



# Soluble serum HLA-G and HLA-A, -B, -C molecules in patients with seasonal allergic rhinitis exposed to pollens

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## ARTICLE INFO

### Article history:

Received 3 March 2009

Received in revised form 19 April 2009

Accepted 23 April 2009

### Keywords:

Allergic rhinitis

Pollens

Pollen season

Serum

sHLA-G

sHLA-A, -B, -C

## ABSTRACT

**Background:** Allergic rhinitis (AR) is characterized by a Th2 polarized immune response and soluble HLA (sHLA) molecules play an immunomodulatory role in this response. Previously, it has been reported that these molecules are increased in sera of patients with pollen-induced allergic rhinitis studied outside the pollen season. To date, however, no study has investigated there in AR patients during the pollen season. **Objective:** The aim of this study was to evaluate serum sHLA-G and sHLA-A, -B, -C levels in both AR patients and healthy controls.

**Methods:** 60 symptomatic allergic patients were enrolled. A group of 50 healthy subjects was included as a control. Serum sHLA-G and sHLA-A, -B, -C levels were determined by an immunoenzymatic method. Allergy severity was assessed by VAS for symptoms and drug use.

**Results:** Allergic patients had significantly higher levels of both sHLA-G ( $p < 0.001$ ) and sHLA-A, -B, -C ( $p = 0.001$ ) than normal controls. In addition, there was a very strong correlation between sHLA-G levels and clinical severity.

**Conclusion:** The present study confirms evidence that serum sHLA-G and sHLA-A, -B, -C molecules are significantly increased in patients with pollen-induced AR also during the pollen season. Moreover, sHLA-G might be considered as a biomarker for assessing clinical severity.

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## 1. Introduction

Allergic rhinitis (AR) is sustained by mucosal inflammation, characterized by mast cell and eosinophil activation. The inflammatory reaction is promoted, maintained, and amplified by T helper (Th)-2 cells [1]. Th2 cells play an important role in the pathogenesis of AR, as IL-4 permits isotype switching to IgE synthesis, while IL-5 allows, mostly in the nasal mucosa, eosinophil accumulation and activation [2,3]. In addition, peripheral blood mononuclear cells of AR patients predominantly produce IL-4 with respect to IFN- $\alpha$  expression by Th-1 cells, the so-called "Th2 polarization" [4]. Moreover, a number of recent studies indicate that regulatory T cells play an important role in controlling the Th2-biased response [5]. Indeed, a defect in T regulatory cells has been evidenced in allergic patients [6]. Therefore, allergic diseases are presently considered as immunoregulatory disorders [5].

In this regard, interest in HLA-G antigens has increased considering that they may be involved in inflammatory diseases [7]. HLA-G is a human, non-classic MHC molecule expressed in human-privileged sites and plays an essential role in the development of maternal tolerance to genetically

different fetal tissues [8]. Soluble HLA-G (sHLA-G) derives from the secretion of soluble isoforms as well as from the shedding of proteolytically cleaved surface isoforms. Several HLA-G-mediated mechanisms have been described. Most of them are immunosuppressive, however it has been reported that there is an induction of a Th2-like cytokine profile through stimulation of IL-3, IL-4, and IL-10 secretion [9]. Numerous studies provided evidence that sHLA-G serum levels are increased in different pathological conditions, including immune-mediated disorders, transplantation, and malignancies [10]. So far, however, only one study has been conducted on patients with pollen-induced allergic rhinitis investigated outside the pollen season. This study showed that they had significantly higher serum sHLA-G levels than healthy controls [11].

Moreover, it is also known that classic HLA class I molecules are characterized by a broad tissue expression and a high degree of polymorphism [12], and are found in the serum of healthy individuals in a soluble form (sHLA-A, -B, -C). The serum level of sHLA-A, -B, -C antigens may increase in physiological and pathological conditions such as pregnancy, acute rejection episodes following organ allografts, autoimmune diseases, viral infections, and tumours [13,14]. sHLA-A, -B, -C molecules may participate in immunoregulatory activities such as inhibition of alloreactive cytotoxic T lymphocyte (CTL) activity, apoptosis of CD8+ T lymphocytes and NK cells and suppression of allogeneic mixed lymphocyte cultures [15,16]. As for sHLA-G, one study

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investigated sHLA class I in allergic disorders and reported that sHLA-I heterodimers are elevated in 64% of patients with atopic dermatitis and that this elevation persisted after clinically effective therapy [17]. Therefore, there are no studies investigating the effect of pollen exposure on serum sHLA molecules. The reason for studying this issue is based on the fact that allergic reaction is strictly dependent on allergen exposure. Indeed, allergic inflammation and symptoms occur only after allergen exposure. Consequently, the aim of the present study was to evaluate patients with pollen-induced allergic rhinitis during the pollen season by measuring serum sHLA-G and sHLA-A, -B, -C molecules and compare these with normal subjects.

