

Original article

Nasal CpG oligodeoxynucleotide administration induces a local inflammatory response in nonallergic individuals

Background: We have previously demonstrated the presence of toll-like receptor 9 in the nasal mucosa of both healthy and allergic individuals. CpG motifs, found in bacterial and viral DNA, elicit strong immunostimulatory effects via this receptor. CpG is known to skew the immune system towards a T helper 1 (Th1) profile, thereby suppressing Th2-driven allergic responses. This study was designed to examine the effects of CpG administration in the human nose.

Methods: Twenty subjects, of whom 10 suffered from seasonal allergic rhinitis (AR), were challenged intranasally with CpG outside pollen season. Symptom scores, nasal airway resistance (NAR), and nasal and pulmonary nitric oxide (NO) levels were assayed prior to challenge and 30 min, 6, 24 and 48 h post challenge. The presence of leukocytes and various cytokines were analyzed in nasal lavage (NAL) fluids before and after CpG exposure.

Results: Increased NAR, nasal NO production and secretion of interleukin (IL)-1 β , IL-6, and IL-8 were seen after CpG exposure. Further analysis revealed that this inflammatory response was more marked in healthy subjects than among patients with AR, although a higher basal inflammatory response was recorded in the allergic group. *In vitro* experiments suggest that the effects induced by CpG are mediated by epithelial cells and neutrophils.

Conclusion: Nasal administration of CpG induces a local airway inflammation, more distinct among healthy than allergic individuals. The reduced responsiveness to CpG in allergic patients might be related to the ongoing minimal persistent inflammation. Results from cytokine analyses reflect the ability of CpG to induce a pro-inflammatory Th1-like immune response.

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Allergens are not always the sole trigger of allergic rhinitis (AR), as respiratory infections, organic compounds, and environmental pollutants are known to cause disease exacerbation (1, 2). Inhalation of lipopolysaccharide (LPS), as a surrogate for bacterial respiratory infection, increases the number of leukocytes within the airway and enhances the secretion of various inflammatory cytokines (3, 4), via activation of toll-like receptor (TLR) 4 (5). In a previous study, we have demonstrated that nasal administration of LPS in conjunction with allergen causes a more marked inflammatory response than both LPS and allergen alone (6).

TLR9, another member of the TLR family, has gained a lot of interest because of its ability to induce a T helper1 (Th1)-biased immune response. An increase in the Th1 response is known to suppress Th2-related activities associated with allergic reactions (7, 8). We have previously demonstrated the presence of TLR9 in nasal biopsies, most prominently in the epithelium and submucosa, but also in leukocytes such as mast cells, dendritic cells, granulocytes, and lymphocytes scattered in the intraepithelial and subepithelial layer (9). The ligand for

TLR9 is CpG oligodeoxynucleotide (ODN), unmethylated CpG motifs present in bacterial and viral DNA (10, 11). The Th1-polarizing ability of CpG ODN has suggested the therapeutic use of these motifs in allergy treatment, as well as making them promising candidates for use as an adjuvant in vaccination (7, 12).

The aim of the present study was to examine the effects of CpG ODN administration in the human nose, focusing on nasal airway resistance (NAR), nitric oxide (NO) production, changes in nasal lavage (NAL) leukocyte numbers and local secretion of different cytokines. Also, the responsiveness to CpG in healthy controls and in patients with pollen-induced AR examined out of season was determined.

Materials and methods

Subjects

Twenty subjects of whom 10 had a history of seasonal AR were included in the study. The allergic patients (two males, eight females) with a medium age of 27 (range: 20–49) and the healthy

volunteers (two males, eight females) with a medium age of 27 (range: 23–48) were all tested outside the pollen season. The AR patients had suffered from AR with moderate-to-severe symptoms during at least two consecutive seasons and they exhibited a positive skin prick test (SPT) to birch and/or timothy pollen (wheal reaction diameter >3 mm). Control subjects were all symptom-free, with no history of AR and a negative SPT to the standard panel of allergens. All participants had been free of medications ≥ 3 months prior to the study. Subjects with a history or signs of persistent AR or chronic rhinosinusitis including nasal polyposis were excluded along with patients with hypertrophy of turbinates, severe septum deviation or a history of airway infection within 2 weeks before the first visit. The study was approved by the Local Ethics Committee and all participants gave their written informed consent.

Skin prick test

SPT was performed with a standard panel of 11 common airborne allergens (ALK Abelló, Hørsholm, Denmark) including pollen (birch, timothy, mugwort, and ragweed), house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), molds (*Cladosporium* and *Alternaria*), and animal allergens (cat, dog, and horse). SPT were performed on the volar side on forearms with saline buffer as negative and histamine chloride (10 mg/ml) as positive controls.

