

Soluble HLA-G Molecule in Patients with Perennial Allergic Rhinitis

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Key Words

Allergic rhinitis • Perennial allergens • Soluble HLA-G

Abstract

Background: Allergic rhinitis (AR) is characterized by T helper 2 polarized immune response. The soluble HLA-G (sHLA-G) molecule has an immunomodulatory activity. However, so far, no study has investigated this activity in perennial AR (PAR). **Objective:** The aim of this study was to evaluate serum sHLA-G levels in PAR patients and in a group of healthy controls. **Methods:** Twenty-five PAR patients were enrolled. A group of 50 healthy non-allergic subjects was considered as control. sHLA-G serum levels were determined by immunoenzymatic method. **Results:** Allergic patients had significantly higher levels of the sHLA-G ($p < 0.001$) molecule than normal controls. Moreover, there was a very strong correlation between sHLA-G and symptom severity and drug use ($r = 0.85$ and 0.793 , respectively). **Conclusion:** The present study provides first evidence that serum sHLA-G levels are significantly increased in PAR patients. Moreover, sHLA-G might be considered as a biomarker for assessing clinical severity.

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Introduction

Perennial allergic rhinitis (PAR) is sustained by a chronic mucosal inflammation, characterized by mast cell and eosinophil activation, and is a consequence of the exposure to perennial allergens, including house dust mite, cat and dog. This inflammatory reaction is orchestrated by T helper (Th) 2 cells [1] and T regulatory cells [2]. Indeed, a defect in T regulatory cells has been evidenced in allergic patients [3]. In this regard, an interest in HLA-G antigens has been shown by the possibility that these antigens may be involved in immune-mediated disorders [4]. HLA-G is a human, non-classical major histocompatibility complex molecule expressed in human-privileged sites and plays an essential role in the development of maternal tolerance to genetically different fetal tissues [5]. Soluble HLA-G (sHLA-G) derives from the secretion of soluble isoforms as well as from the shedding of proteolytically cleaved surface isoforms. HLA-G-mediated mechanisms may be immunosuppressive; however, it has been reported that there is an induction of Th2-like cytokine profile through stimulation of IL-3, IL-4 and IL-10 secretion [6]. In this regard, only one study has been conducted on patients with seasonal allergic rhinitis investigated outside the pollen season, showing that they had significantly higher sHLA-G levels than healthy controls [7].

Therefore, the aim of the present study was to evaluate whether PAR patients may have increased serum sHLA-G levels in comparison with normal subjects.

Methods

Patients

Twenty-five PAR patients (13 males, 12 females, mean age 36.8 years) were enrolled. In order to be enrolled in the study, patients were required to have symptomatic AR due to perennial allergens, such as house dust mite, cat and dog. Patients were monosensitized to a single allergen, documented by positive skin prick test, performed according to validated criteria [8], for the specific allergen, and confirmed by a positive allergen-specific nasal challenge to the causal allergen.

Patients with either acute upper respiratory infections or undergoing specific immunotherapy, or using nasal or oral corticosteroids, antileukotrienes and antihistamines within the previous 4 weeks were excluded. Patients with documented allergy to pollen allergens were excluded as well.

According to validated criteria, in order to make a diagnosis of AR, a history of chronic nasal symptoms and positive results to skin prick test were considered sufficient [9].

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, sHLA-B as well as sHLA-C levels.

Clinical severity was also evaluated, considering nasal symptom perception and drug consumption at the end of the pollen season as previously validated [10]. The patient questionnaire included a validated visual analogue scale (VAS) for quantifying the perception of nasal symptom intensity [11]. Patients had to have a VAS score for nasal symptoms ≥ 5 .

During the pollen season, use of levocetirizine drops (10 or 20, corresponding to a 2.5- or 5-mg concentration) on demand as rescue medication was permitted. However, medication was withdrawn at least 1 week before drawing blood samples. The medication scoring was as follows: not at all (0), occasionally/seldom (1), occasionally/often (2), almost daily (3), continuously with the lowest dose (4), continuously with the maximal dose (5).

The study was conducted after the approval of the local ethics committee; all participants gave their written informed consent.

sHLA-G Molecule Immunoassay

sHLA-G molecules were determined by a commercially available immunoenzymatic assay (EXBIO, Prague, Czech Republic) which has recently been validated in a workshop [12] according to the manufacturer's instructions, and results were expressed as nanograms/milliliter.

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.

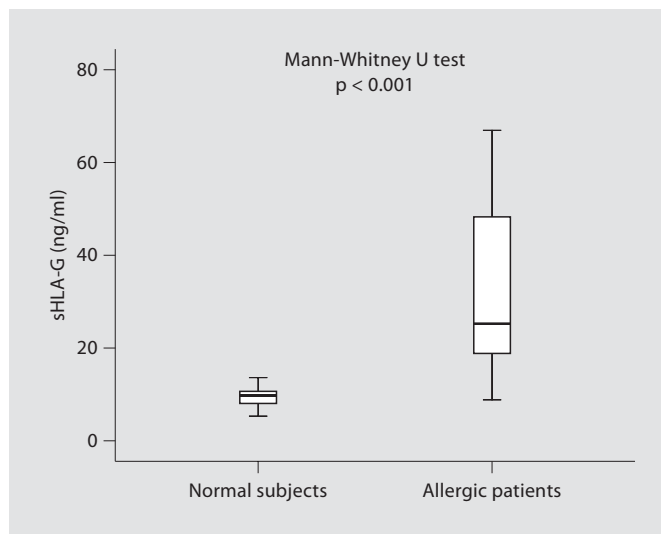


Fig. 1. Serum levels of sHLA-G in the 2 groups of subjects. Box plots are based on the quartile values: boxes represent the interquartile range (50% of central values), whisker lines extend from the highest to the lowest values, excluding outliers (cases with values >1.5 box lengths), and the line across the box indicates the median value.

