL-4 and -5 [57]; the inhibitory effects of CpG ODNs on subepithelial fibrosis may be mediated, at least in part, by direct effects on IL-13.

IL-10 can exert both immunosuppressive and immunostimulatory effects. Although CD4 Th cells engineered to express IL-10 abolished airway eosinophilia and AHR in allergen-sensitised and challenged mice, neutralisation of IL-10 by administration of anti-IL-10 antibody exacerbated AHR and acute airway inflammation [58]. In models of non-allergic inflammation, IL-10 has been shown to be protective against bleomycin-induced pulmonary fibrosis [59]; on the other hand, IL-10 can induce GC hyperplasia and fibrosis in the setting of endotoxin-induced inflammation [60]. The authors [61] and others [62] have found that CpG ODNs induce IL-10 from a variety of cells and that this induction contributes to the protective effects of CpG ODNs in allergic inflammatory responses. More recently, it has been reported that the protective effects of CpG ODNs on subepithelial fibrosis and GC hyperplasia were not associated with an induction of BAL IL-10 chronic asthma [10]. However, in other studies using murine models of chronic asthma, it was found that CpG ODNs induced increased IL-10 production from splenocytes, when challenged with the allergen in context [11]. IL-10 appears to play a role in mediating the protective effects of CpG DNA on chronic airway inflammation.

Airway remodelling in asthma has been linked with viral infections [63,64]. This may be particularly relevant to the asthma phenotype that follows childhood respiratory syncytial virus (RSV) bronchiolitis. Sorkness *et al.* have demonstrated that administration of IFN- γ is effective in preventing post-viral bronchiolitis and chronic airway changes [65], and others have considered the use of CpG ODN to protect against viral-induced airway pathology. CpG ODN inhibits RSV-induced epithelial responses [66] and has been used to promote protective responses in a RSV vaccine [67,68].

5. CpG DNA and goblet cell hyperplasia

Excessive mucus production and secretion are common occurrences in human asthma. The inflammatory epithelial changes lead to viscoelastic changes as well, causing abnormalities in mucociliary clearance, which may lead to 'mucus plug' formation seen in fatal asthma. Recent data suggest that these exist even in mild asthma [69]. Although GCs are rarely present in the normal murine airway epithelium, allergen-induced induction of GC hyperplasia has now been established in several murine models of asthma. CpG ODNs have been shown to inhibit GC hyperplasia in murine models of acute inflammation (with limited number of allergen exposures in sensitised mice) in association with reversal of established AHR [32,33]. However, murine models of acute asthma have been criticised for lacking the features of airway remodelling.

The authors have recently reported the effects of CpG ODN-based mucosal immunotherapy in a murine model of chronic asthma, where allergen-sensitised mice were challenged by repeated allergen exposures (RAE) over 6 weeks [11]. Unlike some prior murine models of chronic asthma, the authors demonstrated in this model that mice receiving RAE develop significantly higher GC hyperplasia and AHR relative to mice that received limited allergen exposures. Mice that received immunotherapy with CpG ODNs alone, as well as CpG ODNs along with allergen, prior to receiving RAE, had significantly inhibited development of GC hyperplasia and AHR. In those studies, the authors also characterised the epithelial changes by morphometric analyses and found that CpG ODNs also inhibit the amount of stored mucin in airway cells. Increased amounts of stored mucin (and subsequent GC degranulation) contribute to the pathogenic mechanisms for patients with asthma, including airway narrowing, exacerbations and accelerated decline in lung function [70]. In the authors' studies, as mice treated with CpG ODNs exhibited decreased amount of stored mucin in association with decreased AHR, it is unlikely that the decreased mucin stores were reflective of a preceding degranulation process. More recently, supporting the authors' findings, Cho *et al.* reported that systemic administration of CpG ODNs inhibited the occurrence of GCs in the airway epithelium of mice exposed to RAE [44]. Taken together, these studies suggest that CpG ODNs may be a novel form of therapy to inhibit GC hyperplasia.

Although several Th2 cytokines orchestrate the induction of GC hyperplasia, studies have implicated specifically important roles for IL-4, -5, -9 and -13 [70]. CpG ODNs have been shown to inhibit GC hyperplasia in association with decreased BAL fluid levels of IL-4, -5 and -13 in acute airway inflammation [32]. More recently, CpG-mediated inhibition of GC hyperplasia has been shown to be associated with decreased BAL fluid levels of IL-13 in a murine model of chronic airway inflammation [44]. In addition, CpG ODNs have been reported to inhibit expression of IL-9 in lungs of mice exposed to RAE [71]. Although IL-4, -5 and -9 have not been studied directly in association with GC hyperplasia in murine models of chronic airway inflammation, it is reasonable to speculate that inhibition of GC hyperplasia by CpG ODNs is likely to be mediated by these cytokines.

6. CpG DNA and airway smooth muscle hyperplasia

Airway smooth muscle (ASM), once felt to represent only the effector mechanisms of airflow obstruction, is now considered to have immunomodulatory capabilities and is known to have significant synthetic (a source of biologically active cytokines) and proliferative properties [72]. ASM remodelling is a well-documented pathological finding in the airways of patients with chronic severe asthma [72,73]. More recently, ASM hyperplasia has been shown to be present even in the airways of patients with mild asthma [74]. The inhibitory effects of CpG ODNs on ASM hyperplasia have been reported recently, where systemically administered CpG ODNs inhibited peribronchial smooth muscle layer thickness, in association with AHR [44]. The mechanisms by which CpG DNA may inhibit ASM hyperplasia require further investigation.