Study design

Subjects were challenged intranasally with CpG. One hundred microliters of sterile physiological saline solution containing 10 μ M CpG was applied by an intranasal spray to both nostrils after exsufflation. Phosphorothioate-modified CpG-ODN 'K3' (CpG-B type) was synthesized by DNA Technology (Aarhus, Denmark), and was free of endotoxins. The following oligonucleotide sequences were used (13): forward, 5'-atcgactctcgagcgttctc-3'; reverse, 5'-gagaacgctcgagatcgat-3'. All challenges were performed before the start of pollen season. NAL fluids were obtained before challenge, and after 24 and 48 h. Nasal symptoms were recorded and NAR along with nasal and pulmonary NO levels were measured before and 30 min, 6, 24 and 48 h after CpG administration.

Symptom scores

Subjects were asked to record the severity of total nasal symptoms (itching/sneezing, secretion, and blockage) and total pulmonary symptoms (cough, wheeze, and dyspnea) using an arbitrary four-step scale from 0 to 3 (0 – no; 1 – mild; 2 – moderate; 3 – severe symptoms) (6). The combination of nose and lung symptoms is referred to as total symptom score, which could maximally reach 18.

Nasal lavage

NAL fluid was obtained as previously described (14). Briefly, after clearing excess mucus by exsufflation, a sterile saline solution was aerosolized into the nostrils and nasal fluids were passively collected in a test tube until 7 ml was recovered. Leukocyte and total cell numbers (leukocytes and epithelial cells) were determined using a Bürker chamber (Marienfeld GmbH, Lauda-Königshofen, Germany).

Rhinomanometry

Nasal airway resistance was determined by active anterior rhinomanometry as previously described (6). Briefly, each nasal passage was measured separately using a pneumotachograph (Rhinomanometer; GM Instruments, Kilwinning, UK) and a total resistance was calculated. Total NAR is expressed as $V_{2\text{tot}}$ representing the resistance of nasal airflow according to the polar co-ordinate system of Bross.

Nitric oxide measurements

The levels of exhaled nasal and pulmonary NO were measured by chemiluminescence with an NIOX[®] NO analyser (Aerocrine AB, Solna, Sweden) as described in detail previously (6, 15). The participants had not been engaged in any strenuous physical activities at least 60 min prior to testing. Fractional exhaled NO was sampled in exhaled air from the lungs via the mouth using a tube, and nasal NO was measured by use of a nasal olive. At all times, measurements were performed in seated positions, and three consecutive measurements were performed for each analysis.

Isolation and culture of neutrophils

Human peripheral blood was acquired from healthy volunteers. Neutrophils were isolated using Polymorphprep[™] (Axis-Shield PoC AS, Oslo, Norway) according to the manufacturer's protocol. Contaminating erythrocytes were removed by hypotonic lysis. After washing, the cells were resuspended in RPMI 1640 (PAA, Pasching, Austria) supplemented with 10% FBS (PAN, Aidenbach, Germany). Neutrophils (4×10^6 /ml) were cultured for 4 and 20 h in 48-well culture plates in the absence or presence of CpG (1 μ M). Thereafter, levels of interleukin (IL)-1 β , IL-6, and IL-8 were measured in the culture supernatants by enzyme-linked immunosorbent assay (ELISA).

Cell line and culture conditions

The nasopharyngeal epithelial cell line Detroit-562 (CCL-138), obtained from ATCC (Mansassa, VA, USA), was cultured in MEM with Earl's salts and 2 mM L-glutamine (Gibco, Grand Island, NY, USA), supplemented with 10% FBS, 1 mM sodium pyruvate (Sigma-Aldrich, St Louis, MO, USA), 0.1 mM nonessential amino acids, 50 μ g/ml gentamicin, and 0.25 μ g/ml fungizone (Gibco). The cells were maintained in culture flasks at 37°C in a humidified 5% CO₂ atmosphere. The cells were plated on 24-well culture plates (250 000 cells/well/ml) in complete MEM medium and cultured overnight. CpG (1 μ M) was added, and after a 24-h incubation, supernatants were recovered and analyzed for levels of IL-1 β , IL-6, and IL-8 by the use of ELISA.

ELISA

Levels of IL-1 β (sensitivity level 1 pg/ml), IL-4 (10 pg/ml), IL-5 (3 pg/ml), IL-6 (0.7 pg/ml), IL-8 (3.5 pg/ml), IL-10 (3.9 pg/ml), interferon (IFN)- γ (8 pg/ml), and MIP-1 α (10 pg/ml) were measured in unprocessed NAL fluids or culture supernatants using ELISA plates from R&D Systems (Minneapolis, MN, USA). An IFN- α multi-subtype ELISA (12.5 pg/ml) from PBL Biomedical Laboratories (Piscataway, NJ, USA) was used for the detection of all IFN- α subunits except IFN- α F in NAL fluid. IL-12p70 (4 pg/ml) was measured using an OptEIA ELISA from BD Biosciences (Franklin Lakes, NJ, USA).