## 2. Methods

Sixty allergic patients (25 males and 35 females, mean age 34.9 years) were included in the study. In order to be enrolled in the study, patients were required to have allergic rhinitis due to pollen sensitization, documented by a positive skin prick test, performed according to validated criteria [18], for the specific pollen, and confirmed by a positive allergen-specific nasal challenge to the causal allergen. A negative nasal challenge for *Dermatophagoides* was considered as an inclusion criterion.

Patients either with acute upper respiratory infections, or undergoing specific immunotherapy, or using nasal or oral corticosteroids, antileukotrienes and antihistamines within the previous four weeks were excluded. Patients with documented allergy to non-pollen allergens were also excluded.

According to validated criteria, in order to make a diagnosis of PER, a history of nasal symptoms during at least two consecutive pollen seasons concordant with positive results from a skin prick test were considered as sufficient [19].

Fifty healthy subjects (25 males and 25 females, mean age 33.8 years) were enrolled as controls, all were skin prick test negative and did not suffer from any nasal disorder. Blood samples were collected from patients and controls to determine serum sHLA-G and sHLA-A, -B, -C levels during the pollen season.

### 2.1. Clinical parameters

Clinical severity was also evaluated, by considering nasal symptom perception and drug consumption at the end of the pollen season as previously validated [20]. The patient questionnaire included a validated visual analogue scale (VAS) for quantifying the perception of nasal symptom intensity [21]. Patients had to have a VAS score for nasal symptoms  $\geq 5$ .

During the pollen season, use of levocetirizine drops (ten or twenty, corresponding to a 2.5 or 5 mg concentration) on demand as rescue medication was permitted. However, medication was withdrawn at least one week before drawing blood samples. The medication scoring (MS) was as follows: not at all (0), occasionally/seldom (1), occasionally/often (2), almost daily (3), continuously with the lowest dose (4), and continuously with the maximal dose (5). The study was performed during the spring, the pollen season. The study was approved by the local Ethics Committee and written informed consent was obtained from all participants.

### 2.2. Soluble HLA molecules immunoassay

sHLA-G molecules were measured by a commercially available immunoenzymatic assay (EXBIO, Praha, Czech Republic) according to the manufacturer's instructions and results were expressed as ng/ml. The kit employs the antibody MEM/G-9 and Namb-I Horseradish Peroxidase Conjugate.

sHLA-A, -B, -C molecules were determined by a double determinant immunoassay performed as previously described [21], with minor modifications, utilizing anti-HLA class I monoclonal antibody W6/32 and biotinylated anti-a2-microglobulin monoclonal antibody NAMB-1

and results were expressed as ng/ml. The kit measuring HLA-I however considers all HLA molecules, including classic and non-classic, such as HLA-G and HLA-E, even though class E has never been detected in serum.

### 2.3. Statistical analysis

Descriptive statistics were calculated and reported in terms of frequencies and percentages for qualitative variables and in terms of medians, 25th and 75th quartiles and minimum and maximum values.

Comparisons of quantitative variables between two groups of patients which were not normally distributed were performed by the non-parametric Mann Whitney *U* test.

Analysis of the correlation between quantitative variables was made by the Spearman's correlation coefficient ( $r_s$ ). For the purpose of this analysis, correlation coefficients  $\geq 0.8$  were considered very strong, from 0.6 to 0.79 strong, from 0.4 to 0.59 moderate, from 0.2 to 0.39 weak and less than 0.2 very weak [22].

All statistical analyses were performed with SPSS (Version 13.0). A value of  $p < 0.05$  was considered statistically significant.

