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SERUM LEVELS OF CERTAIN CC AND CXC CHEMOKINES IN BIRCH POLLEN ALLERGIC INDIVIDUALS OUT OF THE POLLEN SEASON

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Abstract. Chemokines play a key role in the regulation of cell trafficking during immune responses. In pollen-allergic individuals, the roles of chemokines have been predominantly studied during active allergic reactions. However, little is known about chemokine levels and their effect on immune responses out of the pollen season when atopic individuals do not show clinical symptoms of allergy. Therefore, the aim of the present study was to investigate the serum levels of CCL11/Eotaxin, CCL17/TARC, CCL22/MDC, CXCL1/GROα, CXCL9/MIG, CXCL10/IP-10, and CXCL11/I-TAC out of the pollen season and to determine whether there are differences between birch pollen-allergic and non-allergic individuals. We observed significantly increased concentrations of CCL11/Eotaxin ($p<0.01$), CCL17/TARC ($p<0.01$), CCL22/MDC ($p<0.01$), CXCL9/MIG ($p<0.05$) and CXCL10/IP-10 ($p<0.05$) in the sera of birch pollen-allergic patients compared to healthy individuals. In contrast, the serum levels of CXCL1/GROα were lower ($p<0.05$) in the allergic group compared to the non-allergic subjects. Furthermore, IFN-γ and IL-17 levels were significantly elevated ($p<0.05$) in the sera of birch-pollen allergic individuals. These results suggest persistent Th17 activity in birch pollen-allergic individuals. The detected differences implicate a role of these chemokines in subclinical allergic responses, which could provide the basis for development of new therapies and strategies for disease monitoring.

Keywords: *allergy, birch pollen, chemokines, cytokines, subclinical allergic responses.*

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1. Introduction

Pollen allergy incidence has increased over the last decades (D'Amato *et al*., 2007; Bryce *et al*., 2010). It has been estimated that approximately 40% of the population in Europe is sensitized to certain type of pollen (D'Amato *et al*., 2007). Furthermore, exposure to pollen is increasing due to longer pollen seasons with higher pollen counts and reduced rainfall in many regions as result of climate change (Shea *et al*., 2008). Hence, pollen allergy has a significant clinical and economic impact in terms of medical consultations and effective therapy, impaired quality of life and work performance (Bauchau and Durham, 2004).

Birch pollen is highly allergenic and causes one of the most prevalent pollinoses in Europe, North America and certain parts of Asia (D'Amato *et al*., 2007). Birch trees grow in the temperate climate zones and release large amounts of pollen during spring. The main birch allergens are the pathogenesis-related class 10 (PR-10) proteins from the European white birch *(Betula pendula)* termed Bet v 1 and its homologues present in pollen of other Fagales species (Bollen *et*

al., 2007). PR-10 proteins comprise the largest group of aeroallergens – a fact that highlights the social and clinical impact of birch pollen allergy. In addition, PR-10 proteins share epitopes with proteins of several fruits, vegetables and nuts and it has been shown that they are among the four most common food allergens (Breiteneder and Ebner, 2000; Ferreira *et al*., 2004).

Defining the molecular mechanisms of allergic responses in clinical and subclinical stage is a key point in development of more effective therapies and identification of markers for monitoring treatment and disease severity. Important modulators of allergic responses are the chemokines (Turner *et al*., 2014). They comprise a group of low molecular weight cytokines that serve as chemoattractants of leukocytes to sites of allergen challenge, inflammation and injury. Chemokines induce integrin-mediated adhesion and cytoskeletal rearrangement in target leukocytes that arrests their rolling and facilitates diapedesis through the endothelium (Constantin *et al*., 2000). Depending on the arrangement of the N-terminal cysteine residues mammalian chemokines are grouped into four subfamilies: CC (with adjacent cysteine residues), CXC (the two cysteines are separated by one amino acid residue), (X)C (the first /and the third/ cysteine is missing), and C3XC (containing 3 amino acid residues between the two N-terminal cysteines) (Zlotnik and Yoshie, 2012). The chemotactic activity of different chemokines plays a complex physiological role: some chemokines participate in the development of the immune system; others are mediators in innate or adaptive immune responses and immune surveillance; certain chemokines have homeostatic functions and constitutively regulate the migration and homing of different cell types; there are chemokines with dual function that play a role in both inflammatory responses and maintenance of homeostasis (Moser *et al*., 2004; Zlotnik and Yoshie, 2012). Aberrant chemokine expression have been implicated in a broad spectrum of diseases including asthma and allergy (Turner *et al*., 2014).. Xiao et al. have showed that plasma CCL17/TARC levels are elevated in subjects with pollinosis and they correlate with disease severity (Xiao *et al*. 2007). CCL22/MDC and CCL17/TARC were up-regulated in bronchial mucosa and skin of atopic individuals during late phase responses to allergen exposure (Pilette *et al*. 2004). Serum levels of CCL22/MDC have been related to disease activity in Elevated levels of the chemokines CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CCL7/MCP-3, CCL11/Eotaxin, CCL13/MCP-4, CCL24/Eotaxin-2, IL-8, and CXCL10/IP-10 have been detected in bronchoalveolar fluid and biopsy samples from asthmatic patients (Ying *et al*., 1999). Increased concentrations of CCL11/Eotaxin, CCL5/RANTES and CCL3/MIP-1α were found in nasal secretions of patients with seasonal allergic rhinitis (Konig *et al*., 2015). Another example is the increased expression of CCL17/TARC in human nasal epithelium after allergen exposure (Terada *et al*., 2001b)patients with atopic dermatitis and mycosis fungoides/Sézary Syndrome (Galli *et al*., 2000; Kakinuma *et al*., 2002). The role of CCL11/Eotaxin in allergic inflammation has been confirmed in several studies and also a correlation between increased eotaxin levels and symptoms of rhinitis have been demonstrated (Minshall *et al*., 1997; Pullerits *et al*., 2000; Terada *et al*., 2001a).