Flow cytometry

Neutrophils and epithelial cells were analyzed for the expression of TLR9 using flow cytometry. Intracellular staining of the cells was performed using the IntraPrep™ Permeabilization Reagent kit (Immunotech, Beckman Coulter, Marseille, France) in accordance with the specifications of the manufacturer. Cells were incubated with TLR9-FITC (clone 26C593; AMS Biotechnology, Abingdon, UK) or appropriate isotype control for 20 min at room temperature, and thereafter washed and resuspended in phosphate buffered saline. Flow cytometry analyses were performed on a Coulter Epics XL flow cytometer (Beckman Coulter). Live cells were gated based on their FSc and SSc properties, and 10 000–15 000 events were collected and analyzed using Expo32 ADC analysis software (Beckman Coulter).

Statistics

Statistical analysis was performed using GRAPHPAD PRISM 5 (GraphPad Software, San Diego, CA, USA). All data are expressed as mean $\pm$ SEM, and n being the number of experiments performed. Paired t -test (when two sets of data were compared) or one-way repeated measures ANOVA together with Dunnett's post-test (for comparisons of more than two data sets) were employed for paired observations. A P -value of <0.05 was considered statistically significant.

Results

Nasal administration with CpG induces a local inflammatory response

A single nasal dose of CpG (10 μ M) induced no clear symptoms, neither during the acute nor during the late

phase (data not shown). An increase in nasal resistance was seen 30 min after CpG challenge (Fig. 1A), and the nasal NO production was enhanced 6 h post administration (Fig. 1B). Pulmonary NO levels and cell numbers were unaffected by CpG (Fig. 1C,D).

Next, NAL fluids were analyzed for the levels of IL-1 β , IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN- α , IFN- γ , and MIP-1 α . The release of IL-1 β , IL-6, and IL-8 was clearly increased 24 h post CpG administration (Fig. 2A–C). Although the levels of IL-12p70 were close to the detection limit, a higher level was shown both 24 and 48 h after provocation (Fig. 2D). A trend towards an IFN- γ induction was also seen, although not statistically significant. Levels of IL-4 and MIP-1 α were unaffected by CpG, whereas IFN- α , IL-5, and IL-10 could not be detected in NAL fluids (data not shown).

To exclude unspecific effects induced by the vehicle, we investigated how nasal administration with 0.9% NaCl affects a range of parameters in eight healthy subjects. Data clearly show that there are no differences before as compared with 6 h after challenge in total symptoms (0.3 ± 0.1 vs 0.3 ± 0.2 units), NAR (18.6 ± 1.6 vs 16.7 ± 2.1 $V_{2\text{ tot}}$), leukocyte numbers in NAL (1.5 ± 0.5 vs $1.2 \pm 0.6 \times 10^4$ cells/ml), and production of MIP-1 α (7.0 ± 0.2 vs 8.8 ± 0.4 pg/ml).

Responses in healthy subjects and in patients with AR

To explore the nature of the CpG-induced local inflammation, the challenged individuals were divided into two

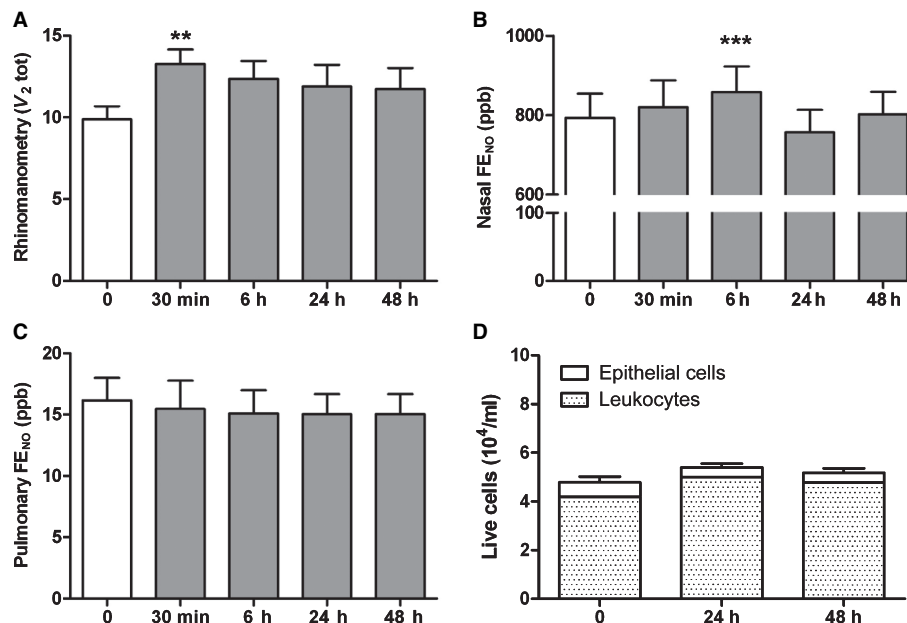


Figure 1. The effects of nasal administration of CpG (10 μ M) were examined prior to challenge and 30 min, 6, 24, and 48 h post challenge. (A) Nasal airway resistance determined by anterior rhinomanometry. (B) Nasal nitric oxide (NO) levels. (C) Pulmonary NO levels. (D) Number of live epithelial cells and leukocytes in nasal lavage fluid. All results are presented as mean $\pm$ SEM ($n = 20$; ** $P < 0.01$; *** $P < 0.001$). FE, fractional exhaled.