## 3. Results

All patients were allergic to Parietaria and/or grasses alone. The median (range) of serum sHLA-G levels was 35.38 [(2).03, 288.21] ng/ml

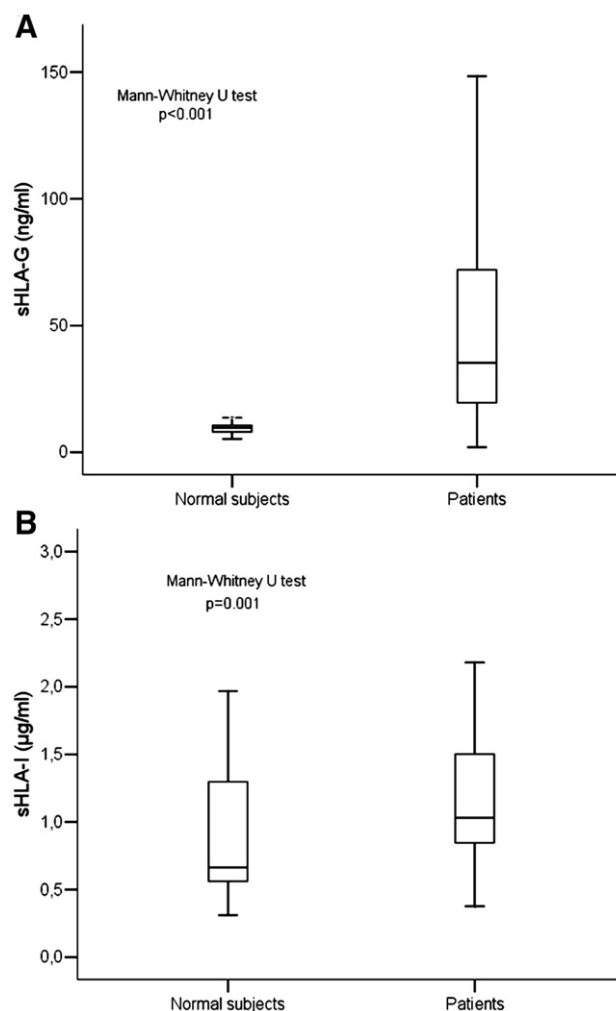


**Fig. 1.** Serum levels of sHLA-A, -B, -C (A) and sHLA-G (B) in the two groups of subjects. Box-plots are based on the quartile values: Boxes represent the interquartile range (contains 50% of central values), Whisker lines extend from the highest to the lowest values, excluding outliers (cases with values more than 1.5 box lengths) and the line across the box indicates the median value.

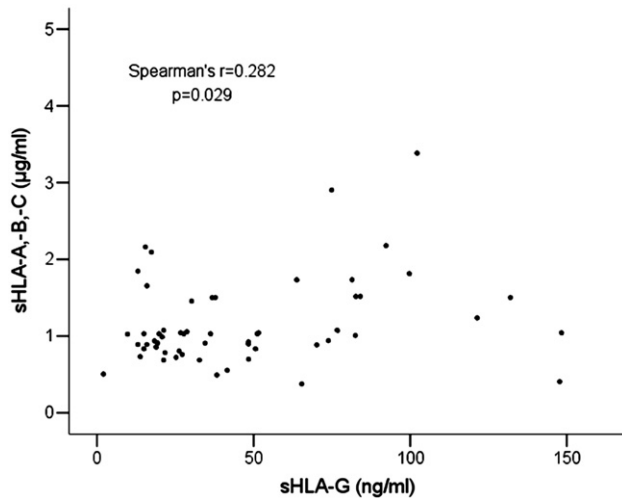


Fig. 2. Correlation between the levels of sHLA-G and sHLA-A, -B, -C in allergic patients.

in allergic patients and 9.76 [(1).34, 20.55) ng/ml in normal subjects, Patients with allergic rhinitis had significantly higher levels than normal subjects,  $p < 0.001$  (Fig. 1A). The median (range) of serum sHLA-A, -B, -C levels was 1.03 (0.38, 6.59)  $\mu\text{g/ml}$  in allergic patients and 0.66 (0.31, 1.97)