In relation to pollinosis, chemokines expression is generally studied in response to allergen challenge and/or during the pollen season. However, investigations on chemokine levels out of the pollen season are scarce. Therefore, our studies were concentrated on evaluation of CC- and CXC-chemokine serum levels in selected group of birch pollen allergic individuals in the absence of allergen challenge, out of the pollen season. We detected significant differences in CXCL1/GROα, CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL11/Eotaxin, CCL17/TARC and CCL22/MDC serum concentrations between birch pollen-allergic and non-atopic individuals together with increase of IL-17 and INF-γ levels in the atopic group. These data contribute to the characterization of subclinical allergic status in birch pollen atopics and stimulates further investigations on chemokine roles in the molecular mechanisms of pollinosis with the aim to identify new therapies, as well as new markers for control of treatment.

2. Materials and methods

***Subjects and study design*:** 32 individuals participated in the study: 20 patients with birch pollen allergy (12 females and 8 males, age range 29-50 years) and 12 non-allergic subjects (8 females and 4 males, age range 22-45 years). All participants were recruited in the period September-December 2013 in collaboration with an allergologist. Atopic subjects were selected based on their case history (birch pollen-induced allergic rhinitis diagnosed at least two years before the study), positive skin prick test to birch pollen (ALK-Abelló Ltd, Hørsholm, Denmark) and detected serum IgE-specific to birch pollen extract (measured by ImmunoCAP, ImmunoDiagnostics Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). Control subjects had negative skin prick tests against 10 standard aeroallergens (birch, grass, mugwort, horse, dog, cat, two house dust mite species and two mould species) (Soluprick, ALK-Abelló Ltd, Hørsholm, Denmark) and no clinical history of allergy. None of the participants was treated with allergen-specific immunotherapy, antihistamines or corticosteroids at least two months before the sampling. The study was performed in accordance to the Declaration of Helsinki ethical guidelines and was approved by the local Ethics committee. All participants signed written informed consent prior to initiation of the study.

***Serum sampling*:** Peripheral venous blood was collected from all participants in the study using Venoject® tubes (Terumo Corporation, Japan). Sampling was performed out of the pollen season in February, 2014 when none of the subjects showed clinical signs of allergy. Blood samples were centrifuged at 1500g for 15 minutes. Isolated sera were transferred to 2 ml cryotubes and stored at -80ºC.

Measurement of chemokine/cytokine concentrations: CXCL1/GROα, CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL11/Eotaxin, CCL17/TARC, CCL22/MDC, IL-17A, IL-10, IL-12, IFN-γ, TNF-α, TGF-β serum levels were measured using Human Common Chemokines/Cytokines Multi-Analyte ELISArray Kits (QIAGEN) with detection limit of 10 pg/ml. All samples including chemokine/cytokine standards were assayed in duplicates according to

manufacturer's instructions. Absorbance at 450 nm was measured with a correction set to 570 nm using Synergy-2 microplate reader (BioTek, USA).

Statistics: Statistical analyses were performed using the StatView software (SAS Institute, USA). The non-parametric Mann-Whitney *U* test was applied to determine differences between the birch pollen-allergic and the control group. *P* values less than 0.05 were considered statistically significant.

3. Results

Increased CC- and CXC-chemokine serum levels in birch pollen-allergic patients out of the pollen season.

