

IP-10 in occupational asthma: review of the literature and case-control study

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Abstract

Objectives. T-helper (Th)2 cytokines are thought to mediate most of the allergic inflammatory responses associated with atopic asthma. But the Th1-related chemokine, interferon (IFN)- γ -induced protein 10 (IP-10)/chemokine (C-X-C motif) ligand (CXCL)10, was the predominant chemokine measured during human allergic pulmonary late-phase reaction. Viral infection and allergens can exacerbate asthma by inducing the accumulation of these chemokines and inflammatory cells in the airway. Short-acting β 2-adrenoreceptor agonists, budesonide and formoterol (all important relievers in asthma exacerbation), such as vitamin D3, vitamin C, have been shown to inhibit airway cells inflammatory responses by modulating these chemokines. Furthermore it has been suggested that Th1-related IP-10 and monokine induced by IFN- γ (MIG)/CXCL9 may be useful inflammatory markers of asthma exacerbation.

Patients and Methods. In this study we have evaluated serum levels of the Th1-related CXC chemokine IP-10, in 8 patients with occupational asthma (OA) during exacerbation due to occupational exposure, and after 2-3 months, when patients were in stable conditions, in comparison with 8 age and gender matched healthy subjects.

Results. A significant increase in the serum levels of IP-10 were found in OA patients with an acute exacerbation in contrast to healthy controls ($p < 0.01$), and in comparison with same OA patients after 2-3 months, when they were without any respiratory symptoms or disorders.

Conclusions. These results suggest that the Th1-related CXC chemokines IP-10 is an useful inflammatory marker of OA exacerbation. However, other studies in larger number of patients are needed. *Clin Ter 2017; 168(2):e151-157. doi: 10.7417/CT.2017.1998*

Key words: asthma, CXCR3 chemokines, IP-10, occupational asthma, Th1 chemokines

Introduction

Asthma is a disease of the airways of the lungs characterized by reversible airflow obstruction, and bronchospasm (1), causing about 489,000 deaths (2) and increased significantly since the 1960s (3).

Asthma is thought to be caused by a combination of genetic and environmental factors (allergens, air pollution, and other environmental chemicals, but also medications such as aspirin and beta blockers) (4-7). Onset before age 12 is more likely due to genetic influence, while onset after 12 is more likely due to environmental influence (5).

Asthma is associated with exposure to indoor allergens (8): dust mites, cockroaches, animal dander (fragments of fur or feathers), and mold (9, 10). Certain viral respiratory infections, such as respiratory syncytial virus and rhinovirus (11), may increase the risk of developing asthma when acquired as young children (12). There is a correlation between obesity and the risk of asthma with both having increased in recent years (13, 14).

Asthma as a result of (or worsened by) workplace exposures is a commonly reported occupational disease (15). It is estimated that 10–25% of asthma cases in adults are work-related. A few hundred different agents have been implicated with the most common being: isocyanates, grain and wood dust, colophony, soldering flux, latex, animals, and aldehydes. The employment associated with the highest risk of problems include: those who spray paint, bakers and those who process food, nurses, chemical workers, those who work with animals, welders, hairdressers and timber workers (15).

A non-specific bronchial hyperreactivity test can be used to help diagnose occupational asthma (OA). It involves testing with methacholine, after which the forced expiratory volume in 1 second (FEV₁) of the patient is measured. This test is often used for measuring the intensity of a person's asthma and to confirm that the person needs to be treated for asthma (16-22).

Symptoms include episodes of wheezing, coughing, chest tightness, and shortness of breath (23). These episodes may occur a few times a day or a few times per week. Depending on the person they may become worse at night or with exercise (7). Signs which occur during an asthma attack include the use of accessory muscles of respiration (sternocleidomastoid and scalene muscles of the neck), there may be a paradoxical pulse, and over-inflation of the chest

(24). A blue color of the skin and nails may occur from lack of oxygen (25).

Diagnosis is usually based on the pattern of symptoms, response to therapy over time, and spirometry (26). Asthma is classified according to the frequency of symptoms, FEV₁, and peak expiratory flow rate (27). It may also be classified as atopic or non-atopic, where atopy refers to a predisposition toward developing a type 1 hypersensitivity reaction (28, 29).

Avoidance of triggers is a key component of improving control and preventing attacks. The most common triggers include allergens, smoke (tobacco and other), air pollution, non selective beta-blockers, and sulfite-containing foods (30, 31).

