

Serum-Soluble HLA-G Is Associated with Specific IgE in Patients with Allergic Rhinitis and Asthma

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Abstract—Allergic rhinitis (AR) and allergic asthma (AA) are characterized by T helper (Th)2-polarized immune response. Soluble **human leukocyte antigen G** (sHLA-G) molecule plays an immunomodulatory activity. Previously, it has been reported that children with AR or AA had higher sHLA-G levels in comparison with normal subjects. Thus, the present study aimed at confirming these data in adults and investigating whether there was a relationship between serum sHLA-G levels and serum IgE levels, in patients with AR or AA. One hundred twenty symptomatic patients, suffering from respiratory symptoms, were enrolled: 45 non-allergic and 75 allergic. A group of 44 healthy subjects was considered as control. Serum sHLA-G levels and serum allergen-specific IgE were determined by immunoenzymatic methods. Allergic patients had significantly higher levels of sHLA-G molecules than non-allergic patients and normal controls ($p < 0.0001$). There was no difference between AR and AA. sHLA-G moderately related with allergen-specific IgE both in AR ($r = 0.468$) and AA patients ($r = 0.479$). The present study confirms that serum sHLA-G molecules are significantly increased in allergic disease and demonstrates that sHLA-G levels are related with allergen-specific IgE levels.

KEY WORDS: allergic rhinitis; asthma; serum sHLA-G; serum-specific IgE.

INTRODUCTION

Allergic rhinitis (AR) and allergic asthma (AA) are sustained by a mucosal IgE-dependent inflammation, characterized by mast cell and eosinophil activation. The inflammatory reaction is promoted, maintained, and amplified by activated T helper (Th)2 cells [1]. Moreover, a defect in T regulatory cells has been evidenced in allergic patients [2]. Therefore, allergic diseases are presently considered as immunoregulatory disorders with reduced tolerance toward allergens [2]. These pathophysiologic events promote the production of allergen-specific IgE antibodies.

Interest about human leukocyte antigen G (HLA-G) antigen has been shown by the possibility that it may be involved in the regulation of inflammatory diseases [3]. HLA-G is a human, non-classical MHC molecule expressed

in human-privileged sites and plays an essential role in the development of maternal tolerance to genetically different foetal tissues [4]. Most of these protective mechanisms are immunosuppressive; however, it has been reported that HLA-G molecule is able of inducing Th2-like cytokine profile synthesis through stimulation of IL-3, IL-4, and IL-10 secretion [5]. Previously, it has been evidenced that patients suffering from AR had significantly higher serum soluble HLA-G (sHLA-G) levels than healthy controls [6]. Moreover, a paediatric study showed that atopic asthmatics had significantly higher sHLA-G levels than healthy controls [7]. Recently, it has been reported that children with AR or AA had higher sHLA-G serum levels than healthy controls, without difference between diseases [8]. Therefore, the aim of the present study was to evaluate adult patients with AR or AA, measuring serum sHLA-G molecule; to compare both AR and AA patients with non-allergic patients and healthy subjects; and to correlate sHLA-G with allergen-specific IgE.

METHODS

Subjects. One hundred sixty-four subjects, 44 healthy subjects (20 males, mean age 41.9 years, range 18–65) and

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120 patients (53 males, mean age 39.4 years, range 18–64), were included in the study. Allergic rhinitis was diagnosed according to validated criteria proposed by ARIA guidelines [9], and asthma was diagnosed on the basis of the Global Initiative for Asthma Guidelines (www.ginasthma.com). On the basis of the diagnosis, 45 patients (17 males) had non-allergic rhinitis and/or asthma, whereas 75 patients (36 males) had AR (34) or AA (41).

Patients with acute upper respiratory infections or either undergoing specific immunotherapy or using nasal medications, inhaled or oral corticosteroids, antileukotrienes, and antihistamines, within the previous 4 weeks were excluded.

Blood samples were collected from patients and controls to determine serum sHLA-G and allergen-specific IgE (sIgE).