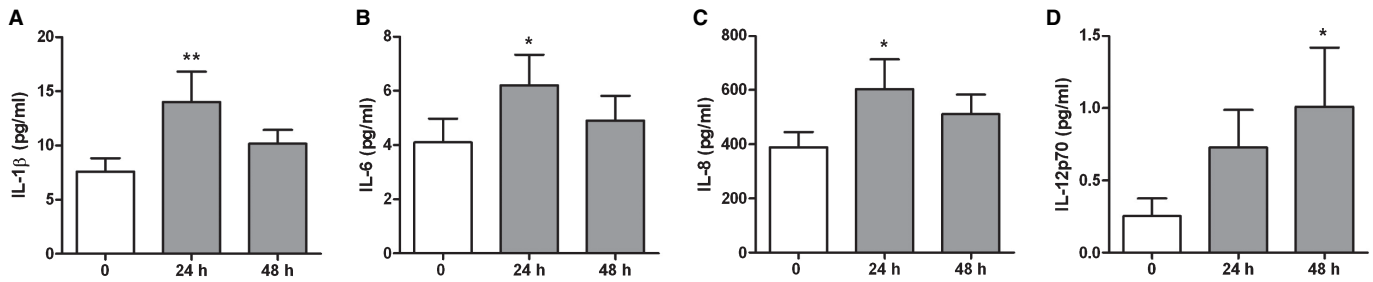


Figure 2. Levels of (A) IL-1 β , (B) IL-6, (C) IL-8, and (D) IL-12p70 in nasal lavage fluids determined before and 24 and 48 h after nasal administration of CpG (10 μ M) by the use of ELISA. All results are presented as mean $\pm$ SEM ($n = 20$; * $P < 0.05$; ** $P < 0.01$).