There is no cure for asthma (7). Symptoms can be prevented by avoiding triggers, such as allergens and irritants, and by the use of inhaled corticosteroids (32, 33). Long-acting beta agonists or antileukotriene agents may be used in addition to inhaled corticosteroids if asthma symptoms remain uncontrolled (34, 35). Treatment of rapidly worsening symptoms is usually with an inhaled short-acting β 2-adrenoreceptor agonists (SABAs) such as salbutamol and corticosteroids taken by mouth (36). In very severe cases, intravenous corticosteroids, magnesium sulfate, and hospitalization may be required (37).

Asthma is the result of chronic inflammation of the airways, bronchi and bronchioles, which subsequently results in increased contractability of the surrounding smooth muscles. This among other factors leads to bouts of narrowing of the airway and the classic symptoms of wheezing. The narrowing is typically reversible with or without treatment (38). Typical changes in the airways include an increase in eosinophils and thickening of the lamina reticularis. Chronically the airways' smooth muscle may increase in size along with an increase in the mucosa of the numbers of mucous glands, T lymphocytes, macrophages, and neutrophils, and other components of the immune system (cytokines, chemokines, histamine, and leukotrienes) (11).

Here we review the chemokine (C-X-C motif) receptor (CXCR)3 chemokines in asthma, and we study whether IP-10 could be a marker of exacerbated OA.

Interferon (IFN)- γ -induced protein 10 (IP-10) in inflammation

IP-10 is a chemokine able to induce integrin activation and to generate directional migration of a lot of cell types [activated T cells, monocytes, and natural killer (NK) cells]; therefore it can regulate inflammation at several levels (39).

IP-10 can also induce apoptosis of pancreatic beta cells and inhibit the proliferation of both epithelial and endothelial cells (40, 41).

IP-10 has other proinflammatory functions, as induction of molecules [like interleukin (IL)-8 and chemokine (C-X-C motif) ligand (CXCL)-5], and up-regulation of costimulatory cell surface molecules (CD54, CD80, CD86, etc), on monocytes.

Under the influence of IFN- γ , which is itself dependent on the IL-12 cytokine family, IP-10 is secreted by several cell types, including T lymphocytes, monocytes, splenocytes, fibroblasts, keratinocytes, thyrocytes, preadipocytes, etc. The

detection of high levels of IP-10 in peripheral liquids can be a marker of host immune response, especially T helper (Th)1 orientated T-cells. Th1 lymphocytes, that are recruited, may be responsible for the enhancement of the production of IFN- γ and tumour necrosis factor (TNF)- α ; they in turn stimulate IP-10 secretion from the above mentioned cells, therefore creating an amplification feedback loop (42).

Circulating levels of IP-10 are increasing with age. Moreover, it was shown that the serum and/or the tissue expressions of IP-10 are increased in organ specific autoimmune diseases (43), such as type 1 diabetes (T1D) (44), Graves' disease (GD), or Graves' ophthalmopathy (GO) (45-47), autoimmune thyroiditis (48-53), or systemic rheumatological disorders like rheumatoid arthritis (RA) (54), systemic sclerosis (SSc) (55-57), psoriasis or psoriatic arthritis (58-62), sarcoidosis (63, 64), HCV-related cryoglobulinemia (65-69), other HCV immune mediated disorders (70, 71), lupus (72, 73), and also in cancers (74-82).

Asthma and IP-10

Segmental antigen bronchoprovocation has long been used as a model to study allergic pulmonary inflammatory responses. The preferential recruitment of Th 2 lymphocytes is among the characteristics of the resulting cellular infiltrate. A first study (83) tested the hypothesis that the chemokines would be released at sites of segmental allergen challenge. Segmental allergen challenge with saline or allergen was performed in 10 adult allergic subjects with asthma, who were off medications. Bronchoalveolar lavage (BAL) was performed at both the saline- and allergen-challenged sites 20 hours after challenge. BAL fluids were analyzed for total cell counts and chemokines.

These data suggest that among the chemokines measured in this study, IP-10 is the predominant chemokine detected 20 hours after saline challenge, likely representing baseline production of a chemokine that favors Th1 cell recruitment. At antigen-challenged sites, levels of both chemokine C-C (motif) receptor type 4 (CCR4) and CXCR3 active chemokines, but not CCR8 active chemokines, are markedly increased and are produced at levels that are likely to have biologic significance. Given the preferential accumulation of Th2 cells at these antigen-challenged sites, the increased production of CCR4-active chemokines might contribute to this response (83).