Comparisons of quantitative variables between 2 groups of patients were performed by means of the non-parametric Mann-Whitney U test as the considered variables were not normally distributed.

Analysis of the correlation between quantitative variables was made by means of Spearman's correlation coefficient.

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

Results

The median of sHLA-G levels was 25.21 ng/ml (range 8.82–285.08) in perennial allergic patients and 9.76 ng/ml (1.34–20.552) in normal subjects. The difference was highly significant ($p < 0.001$; fig. 1).

In addition, in perennial allergic patients, there was a strong correlation between levels of sHLA-G and the VAS ($r = 0.850$, $p < 0.001$) as reported in figure 2.

There was also a significantly strong correlation between levels of sHLA-G and drug use ($r = 0.793$, $p < 0.001$; fig. 3).

Finally, there was also a strong correlation between the VAS and drugs ($r = 0.664$, $p = 0.001$).

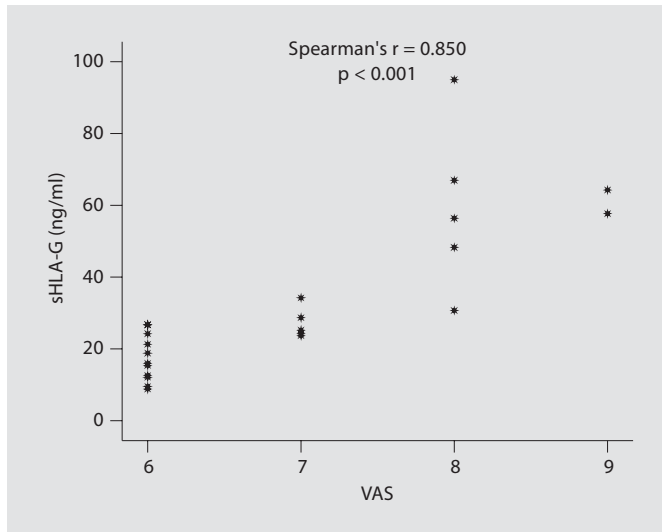


Fig. 2. Correlation between serum sHLA-G levels and the VAS of nasal symptoms in allergic patients.

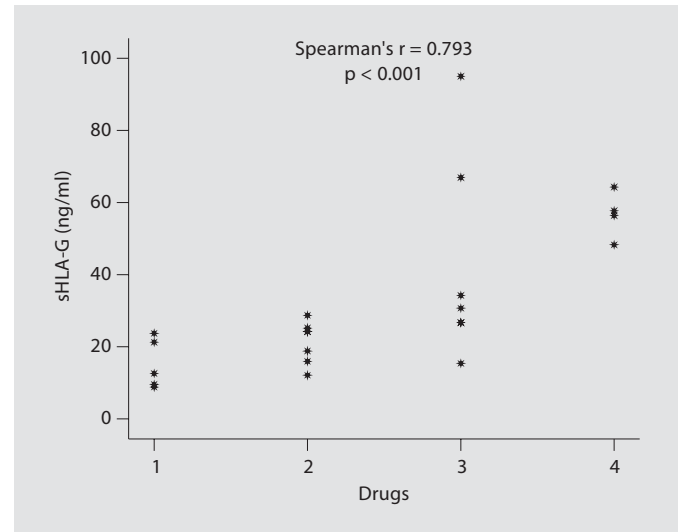


Fig. 3. Correlation between serum sHLA-G levels and drug use in allergic patients.

Discussion

sHLA-G molecules might play an important role in the mechanisms of immune tolerance to allergens as, like their surface membrane-bound counterparts, they have immunosuppressive properties. AR is characterized by an inflammatory response consequent to allergen exposure. Therefore, PAR is characterized by a persistent inflammation which could affect the immune response to allergens. The present study aimed at evaluating the role of the sHLA-G molecule in a group of patients with PAR.

Results obtained first showed that a significant increase in the sHLA-G molecule is detectable in patients with PAR in comparison with normal subjects. These findings confirm previous preliminary reports in patients with pollen-induced AR, evaluated outside the pollen season [7]. On the other hand, there was a very strong correlation between clinical severity, assessed by the VAS and drug use, and sHLA-G values. This issue is very important as it allows to define the clinical relevance of the findings. Indeed, sHLA-G might be considered as a biomarker for evaluating the clinical severity. Moreover, this outcome confirms a previous finding that evidenced the clinical relevance of serum sHLA-G as a possible biomarker for monitoring sublingual immunotherapy [10].

It might be hypothesized that sHLA molecules in these patients derive from immune cells involved in the under-

lying allergic reaction. Indeed, as previously reported [6], sHLA-G might be involved in maintaining the Th2-polarized immune response in allergic patients and in tolerogenic defective mechanisms. In conclusion, the present study has provided the first evidence of an increase in sHLA-G in patients with PAR. In addition, sHLA-G molecules might be considered a biomarker for assessing clinical severity. These preliminary findings need to be confirmed by further functional studies to clarify the role of sHLA-G contribution in allergic reaction.

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