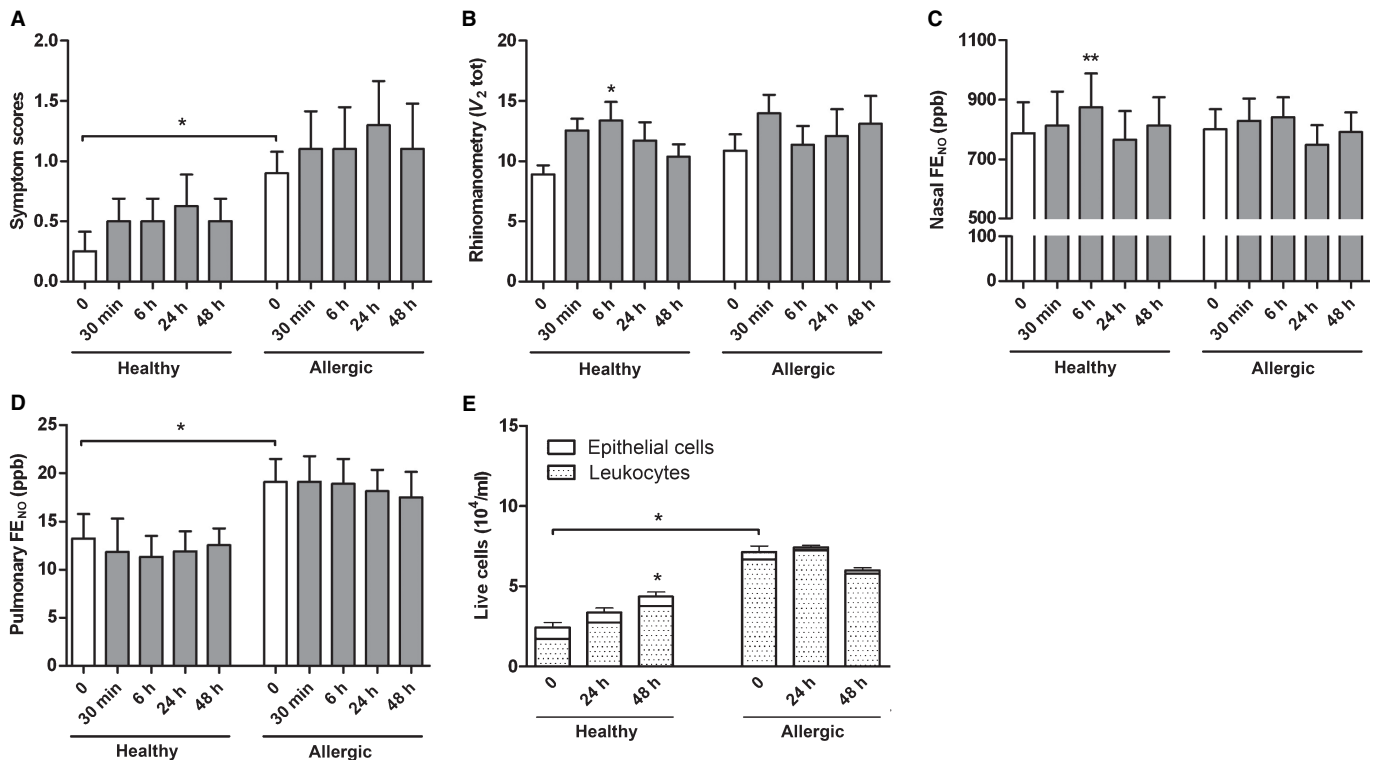


Figure 3. Patients with intermittent allergic rhinitis ($n = 10$) and healthy controls ($n = 10$) were examined prior to and 30 min, 6, 24, and 48 h post nasal administration of CpG (10 μ M). (A) Total symptom score, representing the cumulative score from nose and lungs. (B) Nasal airway resistance determined by anterior rhinomanometry. (C) Nasal nitric oxide (NO) levels. (D) Pulmonary NO levels. (E) Number of live epithelial cells and leukocytes in nasal lavage fluid. All results are presented as mean $\pm$ SEM (* $P < 0.05$; ** $P < 0.01$). FE, fractional exhaled.

groups with and without a history of AR. The allergic patients reported more symptoms during basal conditions (prechallenge) than the healthy individuals, but neither group exhibited significant increases in their symptoms following CpG challenge (Fig. 3A). The nasal resistance increased as a result of CpG exposure in the healthy group reaching its maximum after 6 h, whereas no such increase could be recorded among the allergic patients (Fig. 3B). Analogously, the nasal NO production increased in the healthy group 6 h post CpG challenge, while this was not seen in the allergic group (Fig. 3C). A higher

basal production of NO in the lungs could be recorded among the allergic patients, but no further increase was seen after CpG administration in either of the groups (Fig. 3D). The number of total cells (leukocytes and epithelial cells) increased 24 and 48 h after CpG in NAL obtained from the healthy individuals. A corresponding increase was not seen in the allergic group (Fig. 3E).

The secretion of IL-1 β , IL-6, and IL-8 was increased in the healthy group 24 h after the CpG administration, whereas no effects of CpG were seen among the allergic patients (Fig. 4A–C). However, a trend towards a higher

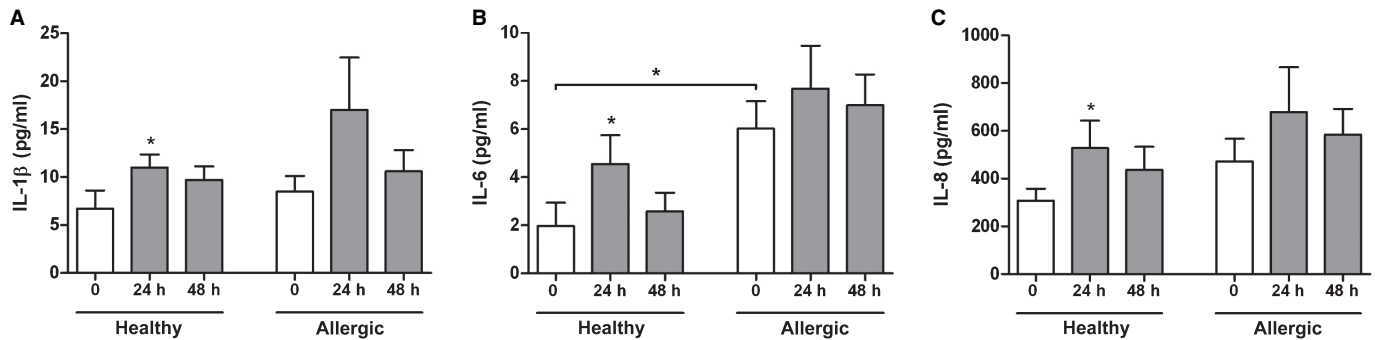


Figure 4. Levels of (A) IL-1 β , (B) IL-6, and (C) IL-8 in nasal lavage fluids of patients with intermittent allergic rhinitis ($n = 10$) and healthy volunteers ($n = 10$) determined before and 24 and 48 h after nasal administration of CpG (10 μ M) by the use of ELISA. All results are presented as mean $\pm$ SEM (* $P < 0.05$).

basal cytokine release was seen in the allergic as compared with the healthy participants, particularly for IL-6. The IL-12p70 secretion tended to be higher in the allergic group after CpG challenge, but did not reach statistical significance ($P = 0.06$). A similar trend was seen for IL-4 ($P = 0.12$) and IFN- γ ($P = 0.18$). Levels of MIP-1 α were the same in both groups, and they were unaffected by the treatment (Table 1).

In vitro cytokine secretion by neutrophils and epithelial cells

To investigate the cellular source behind the cytokine production in response to CpG, neutrophils isolated from peripheral blood, and Detroit-562, a nasopharyngeal epithelial cell line, were used. Both cell types were found to express TLR9 (Fig. 5A,B). CpG gave rise to a significant secretion of IL-1 β , IL-6, and IL-8 by neutrophils after 4 and 20 h (Fig. 5C–E). In epithelial cells, high levels of IL-6 and IL-8 were detected (Fig. 5F,G), whereas IL-1 β was absent (data not shown). However, only IL-8 release was affected by CpG.