To clarify whether there are differences in the chemokine levels between birch pollen-allergic and non-allergic individuals out of the pollen season, peripheral blood samples were collected from 20 allergic patients and 12 healthy controls within several days in February 2014. Figure 1 shows the measured CC-chemokine levels. Figure 2 displays CXC-chemokines serum concentrations. We observed significantly increased concentrations of CCL11/Eotaxin, CCL17/TARC, CCL22/MDC, CXCL9/MIG and CXCL10/IP-10 in the sera of birch pollen-allergic patients compared to healthy individuals. A pronounced difference in the serum concentration of CXCL11/I-TAC between the allergic and the control group was not observed (Figure 2, D). In contrast, the serum levels of CXCL1/GROα showed a significant decrease in the allergic group compared to the non-allergic subjects (Figure 2, A). CXCL1 belongs to the group of ELR^+ CXC-chemokines associated with selective recruitment of neutrophils and development of allergic airway diseases (Park *et al*. 2006). The lower levels of CXCL1 in birch pollen-allergic individuals are not surprising considering our previous data showing reduced serum levels of certain CC-chemokines in ragweed-allergic subjects out of the pollen season (Kostova *et al*. 2015). Reduced chemokine levels out of the season could represent a protective physiological mechanism that suppresses allergic inflammatory reactions in the absence of allergen challenge.

Cytokine profile of birch pollen-allergic subjects in the absence of allergen challenge.

In addition to CC- and CXC-chemokine levels measurements, the serum concentrations of several cytokines were analyzed. Figure 3 shows the serum concentrations of INF-γ, TGF-β and TNF-α, while Figure 4 presents the measured levels of IL-10, IL-12 and IL-17 in birch pollen-allergic subjects and non-allergic controls. Significant differences in regulatory (TGF-β and IL-10) and pro-inflammatory (IL-12 and TNF-α) cytokine levels were not detected. Interestingly, IL-17 and INF-γ levels were increased in the sera of allergic individuals, which suggest persistent Th17 activity. Elevated INF-γ could be due to activation of Th1 cells in response to prior active allergic state – a mechanism that skews the cellular response from Th2 to Th1. Further investigations are needed to support this hypothesis. IL-4 serum levels in birch pollen-allergic patients were similar to those in non-allergic controls (data not shown).

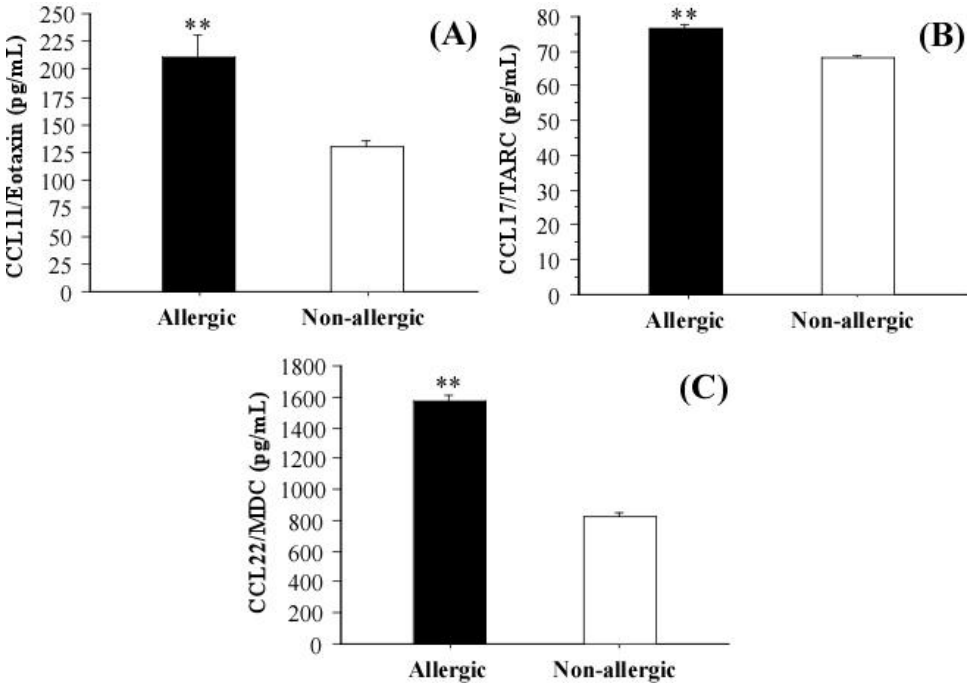

Fig. 1. Concentrations of CCL11/Eotaxin (**A**), CCL17/TARC (**B**) and CCL22/MDC (**C**) in serum samples of birch pollen-allergic (n=20) and non-allergic (n=12) individuals. The results show chemokine levels out of the pollen season and are presented as mean ±SD.