The study was performed with approval of the local ethics committee, and written informed consent by the parents of allergic children was obtained.

Soluble HLA Molecule Immunoassay

sHLA-G molecules were measured by commercially available immunoenzymatic assay (EXBIO, Praha, Czech Republic). The assay was performed according to the manufacturer's instructions. Results were reported as a concentration of sHLA-G (ng/ml) in samples. Limit of detection was 3 units/ml; the percentage of coefficient of variation (CV%) was 7.6 % for intra-assay variation and 5.8 % for inter-assay variation.

Allergen-Specific IgE Measurement

Serum levels of allergen-specific IgE were detected using the immunofluorometric assay procedure (ImmunoCAP; Thermo Fisher Scientific) in peripheral blood samples from patients. The allergen of interest, covalently coupled to ImmunoCAP, reacted with the sIgE in the patient sample. After washing, non-specific IgE enzyme-labelled antibodies against IgE were added to form a complex. After incubation, unbound enzyme anti-IgE was washed away, and the bound complex was then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate was measured. Quantitative sIgE concentrations were expressed in kilo units of A per litre (where A means allergen-specific antibodies) according to the traceable calibration to the second IRP WHO for human IgE. sIgE levels were considered positive over 0.35 kUA/l.

Statistical Analysis

Descriptive statistics were first performed, and quantitative parameters were reported as median, minimum, and maximum values with first and third quartiles (interquartile range). The non-parametric Kruskal-Wallis rank test was performed to evaluate the analysis of variance between groups of subjects. The non-parametric Wilcoxon test was performed for *post hoc* comparisons. Relationships were analysed using the Spearman test. All tests were two-sided, and a *p* value less than 0.05 was considered statistically significant. The package “S-Plus” (Mathsoft Corp.) was used for all the analyses.

RESULTS

Figure 1 shows that sHLA-G levels significantly changed (Kruskal-Wallis test; $p < 0.0001$) among the groups of subjects. The median (range, 25th–75th) of serum sHLA-G levels was 24.68 ng/ml (25th–75th, 9.5–65.95 ng/ml) in allergic patients, 7.03 ng/ml (25th–75th, 4.88–9.68 ng/ml) in non-allergic patients, and 4.41 ng/ml (25th–75th, 2.77–6.55 ng/ml) in normal subjects.

The *post hoc* analysis reveals that non-allergic subjects have higher levels than normal controls ($p = 0.003$), and allergic patients have higher levels than both healthy subjects and non-allergic patients ($p < 0.0001$ for both).

On the other hand, there was no difference between AR and AA patients, as reported in Fig. 2.

There was a significant and moderate relationship between HLA-G levels and allergen-specific IgE levels (concerning the causal allergen, in case of two or more causal allergens, the most clinically relevant allergen was considered) both in AR ($r = 0.468$ and $p = 0.003$) and AA ($r = 0.479$ and $p = 0.006$) patients, as shown in Fig. 3.

DISCUSSION

Allergic disorders are characterized by Th2-polarized inflammation. Th2-derived cytokines, such as IL-4 and IL-13, are the main pathogenic factors able to induce, maintain, and amplify IgE production and allergic inflammation [10]. In this regard, sHLA-G molecules might play an important role in the mechanisms of immune tolerance toward allergens as, like their surface membrane-bound counterparts, they have immunosuppressive properties. In fact, sHLA-G molecules inhibit T cell proliferation induced by allogeneic dendritic cells [11] and induce apoptosis of T and NK CD8+