Discussion

Several studies have demonstrated the immunostimulatory properties of CpG *in vitro* as well as *in vivo* (16).

However, there are no reports exploring the effects of nasal exposure to CpG in the human system. The present study shows that CpG induces a local inflammatory response in the human nose. An increase in NAR, nasal NO production, and secretion of IL-1 β , IL-6, and IL-8 was seen as a result of CpG exposure. This inflammatory response was more prominent in healthy subjects than in patients with a history of AR. Moreover, *in vitro* experiments suggest that the CpG-induced effects are mediated mainly by nasal epithelial cells and neutrophils.

The mucosal surfaces are an important site where microbes and TLRs interact and subsequently mount crucial innate and adaptive immune responses (17, 18). Nasal challenge with LPS (via TLR4), which is present in high levels in organic dust, induces a local cellular infiltration and increases secretion of IL-1 β , IL-6, IL-8, and TNF- α , without affecting nasal resistance or airway NO levels (3, 4, 6). Intranasal administration of Imiquimod, an imidazoquinoline that binds TLR7, has been shown to rapidly induce high levels of IFN- α and TNF- α in primates (19). The present study is the first to demonstrate that administration of the TLR9 ligand CpG ODN affects both clinical parameters and cytokine release in the human nose. To rule out the possibility that the effects were induced by the vehicle, healthy subjects were treated with physiological saline (0.9% NaCl). However, no increases in symptoms, NAR,

Table 1. Cytokine release in nasal lavage fluids obtained from healthy ($n = 10$) and allergic subjects ($n = 10$) prior to and 24 and 48 h post CpG challenge

pg/ml	Healthy subjects			Allergic patients		
	Before	24 h	48 h	Before	24 h	48 h
IL-1 β	6.7 $\pm$ 1.9	11.0 $\pm$ 1.4*	9.7 $\pm$ 1.4	8.5 $\pm$ 1.6	17.0 $\pm$ 5.4	10.6 $\pm$ 2.2
IL-4	2.6 $\pm$ 0.9	3.8 $\pm$ 1.1	2.4 $\pm$ 1.0	3.6 $\pm$ 0.5	4.7 $\pm$ 0.6	3.1 $\pm$ 0.3
IL-6	2.0 $\pm$ 1.0	4.5 $\pm$ 1.2**	2.6 $\pm$ 0.8	6.0 $\pm$ 1.1	7.7 $\pm$ 1.8	7.0 $\pm$ 1.3
IL-8	306.9 $\pm$ 50.9	528.2 $\pm$ 115.4*	437.3 $\pm$ 96.7	471.7 $\pm$ 95.9	679.6 $\pm$ 188.2	585.5 $\pm$ 107.2
IL-12p70	0.4 $\pm$ 0.2	0.2 $\pm$ 0.1	0.7 $\pm$ 0.4	0.2 $\pm$ 0.1	1.2 $\pm$ 0.4	1.3 $\pm$ 0.7
IFN- γ	7.7 $\pm$ 3.1	6.6 $\pm$ 2.7	5.9 $\pm$ 2.6	7.4 $\pm$ 4.6	33.6 $\pm$ 17.8	23.3 $\pm$ 16.3
MIP-1 α	35.3 $\pm$ 0.9	37.7 $\pm$ 1.5	35.4 $\pm$ 0.8	35.2 $\pm$ 0.9	37.7 $\pm$ 1.8	36.0 $\pm$ 1.0

Data depicted as mean $\pm$ SEM.

* $P < 0.05$; ** $P < 0.01$.