CXCR3 binding chemokine IP-10 markedly enhances antigen-specific Th1 recall responses in healthy humans, suggesting a role for this pathway in the maintenance of clinical tolerance to environmental allergens as well as a potential therapeutic role for CXCR3 ligands in re-balancing the Th2-dominated responses that underlie generation and maintenance of allergic disorders. A study (84) investigated the capacity of CXCR3 ligands to modulate allergen-driven IFN- γ production by healthy and allergic individuals characterized by Th1 and Th2 immunity-dominated allergen specific responses, respectively. Exogenous CXCR3 ligands up-regulated antigen-dependent IFN- γ production from healthy individuals' peripheral blood mononuclear cells up to 120-fold, while allergic individuals were strikingly hyporesponsive to CXCR3 ligands suggesting CXCR3-ligation preferentially augments ongoing Th1 over Th2 responses

and that reduced capacity of allergic individuals to respond to CXCR3 ligands promotes the maintenance of human allergic disorders (84).

In a third study (85) BAL was performed in 15 nonsmoking mild atopic asthmatics before and 24 h after a fibreoptic segmental allergen challenge, and chemokines related to T-cell recruitment were assayed by ELISA. The Th2-related CCR4 ligands, MDC and TARC, were increased in BAL after challenge. There were no alterations in monokine induced by interferon (MIG) or macrophage inflammatory protein 1- α (MIP-1- α); whereas a significant increase in IP-10 was observed, which did not correlate with the T-cell influx (85).

A further study (86) investigated the modulation of nuclear factor- κ B (NF- κ B) and the mitogen-activated protein kinase (MAPK) pathway on the *in vitro* release of chemokines upon the interaction of human bronchial epithelial BEAS-2B cells and eosinophils. The interaction of eosinophils and BEAS-2B cells was found to up-regulate the gene expression of the chemokines interleukin (IL)-8, monocyte chemoattractant protein (MCP)-1, MIG, regulated upon activation normal T cell expressed and secreted (RANTES) and IP-10 expression in BEAS-2B cells, and to significantly elevate the release of the aforementioned chemokines, except RANTES in a coculture of BEAS-2B cells and eosinophils (86).

Statins have anti-inflammatory effects on immune cells. A study (87) investigates the immunomodulatory effects of fluvastatin on peripheral blood mononuclear cells (PBMCs) after allergen-specific and non-allergen-specific stimulation in patients with asthma and in healthy subjects suggesting that fluvastatin has inhibitory effects on cytokine and chemokine production, and thus might be used as a potential therapeutic agent in severe asthma (87).

Ketotifen is a mast cell stabilizer and useful in younger children with allergic diseases such as asthma and allergic rhinitis. A study (88) investigated the effects of ketotifen on the expression of Th1- and Th2-related chemokines of human monocytes *in vitro* and *ex vivo*. Ketotifen significantly down-regulated lipopolysaccharide (LPS)-induced MDC, MIG and IP-10 ($p < 0.05$, each comparison) in THP-1 cells and human primary monocytes in a dose-dependent manner. SB203580 [p38 MAPK inhibitor] suppressed LPS-induced MDC and IP-10 expression, and PD98059 (ERK-MAPK inhibitor) could only suppress LPS-induced IP-10, but not MDC expression. These data demonstrated that ketotifen is effective in down-regulating LPS-induced MDC, MIG and IP-10, which play important roles in the pathogenesis of airway inflammation (88).

Th2 cytokines are thought to mediate most of the allergic inflammatory responses associated with atopic asthma, but the Th1-related chemokine, IP-10, was the predominant chemokine measured during human allergic pulmonary late-phase reaction. A study (89) aimed to evaluate the role of Th1- and Th2-related chemokines in the pathogenesis of asthma exacerbation. A significant increase in the plasma levels of IP-10 and MIG were found in patients with an acute exacerbation in contrast to patients with stable asthma. Plasma levels of IP-10 and MIG were significantly higher in patients during an acute asthma exacerbation than during a subsequent convalescent period. These results suggest

that Th1-related CXC chemokines IP-10 and MIG may be useful inflammatory markers of asthma exacerbation in children (89).

Low vitamin C and reduced α -carotene intake are associated with increased asthma risk in children. In addition, mean serum vitamin A concentrations are significantly lower in asthmatic children than in controls. All-trans retinoic acid (ATRA) is a derivative of vitamin A. A study (90) evaluated whether ATRA and ascorbic acid effect Th1- and Th2-related chemokine expression in monocytes. To test this, THP-1 cells were pre-treated with ATRA or ascorbic acid and stimulated by LPS or poly I:C. Supernatants were measured for Th2-related (MDC) and Th1-related (IP-10) chemokine concentrations by ELISA. After stimulation, ATRA significantly down-regulated MDC and IP-10 in a dose-dependent manner. RT-PCR showed ATRA inhibited IP-10 expression through decreasing the level of transcription. These results demonstrated ATRA suppressed Th2- and Th1-related chemokines expression in THP-1 cells (90).