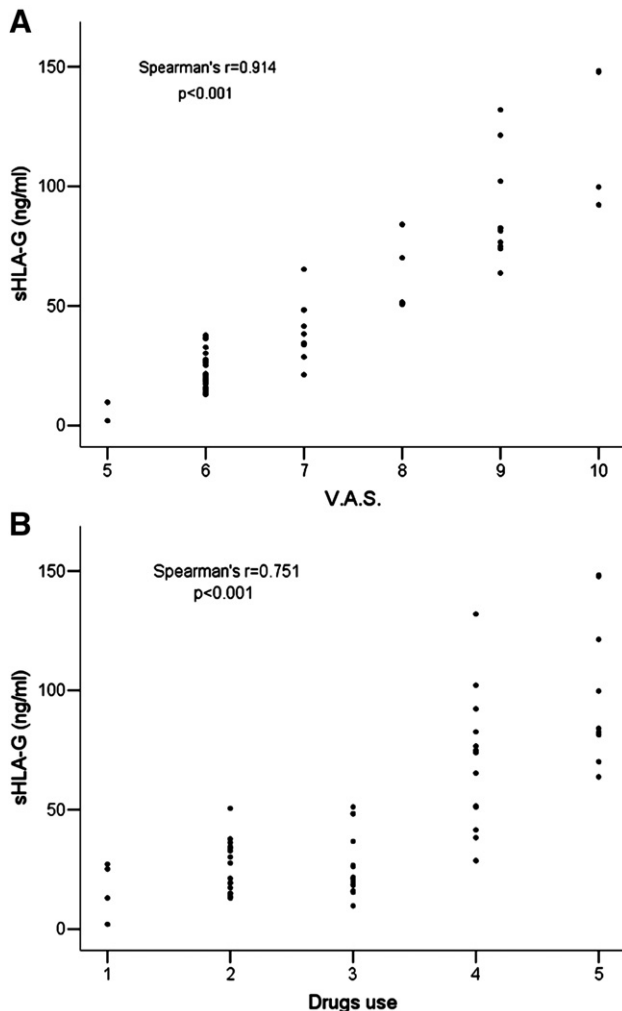


Fig. 3. Correlation between serum sHLA-G levels and V.A.S. of nasal symptoms (upper) and between serum sHLA-G levels and drug use (lower).

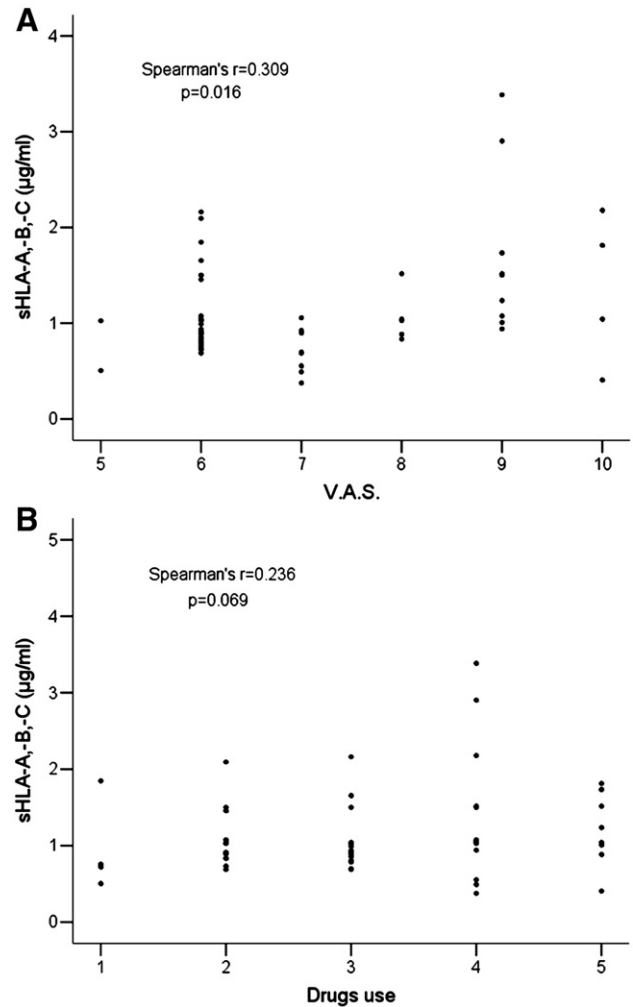


Fig. 4. Correlation between serum sHLA-A, -B, -C levels and V.A.S. of nasal symptoms (upper) and between serum sHLA-A, -B, -C levels and drug use (lower).

$\mu\text{g/ml}$  in normal subjects, Patients with allergic rhinitis had significantly higher levels than normal subjects,  $p = 0.001$  (Fig. 1B). There was a weak relationship between serum sHLA-G levels and serum sHLA-A, -B, -C levels ( $r = 0.282$ ;  $p = 0.029$ ) as reported in Fig. 2.

Instead, there was a significant, very strong correlation between serum sHLA-G levels and V.A.S. for nasal symptoms ( $r = 0.914$ ;  $p < 0.001$ ) and a strong correlation between serum sHLA-G levels and drug consumption assessed by monitoring ( $r = 0.751$ ;  $p < 0.001$ ) (Fig. 3). Finally, a significant, even though weak, positive relationship between sHLA-A, -B, -C serum values and symptom severity assessed by VAS was observed ( $r = 0.309$ ;  $p = 0.016$ ) as reported in Fig. 4. Instead, there was not a significant relationship between sHLA-A, -B, -C serum values and drug use, assessed by medication scoring as shown in Fig. 4.