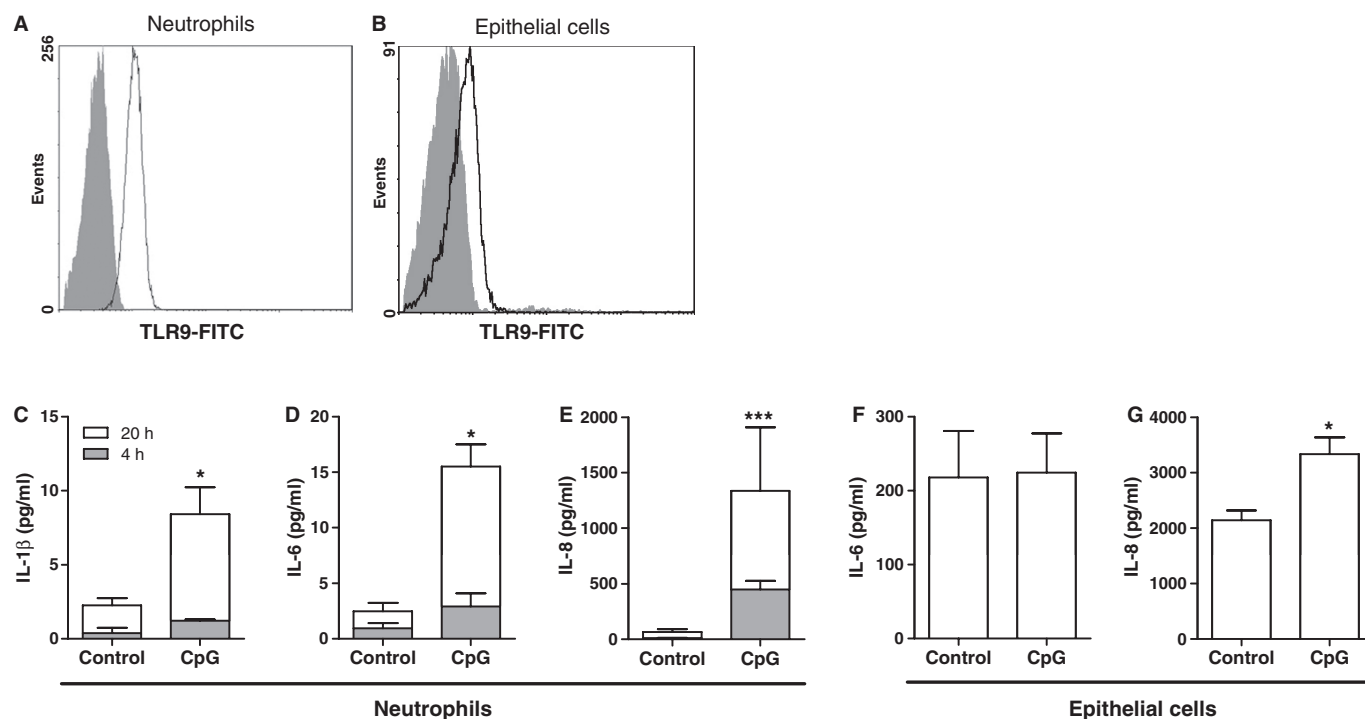


Figure 5. (A–B) Expression of TLR9 in neutrophils and epithelial cells. Cells were stained with TLR9-FITC (open histogram) or isotype control (filled histogram) and analyzed by flow cytometry. (C–E) Neutrophils were cultured for 4 and 20 h, and (F–G) epithelial cells for 24 h, in the absence or presence of CpG (1 μ M). Thereafter, levels of IL-1 β , IL-6, and IL-8 in the supernatants were determined by the use of ELISA. All results are presented as mean $\pm$ SEM ($n = 3$ –5; * $P < 0.05$; *** $P < 0.001$).

leukocyte numbers, or cytokines were found. Previous studies in murine and primate models of asthma show that immunostimulatory sequences (ISS), containing CpG motifs, induce Th1 cytokines and affect allergic airway symptoms, including inhibition of airway eosinophilia and hyperresponsiveness (20–24). A randomized phase 2 trial of a vaccine consisting of Amb a 1, a ragweed-pollen antigen, conjugated to a TLR9 agonist, appeared to offer long-term clinical efficacy in the treatment of ragweed AR by suppressing the seasonal rise in IgE and improving rhinitis scores, nasal symptoms, and quality of life (25). Another carefully performed clinical trial, demonstrated that 1018 ISS inhalation induced the expression of IFN- γ and IFN-related genes, without inhibiting allergen-induced bronchoconstriction, hyperresponsiveness, or sputum-derived eosinophils (26).

In the present study, CpG was found to induce an inflammatory response in the nose that persisted for 48 h. This was manifested by an increase in nasal resistance as a measure of nasal obstruction during respiration, an increase in infiltrating leukocytes, production of cytokines, and an induction of NO in the nose. Nitric oxide, which is detected in exhaled air, plays a key role in the nonspecific host defense by its antimicrobial activity, and has been shown to increase in the upper airways during respiratory tract infections (27). Sources behind the nasal NO production include the paranasal sinuses, epithelial

cells, macrophages, and neutrophils (15, 27, 28). Moreover, nasal application with CpG did not seem to affect lung functions as the pulmonary NO levels remained unchanged during the study period. Previous work carried out by Kodama et al. (16) has shown that nasal administration with CpG in mice caused no signs of local or systemic inflammation. The number of CD8 $^{+}$ T cells was increased but no tissue damage could be recorded. In contrast, when CpG was co-administered with P6, an outer membrane protein of nontypeable *Haemophilus influenzae*, a P6-specific mucosal and systemic immune response was seen (16). In line with this, other groups have shown intranasal delivery of CpG ODN, as an adjuvant in mice, to induce strong mucosal and systemic immune responses to co-administered viral antigens including glycoprotein B of herpes simplex virus-1 and hepatitis B surface Ag (29–31).

In humans, plasmacytoid dendritic cells (PDCs) and B cells have been described as the primary cell types expressing TLR9, and these cells are known to respond directly to CpG ODN stimulation (11, 32). CpG promotes B-cell activation and stimulates PDCs to produce IFN- α (16). Based on the capability to activate PDCs and B cells, three structurally different classes of CpG-ODNs have been described (33): CpG-A, which induces large amounts of IFN- α by PDCs; CpG-B, which promotes B-cell activation but is weak in inducing IFN- α secretion by PDCs; and CpG-C, which combines the stimulatory

attributes of CpG-A and CpG-B (11, 34). The CpG used in this study is of type B. As the B-cell numbers are comparably low in the nasal mucosa, we propose that TLR9-expressing epithelial cells and neutrophils that are present in high numbers, have a role in this response (9, 35, 36).