Exposure to ubiquitous allergens early in life, even before birth, may influence the incidence of allergic diseases later in life. During pregnancy, the fetomaternal interface is surrounded by high levels of Th2-like cytokines, possibly favouring the development of Th2-like immune responses in the offspring. The aim of one study (91) was to evaluate the relation between cord blood (CB) IgE antibodies, Th1- and Th2-like cytokines and chemokines, maternal allergy and development of allergic disease during the first 2 years of life in the offspring. The CB cytokine and chemokine levels from children of 20 allergic and 36 non-allergic women were determined by a multiplexed Luminex assay and ELISA. The results suggested that development of allergic disease is associated with a more marked Th2-like deviation already at birth, shown as increased levels of CB IgE and MDC and higher ratios of MDC to IP-10 and I-TAC (91).

As indicated in the Global Initiative for Asthma guidelines, SABAs are important relievers in asthma exacerbation. Regulated on activation, normal T expressed and secreted (RANTES) is a chemokine which plays a role in attractant of eosinophils, mast cells, and basophils toward the site of allergic inflammation. Bronchial epithelial cells are first-line barriers against pathogen invasion. A study (92) evaluated, whether SABAs have regulatory effects on the expression of IP-10 and RANTES in bronchial epithelial cells. BEAS-2B cells, the human bronchial epithelial cell lines, were pretreated with procaterol (one of the SABAs) or dibutyryl-cAMP (a cyclic AMP analog) at different doses for 1 hour and then stimulated with poly I:C (10 μ g/mL). Supernatants were collected 12 and 24 hours after poly I:C stimulation to determine the concentrations of IP-10 and RANTES by ELISA. In some cases, the cells were pretreated with selective β 2-adrenoreceptor antagonist, ICI-118551, 30 min before procaterol treatment. Procaterol significantly suppressed poly I:C-induced IP-10 and RANTES in BEAS-2B cells in a dose-dependent manner. ICI-118551, a selective β 2-adrenoreceptor antagonist, could significantly reverse the suppressive effects. Dibutyryl-cAMP could confer the similar effects of procaterol on poly I:C-induced IP-10 and RANTES expression. These data suggest that SABAs could suppress poly I:C-induced IP-10 and RANTES expression in bronchial epithelial cells (92, 93).

The association between vitamin D deficiency and asthma epidemic has been recognized. TNF- α and chemokines play important roles in the pathogenesis of asthma. A further study (94) investigated whether vitamin D has immunoregulatory function on TNF- α and chemokines expression in human monocytes pretreating the human monocytic cell line, THP-1 cells and human primary monocytes with various concentrations of 1 α ,25-(OH)(2)D(3) for 2 hours before stimulation with LPS. This study suggests 1 α ,25-(OH)(2)D(3) could significantly suppress TNF- α and Th1-related chemokine IP-10, which plays important roles in the pathogenesis of severe refractory asthma (94).

Viral infection can exacerbate asthma by inducing the accumulation of inflammatory cells in the airway. It has been previously reported that double-stranded RNA (dsRNA), a viral product and ligand of the Toll-like receptor-3, activates the transcription factors NF- κ B and interferon regulatory factor (IRF)3 and upregulates the expression of inflammatory chemokines in airway epithelial cells. A study (95) examined the effects of the glucocorticoid fluticasone propionate (FP) on the expression of the inflammatory chemokines RANTES, IL-8 and IP-10. The airway epithelial cell line BEAS-2B was treated with FP and dsRNA-induced expression of RANTES, IL-8 and IP-10 protein and mRNA were significantly and dose-dependently (10^{-9} to 10^{-6} M) inhibited. This study suggests FP inhibits the dsRNA-stimulated expression of inflammatory chemokines in airway epithelial cells (95).

Combination therapy with budesonide and formoterol reduces exacerbations of asthma, which are closely associated with human rhinovirus (RV) infections in both children and adults. These data suggest that budesonide and formoterol inhibit virus-induced inflammatory responses of airway epithelial cells. To test this hypothesis, bronchial epithelial (BE) cells were obtained from airway brushings of 8 subjects with moderate-to-severe allergic asthma and 9 with neither asthma nor respiratory allergies (96). Cultured BE cells were incubated for 24 hours with budesonide (1.77 μ M), formoterol (0.1 μ M), both, or neither, and then inoculated with RV-16 (5×10^6 plaque forming units [PFU]/mL). After 24 hours, viral replication (RV RNA), cytokine secretion and mRNA expression were analyzed suggesting that budesonide and formoterol can inhibit BE cell inflammatory responses *in vitro* without interfering with viral replication or production of IFNs, by modulating chemokines (96).