#### 4. Discussion

Allergic rhinitis is characterized by a Th2-polarized inflammation. Th2-derived cytokines, such as IL-4 and IL-13, are the main pathogenic factors which are able to induce, maintain and amplify the allergic inflammation [23]. In addition, a deficit in Tregs characterizes allergic disorders, but this defect may be replaced by specific immunotherapy [6]. In this regard, sHLA-G molecules might play an important role in the mechanisms of immune tolerance toward allergens similar to their surface membrane bound counterparts, which have immunosuppressive

properties. In fact, sHLA-G molecules inhibit T cell proliferation induced by allogeneic dendritic cells [24] and, in analogy to sHLA-A, -B, -C molecules, which induce apoptosis of T and NK CD8+ cells and inhibit cytotoxic T cell activity through CD8 ligation by a Fas/sFasL interaction [23]. The immunoregulatory characteristics of HLA-G have prompted a number of studies aimed at evaluating the expression of sHLA-G in sera from patients affected by a variety of disorders of the immune system as reported by an extensive review [10]. To date, however, only one study has been conducted on patients with pollen-induced allergic rhinitis investigated outside the pollen season, and demonstrated that they had significantly higher serum sHLA-G and sHLA-A, -B, -C levels than healthy controls [11].

The present study was therefore aimed at evaluating the role of sHLA-G and sHLA-A, -B, -C molecules in a group of patients with pollen-induced allergic rhinitis evaluated during the pollen season, when both allergic inflammation and symptoms occur.

Results obtained first show that a significant increase of sHLA-G and sHLA-A, -B, -C molecules is detectable in patients with allergic rhinitis in comparison with normal subjects and that this increase is more evident for the sHLA-G molecule. These findings confirm a previous report demonstrating elevated levels of both soluble molecules in pollen-induced rhinitic patients evaluated outside the pollen season [11].

In addition, there was a slight significant relationship between sHLA-G and sHLA-A, -B, -C, partially confirming the previous results [11].

On the other hand, there was a very strong correlation between clinical severity, drug use, and sHLA-G values. This issue is very important as it allows for the definition of clinical relevance of the findings. Indeed, sHLA-G might be considered as a biomarker for evaluating clinical severity. Moreover, this outcome confirms two previous studies that evidenced the clinical relevance of serum sHLA-G as a possible biomarker for monitoring sublingual immunotherapy [20,25].

However, there was no difference when we compared the medians of sHLA-G serum levels in the two groups, outside or during the pollen seasons. This fact might be confusing as patients evaluated outside the pollen season were symptoms-free, whereas patients studied in season were symptomatic and a relationship between sHLA-G levels and symptom severity was demonstrated. A possible explanation for these conflicting results might stem from the immunological characteristics of patients. In fact, the immune response to allergens is typically allergen-specific. It has been previously reported that the clinical and inflammatory responses depend on the type of causal allergen [26]. In other words, each single type of pollen induces a different grade of clinical, functional, and inflammatory response [26]. In this regard, it has been previously reported that birch allergy is characterized by a more severe immune impairment in comparison with grass or Parietaria allergy [27,28]. In a previous study, which was conducted outside the pollen season, 21 out of 49 enrolled patients were allergic to birch [11]. On the contrary, the present study considered only patients allergic to grasses and/or Parietaria. Actually, considering the allergy type, patients with birch allergy showed the highest serum levels of sHLA-G. This fact might therefore partially explain these conflicting findings. Moreover, a limitation of this study is that the groups considered were different, such as the same patients were not evaluated outside and during the season.

Moreover, it is to consider that the assay for measuring sHLA-A, -B, -C actually doses all soluble HLA molecules, including G and E. On the other hand, the quantity of HLA-G was remarkably lower (about 3.5%) than that of HLA class I. Therefore, it appears reasonable to consider the reported level practically corresponding to the quote of HLA-A, -B, -C alone.

From a functional point of view, it might be hypothesized that sHLA molecules in these patients derive from immune cells involved in the underlying allergic reaction, as previously reported [9], sHLA-G might

be involved in maintaining the Th2-polarized immune response in allergic patients and in tolerogenic defective mechanisms. Functional studies therefore need to clarify the role of sHLA contribution in allergic reaction.

In conclusion, the present study provides the first evidence of a contextual and correlated increase of sHLA-G and sHLA-A, -B, -C molecules in patients with rhinitis due to pollen allergy evaluated during the pollen exposure. In addition, sHLA-G molecules might be considered biomarkers for assessing allergy severity in relationship with clinical parameters. These preliminary findings need to be confirmed by further studies in other allergic disorders.

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