The inflammatory response to infectious stimuli involves the secretion of a variety of cytokines (37). In the present study, generation of IL-1 β , IL-4, IL-6, IL-8, IL-12p70, IFN- γ , and MIP-1 α could be demonstrated in NAL fluids, whereas IFN- α , IL-5, and IL-10 could not be detected. Moreover, upon administration with CpG, levels of IL-1 β , IL-6, IL-8 were significantly increased 24 h post challenge in healthy, but not in allergic individuals. A trend towards an increased secretion of IL-4, IL-12p70, and IFN- γ was seen in the allergic group after exposure to CpG. Even though not reaching significant levels, these cytokines might be causally linked. In contrast to the key role of IL-4 in inducing Th2-cell differentiation, IL-4 also has the potential to promote Th1 responses. Studies have shown that IL-4-mediated conditioning of DCs generates IL-12p70 and consequently instructs IFN- γ production by T cells (38–40). This aspect of immune regulation may be of importance in Th2-related diseases such as asthma and AR. The cytokine levels in unprocessed NAL were overall low, but the induction by CpG may still be physiologically relevant. In NAL fluids, neutrophils are the dominating cells reflecting their continuous migration into the nasal cavity because of the constant exposure to inhaled particles (41). They have previously been described to release IL-6 and IL-8 upon CpG ODN stimulation (35). Also, nasal epithelial cells have been reported to express TLR9 (9, 36, 42). Present *in vitro* experiments propose IL-1 β , IL-6, and IL-8 to emanate mainly from neutrophils and the epithelium. However, a recent study has shown a cross talk between epithelial cells and polymorphonuclear leukocytes upon TLR4-mediated activation by *Candida albicans* (43). Therefore, the present data should be interpreted with caution as physical interactions between neutrophils and epithelial cells might affect the outcome of the response. In addition, as cell lines do not fully reflect the responses of primary cells, it should be taken into account that the reality in the nose might be slightly different. Further, the cytokine profile seen upon CpG administration suggests the involvement of a pro-inflammatory and a Th1-type (IL-1 β , IL-6, IL-8, and IL-12p70), rather than a Th2-type (IL-4, IL-5, and IL-10), immune response (11, 44).

The allergic subjects displayed higher basal symptom scores, pulmonary NO production, NAL leukocyte numbers, and cytokine levels as compared with the healthy participants. On the other hand, the allergic patients seemed to be almost anergic by not significantly responding to CpG provocation. The ongoing so-called minimal persistent inflammation seen among the allergic individuals might result in a higher activation threshold,

thus providing an explanation to the differences seen between the two groups (45, 46). We have previously demonstrated the presence of TLR9 in the nasal mucosa and NAL neutrophils, and that the expression is identical in healthy controls and allergic patients outside as well as during pollen season (9). However, a recent study has demonstrated a decreased expression of TLR9 in sinonasal epithelial cells of patients with chronic rhinosinusitis as compared with cells from healthy donors (42). Thus, a damage of TLR9-expressing nasal epithelial cells caused by the persistent inflammation in the allergic subjects cannot be excluded, thereby explaining the reduced responsiveness to CpG. Another reason might be related to the differences in the set of responder cells, or level of responder cells, between the allergic and nonallergic nasal mucosa. Hartmann et al. (33) have shown that the number of PDCs in the nasal mucosa of allergic patients is lower than the numbers found in healthy individuals. A similar phenomenon might be true also for other cell types. It should also be noted that the allergic patients were examined out of season. The magnitude of the allergic Th2 response would probably be minimal during this period, and possible effects of CpG might consequently be masked by this fact. Nevertheless, several preclinical and early clinical studies suggest that CpG ODN has a high potential as a novel therapeutic alternative for treatment of allergic asthma and related diseases (47). Even though the allergic subjects were anergic to nasal CpG challenge, it is possible that a synergistic response can be induced upon co-administration with allergen. This can occur in a similar manner as for mice that are unresponsive to CpG but have been shown to give rise to a profound immune activation upon simultaneous administration with viral or bacterial antigens (16, 29–31).

To summarize, the present study demonstrates that CpG induces a local inflammatory response in the human nose, a response more marked in healthy than in allergic individuals and with a cytokine pattern skewed towards a Th1 profile. Immune activation by CpG ODN has been shown by several groups to be advantageous in animal models as a vaccine adjuvant for infectious diseases and for the treatment of asthma/allergy based on its propensity to induce a Th1-dominated immune response (7, 12). Hence, the present results warrant further clinical studies with nasal application of CpG during bacterial or viral infections and during symptomatic allergic airway disease to investigate the potential of CpG to augment both humoral and cell-mediated immune responses.

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