Patients and Methods

The aim of the present study is to evaluate the role of Th1 chemokine IP-10 in the pathogenesis of OA exacerbation.

Serum levels of the Th1-related CXC chemokine IP-10 were measured in 8 patients with OA during exacerbation due to occupational exposure, and after 2-3 months, after standard therapy with β_2 -agonists, and corticosteroids, when patients were in stable conditions without any respiratory symptoms or disorders (and without no therapy from at least 3 weeks), in comparison with 8 healthy subjects.

Among the 8 patients with OA, all were affected by grain OA (bakers). The criteria for OA were studied, and fully established as previously reported (15, 20-22).

All OA patients were not affected by disorders that might alter IP-10 levels, such as: a) thyroid autoimmune disorders; b) other autoimmune disorders; c) evidence of infectious diseases in the last three months; d) treatment with drugs known to interfere with immune system, namely cytokines, IFN, non-steroidal anti-inflammatory drugs (NSAIDs), amiodarone, lithium; e) pregnancy and lactation over the previous 6 months; f) presence of acute or chronic systemic diseases.

The control group consisted of 8 healthy subjects, matched by age and gender with 8 OA patients (6 males, 2 females, age range 22-54 years), extracted from a random sample of the general population from the same geographic area in whom a complete clinical work-up was available, and excluded the presence of asthma, or disorders that might alter CXCL10 levels, such as: a) thyroid autoimmune disorders; b) other autoimmune disorders; c) evidence of infectious diseases in the last three months; d) treatment with drugs known to interfere with immune system, namely cytokines, IFN, non-steroidal anti-inflammatory drugs (NSAIDs), amiodarone, lithium; e) pregnancy and lactation over the previous 6 months; f) presence of acute or chronic systemic diseases.

All study subjects gave their informed consent to the study, which was approved by the local Ethical Committee.

Serum IP-10 levels were assayed by a quantitative sandwich immunoassay using a commercially available kit (R&D Systems, Inc., Minneapolis, MN, USA) (48, 59, 60) with a sensitivity ranging from 0.40-4.51 pg/mL and a mean minimum detectable dose of 1.61 pg/mL. The intra- and inter-assay coefficients of variation were 3.2% and 6.5%.

Data analysis Values are given as mean $\pm$ SD for normally distributed variables, otherwise as median and [interquartile range]. Mean group values were compared by using one-way analysis of variance (ANOVA) for normally distributed variables (age and body mass index), otherwise by the Mann-Whitney *U* or Kruskal-Wallis test. Proportions were compared by the χ^2 test. *Post-hoc* comparisons on normally distributed variables were carried out using the Bonferroni-Dunn test.

Results

A significant increase in serum IP-10 levels were found in OA patients with an acute exacerbation in contrast to healthy controls ($p < 0.01$) (Tab. 1). Serum levels of IP-10 were significantly higher ($p < 0.05$) in OA patients with an acute exacerbation, than in the same OA patients after 2-3 months, when patients were in stable conditions without any respiratory symptom or disorders.

Conclusion

Th2 cytokines are thought to mediate most of the allergic inflammatory responses associated with atopic asthma, but the Th1-related chemokine, IP-10, was the predominant chemokine measured during human allergic pulmonary late-phase reaction. Viral infection and allergens can exacerbate

Table 1: Serum IP-10 in OA patients with exacerbation, the same OA patients after 2-3 months in stable conditions, or healthy controls.

	Controls	Patients with exacerbated OA	The same patients after 2-3 months	P value
Male/female	6/2	6/2	6/2	ns
Age (years)	37±11	37±11	37±11	ns
IP-10 (pg/ml)	65±31	109±37*°	71±35	<0.01

ns= not significant, p>0.05

* p <0.01 vs controls

° p<0.05 vs the same patients after 2-3months

asthma by inducing the accumulation of these chemokines and inflammatory cells in the airway. SABAs, budesonide and formoterol (all important relievers in asthma exacerbation), such as vitamin D3, vitamin C, have been shown to inhibit airway cells inflammatory responses by modulating these chemokines. Furthermore it has been suggested that Th1-related IP-10 and MIG may be useful inflammatory markers of asthma exacerbation.

Our experimental study suggests that the Th1-related CXC chemokine IP-10 is an useful inflammatory marker of OA exacerbation in patients with wood dust OA. These results agree with those obtained in patients with exacerbated asthma, of not occupational origin (89). However, other studies in larger number of OA patients are needed.

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