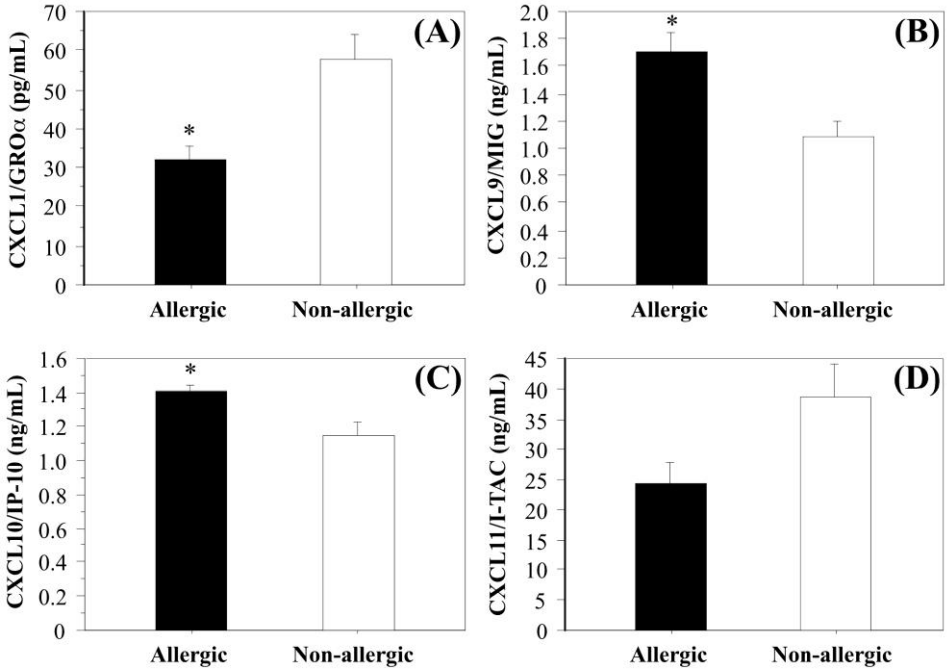

Fig. 2. CXC-chemokine levels out of the pollen season. CXCL1/GROα (**A**), CXCL9/MIG (**B**), CXCL10/IP-10 (**C**) and CXCL11/I-TAC (**D**) serum concentrations in birch pollen-allergic (n=20) and non-allergic (n=12) individuals.
The results are shown as mean ±SD.

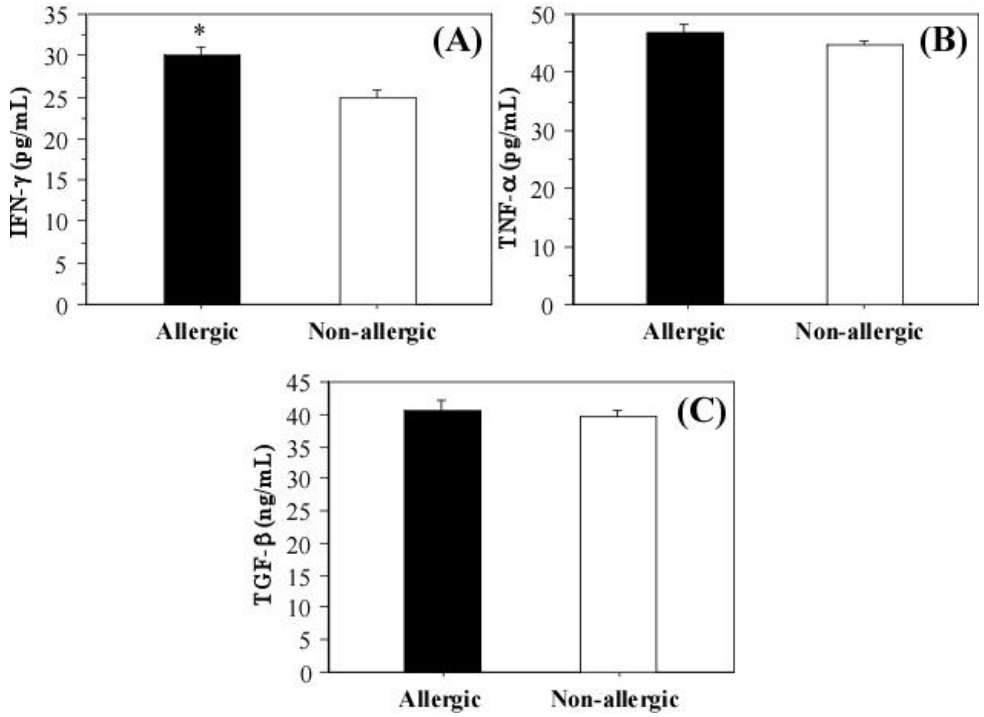

Fig. 3. IFN-γ (**A**), TNF-α (**B**) and TGF-β (**C**) serum concentrations in birch pollen-allergic individuals (n=20) and non-allergic (n=12) controls. Data are shown as mean ±SD and represent cytokine values out of the pollen season.