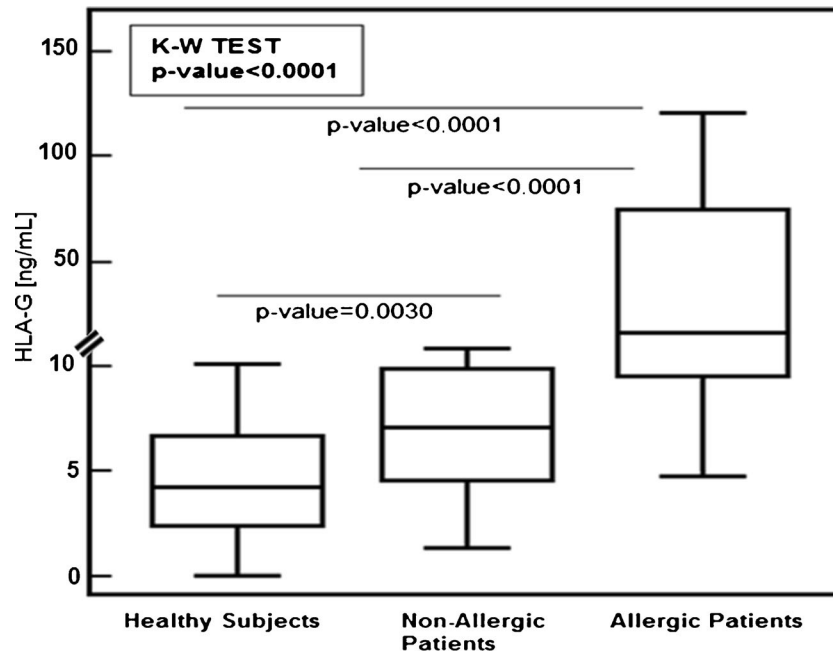


Fig. 1. Serum sHLA-G (ng/ml) distribution in allergic and non-allergic patients and healthy subjects. Values are represented as medians (*horizontal lines*), quartiles (25th and 75th percentiles, *boxes*), and ranges (*vertical lines*). $p < 0.0001$, Kruskal-Wallis test (p values of individual group comparisons are given in the figure).

cells and inhibition of cytotoxic T cell activity through CD8 ligation by Fas-sFasL interaction [12].

So far, however, only one study has been conducted on children with allergic asthma, showing that they had

significantly higher serum sHLA-G levels than healthy controls [7]. However, this study reported that non-allergic asthmatic children had normal sHLA-G values [7]. A further paediatric study partially confirmed these findings

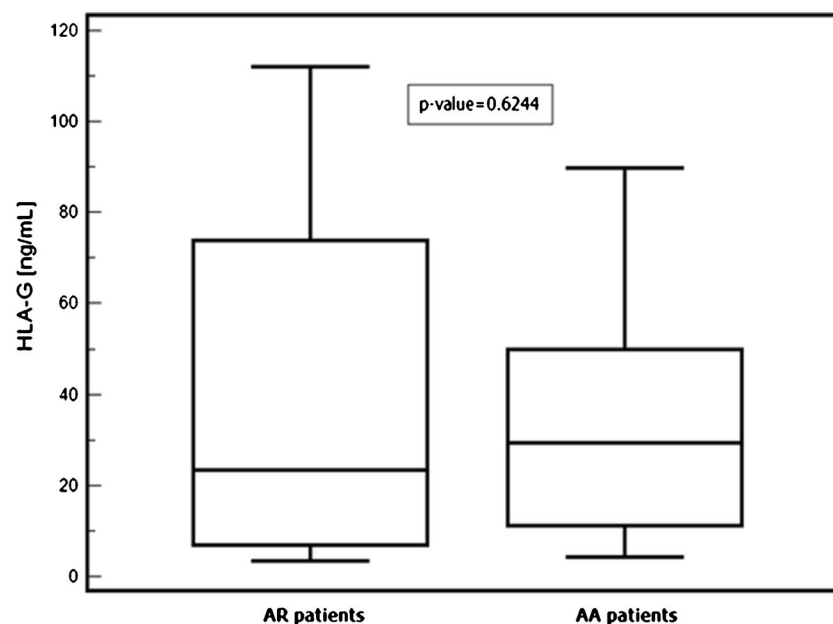


Fig. 2. Serum sHLA-G (ng/ml) distribution in allergic patients, evaluated separately in patients with allergic rhinitis (AR) and allergic asthma (AA). Values are represented as medians (*horizontal lines*), quartiles (25th and 75th percentiles, *boxes*), and ranges (*vertical lines*).