7. Expert opinion and summary

Interest in the investigation of chronic asthma and airway remodelling is rapidly growing. Development of therapeutic agents addressing persistent AHR remains a major research challenge. We have recently reported the protective effects of CpG DNA in airway remodelling using a murine model of chronic asthma. These studies provide exciting data and are encouraging for the further development of CpG ODNs as immunotherapeutic agents for asthma. Based on these studies, we propose a model in which CpG DNA may protect against airway remodelling changes in concert with T regulatory cells, as well as other as yet unidentified mediators of chronic inflammation (Figure 1). Although our understanding of the mechanisms that mediate this protection remain incomplete at this time, CpG DNA seems to be a novel therapeutic agent that holds promise for the immunomodulation of acute as well as chronic persistent asthma.

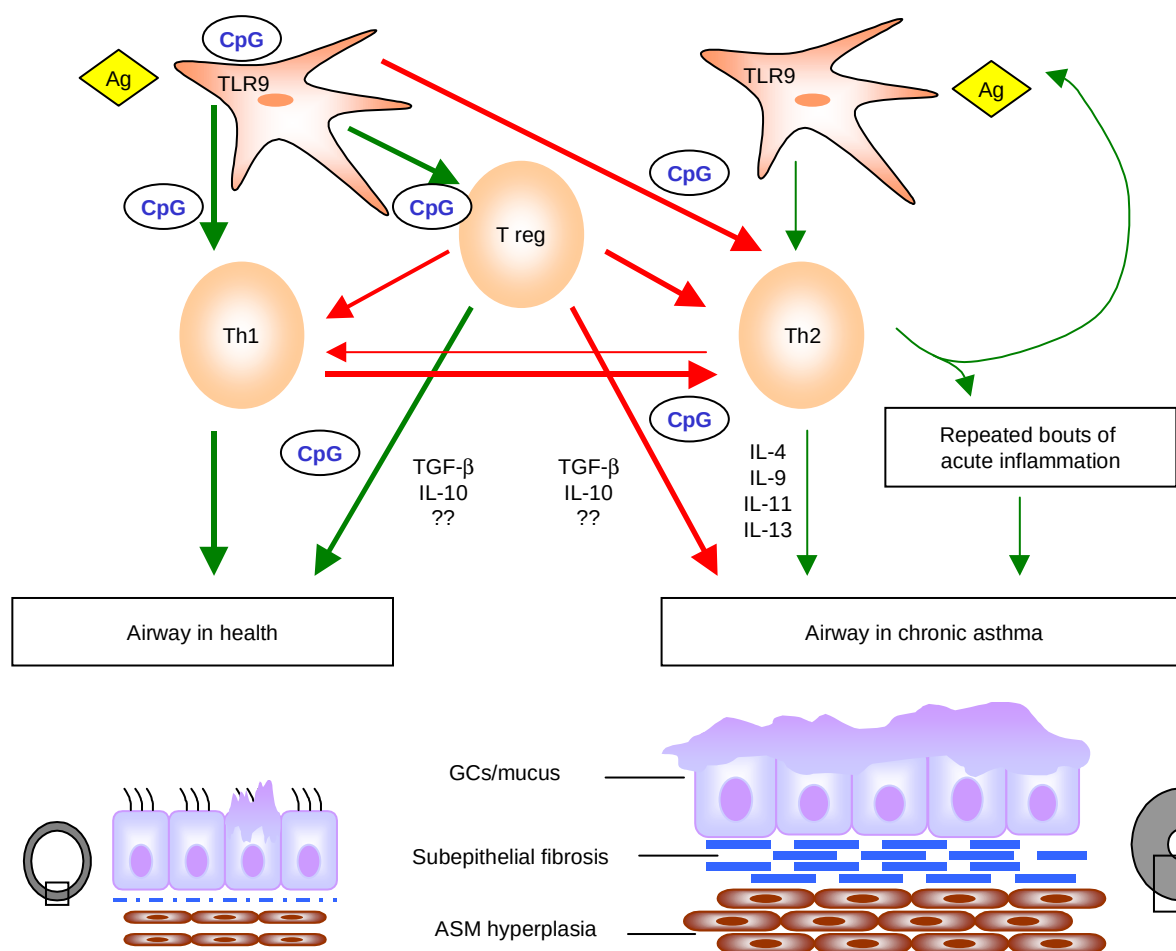


Figure 1. Proposed model for the role of CpG DNA in airway remodelling. APCs (dendritic cells and B cells) once exposed to allergen (outlined on the right) polarise the naive CD4⁺ cells to Th2-type cells, which then create a Th2 cytokine milieu that elicits (green arrows) the chronic epithelial changes seen in chronic asthma. The naive CD4⁺ cells, however, when exposed to allergen in the presence of CpG DNA (outlined on the left), differentiate into Th1-type cells and/or T regulatory cells; these may then induce mucosal tolerance to airway allergens, such that atopic Th2 responses are inhibited (red arrows), thus inhibiting the airway remodelling changes. CpG may both directly induce tolerising cytokine responses (such as IL-10 from regulatory cells) and inhibit Th2-type lymphocytes.

Ag: Antigen; APC: Antigen-presenting cell; ASM: Airway smooth muscle; IL: Interleukin; TGF: Transforming growth factor; Th: T helper; TLR: Toll-like receptor; T reg: Regulatory T cells.

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