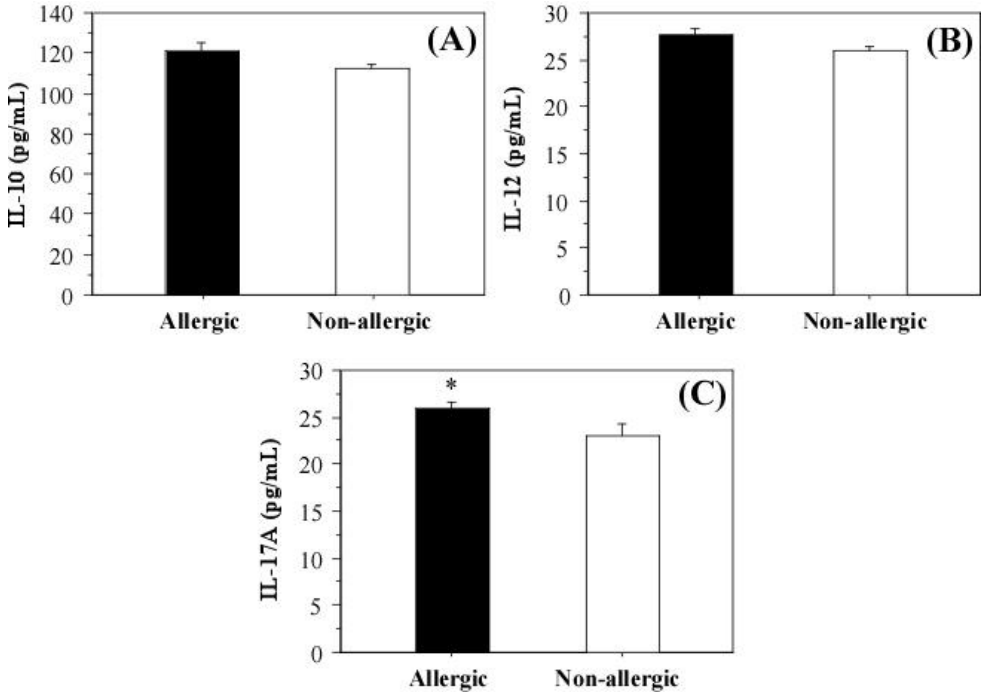

Fig. 4. IL-10 (**A**), IL-12 (**B**) and IL-17A (**C**) serum concentrations in birch pollen-allergic individuals (n=20) and non-allergic (n=12) controls measured out of the pollen season. The results are present as mean ±SD.

4. Discussion

Cytokines, in particular chemokines, are important mediators of allergic responses. Their levels and functions have been generally studied during active allergic reactions following allergen exposure. To date, little is known about chemokine serum concentrations out of the pollen season in the absence of allergen challenge. Our group has previously shown that ragweed-allergic subjects have decreased serum levels of the inflammatory chemokines CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES out of the pollen season (Kostova *et al*., 2015). Based on these data we have suggested that reduction of CC-chemokines concentration under the physiologic levels represents a protective mechanism against initiation of allergic inflammatory reaction. The present study demonstrates elevated serum concentration of several other CC-chemokines out of the pollen season. We found significantly higher levels of CCL11/Eotaxin, CCL17/TARC and CCL22/MDC in birch pollen-allergic individuals compared to non-allergic controls. This finding is not contradictory to our previous results and brings new information on different chemokines roles in pollinosis. CCL11/Eotaxin, CCL17/TARC and CCL22/MDC are members of the subgroup of chemokines with dual function (Zlotnik and Yoshie, 2012). Hence, under specific conditions they serve either as modulators of inflammatory reactions, or as homeostatic mediators. We assume that the increased serum levels of these chemokines in the absence of allergic inflammation could play a regulatory role aiming to restore homeostasis. This might be achieved by altered differential expression of chemokine receptors induced by other molecular factors. CCL17/TARC and CCL22/MDC bind CCR4 – a chemokine receptor that has been associated with human allergen-specific induction of late nasal responses (Banfield *et al*., 2010), which is primarily expressed on Th2 cells that are main players in allergic reactions (Campbell and HayGlass, 2000). Down-regulation of CCR4 expression or blocking of the receptor by physiologically expressed antagonists could explain the lack of allergic reaction in the presence of higher CCL17 and CCL22 serum concentrations. Further experiments are needed to confirm this hypothesis. On the other hand, CCL11/Eotaxin binds different receptors: CCR2, CCR3, CCR5 and CXCR3 (Zlotnik and Yoshie, 2012). CC-receptors 2, 3 and 5 are associated with inflammatory responses, as well as CXCR3 (Zlotnik and Yoshie, 2012). However, it is also well known that CXCR3 agonists could play anti-allergy role (Gangur *et al*., 2003). Thus, one could speculate that increased CCL11 levels out of the pollen season may favor preferential interactions with CXCR3. In addition, it is possible that CXCR3 expression is increased during periods without allergen challenge. This assumption is supported by our data on the CXC-chemokines CXCL9/MIG and CXCL10/IP-10. We show increased serum levels of these chemokines out of the pollen season. CXCL9/MIG, CXCL10/IP-10 and CXCL11/I-TAC also bind to CXCR3 and it has been shown that endogenous production of CXCR3 agonists might be beneficial for allergic individuals by promoting anti-allergic Th1 responses and by interfering with CCR3 mediated pathogenic pathway (Gangur *et al*., 2003). It was reported that CXCR3 ligands are potent natural antagonists of