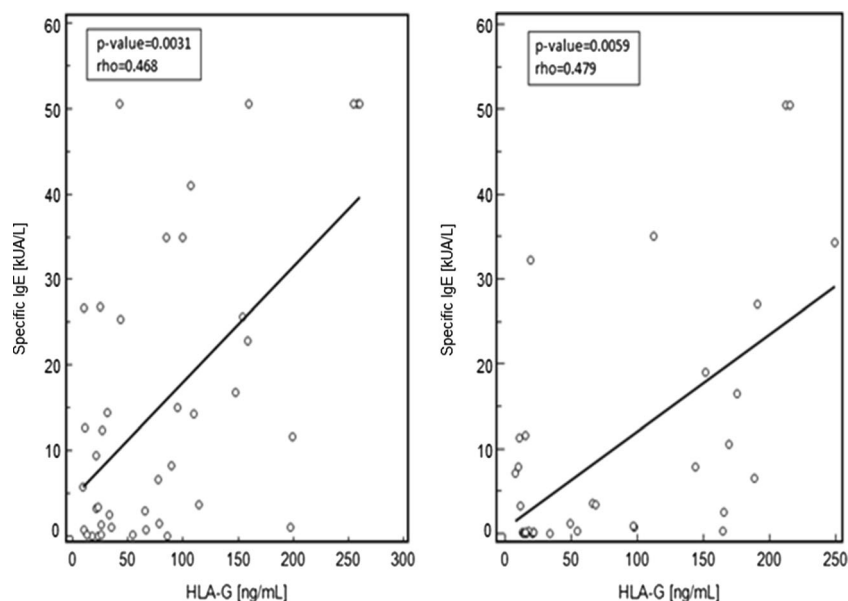


Fig. 3. Relationship between serum sHLA-G and serum allergen-specific IgE in patients with AR (a) and AA (b).

but reported that there was no difference between AR and AA children [8].

The present study was therefore aimed at evaluating the role of sHLA-G molecules in a group of allergic patients comparing with both non-allergic patients and normal subjects, the possible difference between AR and AA, and the possible relationship between HLA-G and allergen-specific IgE.

Findings firstly show that allergic patients have a significant increase of sHLA-G in comparison with non-allergic patients and normal subjects. These findings confirm the previous report demonstrating elevated levels of sHLA-G molecules in pollen-induced rhinitic patients evaluated outside the pollen season [6] and in AR patients due to perennial allergens [13]. Therefore, allergy *per se* is able of inducing increased sHLA-G production in comparison with non-allergic disorders.

Secondly, this study confirms that there is no difference between AR and AA patients. This finding might suggest that sHLA-G might be a biomarker of allergic reaction. This hypothesis is reinforced by the relationship between sHLA-G and IgE. Functional studies exploring the role of sHLA contribution in allergic reaction are ongoing (submitted paper). In addition, it has been reported that sHLA-G levels are related with symptoms and drug use in patients with seasonal allergic rhinitis [14]. Moreover, serum sHLA-G levels decrease in patients successfully treated with allergen immunotherapy [15], and this change significantly relates with increased IFN- γ

production [16]. On the other hand, specific IgE is as easily accessible as sHLA-G, since detected in serum, and can be easily measured. However, in contrast to sHLA-G, specific IgE provides clear information on allergic sensitization. Thus, sHLA-G could be also considered as a potential biomarker for assessing allergic reaction severity after specific therapy.

In addition, this study demonstrates that sHLA-G is also increased in patients with non-allergic respiratory symptoms in comparison with healthy subjects. This finding could be explained as sHLA-G does increase in several inflammatory disorders; thus, also non-allergic respiratory symptoms are sustained by inflammatory response able to increase sHLA-G, even though in a lesser extent, than those in allergic reaction.

In conclusion, the present study confirms that sHLA-G is significantly increased also in adult allergic patients, independently of target organ, and relates with sIgE level.

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REFERENCES

1. Romagnani, S. 1995. Biology of human Th1 and Th2 cells. *Journal of Clinical Immunology* 15: 121–129.
2. Umetsu, D.T., and R.H. DeKruyff. 2006. The regulation of allergy and asthma. *Immunological Reviews* 212: 238–255.
3. Carosella, E.D., P. Moreau, S. Aractingi, and Rouass-freiss. 2001. HLA-G: a shield against inflammatory aggression. *Trends in Immunology* 10: 553–555.
4. Pistoia, V., F. Morandi, X. Wang, and S. Ferrone. 2007. Soluble HLA-G: are they clinically relevant? *Seminars in Cancer Biology* 17: 469–479.
5. Kanai, T., T. Fujii, S. Kozuma, T. Yamashita, A. Miki, A. Kikuchi, et al. 2001. Soluble HLA-G influences the release of cytokines from allogeneic peripheral blood mononuclear cells in culture. *Molecular Human Reproduction* 7: 195–2001.
6. Ciprandi, G., B.M. Colombo, P. Contini, P. Cagnati, A. Pistorio, F. Puppo, and G. Murdaca. 2008. Soluble HLA-G and HLA-A,-B,-C serum levels in patients with allergic rhinitis. *Allergy* 63: 1335–1338.
7. Tahan, F., and T. Patiroglu. 2006. Plasma soluble human leukocyte antigen G levels in asthmatic children. *International Archives of Allergy and Immunology* 141: 213–216.
8. Ciprandi, G., M. De Amici, S. Caimmi, A. Marseglia, A. Marchi, A.M. Castellazzi, and G. Marseglia. 2010. Soluble serum HLA-G in children with allergic rhinitis and asthma. *Journal of Biological Regulators* 24: 221–224.
9. Bousquet, J., N. Khaltaev, A.A. Cruz, J. Denburg, W.J. Fokkens, A. Togias, et al. 2008. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA2LEN and AllerGen). *Allergy* 63(Suppl. 86): 8–160.
10. Bachert, C., M. Van Kempen, and P. Van Cauwenberge. 1999. Regulation of proinflammatory cytokines in seasonal allergic rhinitis. *International Archives of Allergy and Immunology* 118: 375–379.
11. Rebmann, V., K. Pfeiffer, M. Pässler, S. Ferrone, S. Maier, E. Weiss, and H. Grosse-Wilde. 1999. Detection of soluble HLA-G molecules in plasma and amniotic fluid. *Tissue Antigens* 53: 14–22.
12. Puppo, F., P. Contini, M. Ghio, S. Brenci, M. Scudeletti, G. Filaci, S. Ferrone, and F. Indiveri. 2000. Soluble human MHC class I molecules induce soluble Fas ligand secretion and trigger apoptosis in activated CD8+ Fas(CD95)+ T lymphocytes. *International Immunology* 12: 195–203.
13. Ciprandi, G., P. Contini, G. Murdaca, A.M. Gallina, and F. Puppo. 2009. Soluble HLA-G molecule in patients with perennial allergic rhinitis. *International Archives of Allergy and Immunology* 150: 278–281.
14. Ciprandi, G., P. Contini, G. Murdaca, G. DeAmici, A.M. Gallina, and F. Puppo. 2009. Soluble serum HLA-G and HLA-A,-B,-C molecules in patients with seasonal allergic rhinitis exposed to pollens. *International Immunopharmacology* 9: 1058–1062.
15. Ciprandi, G., P. Contini, A. Pistorio, G. Murdaca, and F. Puppo. 2009. Sublingual immunotherapy reduces soluble HLA-G and HLA-A,-B,-C serum levels in patients with allergic rhinitis. *International Immunopharmacology* 9: 253–257.
16. Ciprandi, G., P. Contini, D. Fenoglio, M.P. Sormani, S. Negrini, F. Puppo, and F. Indiveri. 2008. Relationship between soluble HLA-G and HLA-A,-B,-C serum levels and IFN-gamma production after sublingual immunotherapy in patients with allergic rhinitis. *Human Immunology* 69: 510–512.

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