CCR3-mediated chemotaxis and CXCR3 agonists are preferentially chemotactic to Th1 cells but not Th2 cells (Campbell and HayGlass, 2000; Cosmi *et al*., 2001; Loetscher *et al*., 2001). Wiley et al. demonstrated that induced by adenovirus-mediated gene transfer expression of human CXCL10/IP-10 at the time of intranasal sensitization attenuates Th2 cytokines expression and eosinophilia by IFN-γ dependent mechanism (Wiley *et al*., 2001). In concordance to these studies, we show CXCL10/IP-10, CXCL9/MIG and IFN-γ elevated serum levels that suggest induction of Th1 response in birch pollen-allergic individuals out of the pollen season that could provide a protective negative feedback leading to suppression of allergic inflammatory responses. It has been demonstrated that Th1 lymphocytes and cytokines like IFN-γ and IL-12 may suppress allergic Th2 responses (Cohn *et al*., 1999; Teixeira *et al*., 2005). IFN-γ reduces the expression of the eotaxin receptor CCR3, which has been shown to induce eosinophil differentiation from hematopoietic progenitor cells (Lamkhioued *et al*., 2003). Consequently, IFN-γ could regulate the differentiation, activation and recruitment of eosinophils. The adoptive transfer of IFN-γ-producing cells into allergen-sensitized recipients protected them from airway eosinophilia (Cohn *et al*., 1999). Furthermore, it has been demonstrated that defective IFN-γ production predisposes to allergy development (Teixeira *et al*., 2005) and resolution of allergic inflammation depends not on reduction of Th2 cytokine production but on normalization of IFN-γ levels (Smart *et al*., 2002). These data emphasize the inhibitory character of IFN-γ on the allergic responses supporting our results and hypothesis for skewing of the immune responses towards Th1 activity out of the pollen season and modulation of chemokine receptors expression.

The anti-allergy effect of Th1 responses and IFN-γ could raise the question why these responses do not provide long-term allergy protection even during the pollen season. However, together with Th1 activity evidenced by elevated IFN-γ, MIG and IP-10 levels we also demonstrate persistent Th17 activity based on significantly increased IL-17 serum concentration. This data leads to the assumption that there is an imbalance in Th1 and Th17 subsets and activities that allows recurrent allergic inflammation during the pollen season. Levels of the regulatory cytokines IL-10 and TGF-β were similar in non-allergic and birch pollen-allergic subjects, which suggest impaired peripheral tolerance due to ineffective regulatory T cells functionality. In fact, supporting our assumption, Soyka et al. have reported decreased suppressive activity of allergen-specific T regulatory cells in subjects with seasonal allergic rhinitis (Soyka *et al*., 2012).

IL-17 has a broad proinflammatory effect and it has been shown to contribute to the development and sustainment of Th2 allergic responses (Nakae *et al*., 2002). A recent study demonstrated significant increase in circulating Th17 cells following allergen inhalation in asthmatics when compared to normal controls (Naji *et al*., 2014). The same report indicates that peripheral blood mononuclear cells (PBMCs) of atopic asthmatics produce significantly more IL-17A *in vitro* than PBMCs from healthy individuals. Confirming previous studies in murine models of allergen-induced airway disease, these data proof the importance of Th17 cells for the pathogenesis of allergic diseases (Kratzer and Pickl, 2016). Our findings further indicate a role of Th17 cells in pollinosis. The

increased serum levels of IFN-γ and IL-17 out of the pollen season suggest imbalance between different T cell subsets that could result in impaired tolerance to allergens. Future research in the field will clarify the mechanisms behind this imbalance paving the way for new therapeutic approaches.

5. Conclusion

In conclusion, the present paper demonstrates new information on CC- and CXC-chemokine serum levels, as well as cytokines with central role for Th1, Th17 and T-regulatory responses out of the pollen season. The decreased serum concentrations of CXCL1/GROα and the elevated levels of CCL11/Eotaxin, CCL17/TARC, CCL22/MDC, CXCL9/MIG and CXCL10/IP-10 could be used for more accurate evaluation of the allergic status of patients with pollen allergy out of the season; to study the mechanisms of subclinical allergic responses; for development of new therapeutic strategies.

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References

1. Banfield G., Watanabe H., Scadding G., Jacobson M.R., Till S.J., Hall D.A., Robinson D.S., Lloyd C.M., Nouri-Aria K.T., Durham S.R., (2010) CC chemokine receptor 4 (CCR4) in human allergen-induced late nasal responses, *Allergy*, 65(9), 1126-1133.
2. Bauchau V., Durham S.R., (2004) Prevalence and rate of diagnosis of allergic rhinitis in Europe, *The European Respiratory Journal*, 24(5), 758-764.
3. Bollen M.A., Garcia A., Cordewener J.H., Wichers H.J., Helsper J.P., Savelkoul H.F., Boekel M.A., (2007) Purification and characterization of natural Bet v 1 from birch pollen and related allergens from carrot and celery, *Molecular Nutrition & Food Research*, 51(12), 1527-1536.
4. Breiteneder H., Ebner C., (2000) Molecular and biochemical classification of plant-derived food allergens, *The Journal of Allergy and Clinical Immunology*, 106(1), 27-36.
5. Bryce M., Drews O., Schenk M.F., Menzel A., Estrella N., Weichenmeier I., Smulders M.J., Buters J., Ring J., Gorg A., Behrendt H., Traidl-Hoffmann C., (2010) Impact of urbanization on the proteome of birch pollen and its chemotactic activity on human granulocytes, *International Archives of Allergy and Immunology*, 151(1), 46-55.
6. Campbell J.D., HayGlass K.T., (2000) T cell chemokine receptor expression in human Th1- and Th2-associated diseases, *Archivum Immunologiae et Therapiae Experimentalis*, 48(6), 451-456.
7. Cohn L., Homer R.J., Niu N., Bottomly K., (1999) T helper 1 cells and interferon gamma regulate allergic airway inflammation and mucus production, *The Journal of Experimental Medicine*, 190(9), 1309-1318.

8. Constantin G., Majeed M., Giagulli C., Piccio .L, Kim J.Y., Butcher E.C., Laudanna C., (2000) Chemokines trigger immediate beta2 integrin affinity and mobility changes: differential regulation and roles in lymphocyte arrest under flow, *Immunity*, 13(6), 759-769.
9. Cosmi L., Annunziato F., Maggi E., Romagnani S., Manetti R., (2001) Chemoattractant receptors expressed on type 2 T cells and their role in disease, *International Archives of Allergy and Immunology*, 125(4), 273-279.
10. D'Amato G., Cecchi L., Bonini S., Nunes C., Annesi-Maesano I., Behrendt H., Liccardi G., Popov T., van Cauwenberge P., (2007) Allergenic pollen and pollen allergy in Europe, *Allergy*, 62(9), 976-990.
11. Ferreira F., Hawranek T., Gruber P., Wopfner N., Mari A., (2004) Allergic cross-reactivity: from gene to the clinic, *Allergy*, 59(3), 243-267.
12. Galli G., Chantry D., Annunziato F., Romagnani P., Cosmi L., Lazzeri E., Manetti R., Maggi E., Gray P.W., Romagnani S., (2000) Macrophage-derived chemokine production by activated human T cells *in vitro* and *in vivo*: preferential association with the production of type 2 cytokines, *European Journal of Immunology*, 30(1), 204-210.
13. Gangur V., Birmingham N.P., Thanesvorakul S., Joseph S., (2003) CCR3 and CXCR3 as drug targets for allergy: principles and potential, *Current Drug Targets-Inflammation and allergy*, 2(1), 53-62.
14. Kakinuma T., Nakamura K., Wakugawa M., Mitsui H., Tada Y., Saeki H., Torii H., Komine M., Asahina A., Tamaki K., (2002) Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis, *Clinical and Experimental Immunology*, 127(2), 270-273.
15. Konig K., Klemens C., Eder K., San Nicolo M., Becker S., Kramer M.F., Groger M., (2015) Cytokine profiles in nasal fluid of patients with seasonal or persistent allergic rhinitis, *Allergy, Asthma, and Clinical Immunology*, 11(1), 26 p.
16. Kostova Z., Batsalova T., Moten D., Teneva I., Dzhambazov B., (2015) Ragweed-allergic subjects have decreased serum levels of chemokines CCL2, CCL3, CCL4 and CCL5 out of the pollen season, *Central European Journal of Immunology*, 40(4), 442-446.
17. Kratzer B., Pickl W.F., (2016) Years in Review: Recent Progress in Cellular Allergology, *International Archives of Allergy and Immunology*, 169(1), 1-12.
18. Lamkhioued B., Abdelilah S.G., Hamid Q., Mansour N., Delespesse G., Renzi P.M., (2003) The CCR3 receptor is involved in eosinophil differentiation and is up-regulated by Th2 cytokines in CD34+ progenitor cells, *Journal of Immunology*, 170(1), 537-547.
19. Loetscher P., Pellegrino A., Gong J.H., Mattioli I., Loetscher M., Bardi G., Baggiolini M., Clark-Lewis I., (2001) The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3, *The Journal of Biological Chemistry*, 276(5), 2986-2991.
20. Minshall E.M., Cameron L., Lavigne F., Leung D.Y., Hamilos D., Garcia-Zepada E.A., Rothenberg M., Luster A.D., Hamid Q., (1997) Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis, *American Journal of Respiratory Cell and Molecular Biology*, 17(6), 683-690.
21. Moser B., Wolf M., Walz A., Loetscher P., (2004) Chemokines: multiple levels of leukocyte migration control, *Trends in Immunology*, 25(2), 75-84.

22. Naji N., Smith S.G., Gauvreau G.M., O'Byrne P.M., (2014) T helper 17 cells and related cytokines after allergen inhalation challenge in allergic asthmatics, *International Archives of Allergy and Immunology*, 165(1), 27-34.
23. Nakae S., Komiyama Y., Nambu A., Sudo K., Iwase M., Homma I., Sekikawa K., Asano M., Iwakura Y., (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses, *Immunity*, 17(3), 375-387.
24. Park S.J., Wiekowski M.T., Lira S.A., Mehrad B., (2006) Neutrophils regulate airway responses in a model of fungal allergic airways disease, *Journal of Immunology*, 176(4), 2538-2545.
25. Pilette C., Francis J.N., Till S.J., Durham S.R., (2004) CCR4 ligands are up-regulated in the airways of atopic asthmatics after segmental allergen challenge, *The European Respiratory Journal*, 23(6), 876-884.
26. Pullerits T., Linden A., Praks L., Cardell L.O., Lotvall J., (2000) Upregulation of nasal mucosal eotaxin in patients with allergic rhinitis during grass pollen season: effect of a local glucocorticoid, *Clinical and Experimental Allergy*, 30(10), 1469-1475.
27. Shea K.M., Truckner R.T., Weber R.W., Peden D.B., (2008) Climate change and allergic disease, *The Journal of Allergy and Clinical Immunology*, 122(3), 443-453.
28. Smart J.M., Horak E., Kemp A.S., Robertson C.F., Tang M.L., (2002) Polyclonal and allergen-induced cytokine responses in adults with asthma: resolution of asthma is associated with normalization of IFN-gamma responses, *The Journal of Allergy and Clinical Immunology*, 110(3), 450-456.
29. Soyka M.B., Holzmann D., Akdis C.A., (2012) Regulatory cells in allergen-specific immunotherapy, *Immunotherapy*, 4(4), 389-396.
30. Teixeira L.K., Fonseca B.P., Barboza B.A., Viola J.P., (2005) The role of interferon-gamma on immune and allergic responses, *Memorias do Instituto Oswaldo Cruz*, 100(1), 137-144.
31. Terada N., Hamano N., Kim W.J., Hirai K., Nakajima T., Yamada H., Kawasaki H., Yamashita T., Kishi H., Nomura T., Numata T., Yoshie O., Konno A., (2001a) The kinetics of allergen-induced eotaxin level in nasal lavage fluid: its key role in eosinophil recruitment in nasal mucosa, *American Journal of Respiratory and Critical Care Medicine*, 164(4), 575-579.
32. Terada N., Nomura T., Kim W.J., Otsuka Y., Takahashi R., Kishi H., Yamashita T., Sugawara N., Fukuda S., Ikeda-Ito T., Konno A., (2001b) Expression of C-C chemokine TARC in human nasal mucosa and its regulation by cytokines, *Clinical and Experimental Allergy*, 31(12), 1923-1931.
33. Turner M.D., Nedjai B., Hurst T., Pennington D.J., (2014) Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease, *Biochimica et Biophysica Acta*, 1843(11), 2563-2582.
34. Wiley R., Palmer K., Gajewska B., Stampfli M., Alvarez D., Coyle A., Gutierrez-Ramos J., Jordana M., (2001) Expression of the Th1 chemokine IFN-gamma-inducible protein 10 in the airway alters mucosal allergic sensitization in mice, *Journal of Immunology*, 166(4), 2750-2759.
35. Xiao J.Z., Kondo S., Takahashi N., Odamaki T., Iwabuchi N., Miyaji K., Iwatsuki K., Enomoto T., (2007) Changes in plasma TARC levels during Japanese cedar pollen season and relationships with symptom development, *International Archives of Allergy and Immunology*, 144(2), 123-127.
36. Ying S., Meng Q., Zeibecoglou K., Robinson D.S., Macfarlane A., Humbert M., Kay A.B., (1999) Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES,

monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (Intrinsic) asthmatics, *Journal of Immunology*, 163(11), 6321-6329.

37. Zlotnik A., Yoshie O., (2012) The chemokine superfamily revisited, *Immunity*, 36(5), 705-716.