

Original article

Soluble HLA-G and HLA-A,-B,-C serum levels in patients with allergic rhinitis

Background: Allergic rhinitis (AR) is characterized by Th2-polarized immune response. Soluble HLA (sHLA) molecules play an immunomodulatory activity. So far, however, no study investigated them in AR.

Objective: The aim of this study was to evaluate sHLA-G and sHLA-A,-B,-C serum levels in AR patients with pollen allergy and in a group of healthy controls.

Methods: Forty-nine AR patients were enrolled. A group of healthy nonallergic subjects was considered as control. sHLA-G and sHLA-A,-B,-C serum levels were determined by immunoenzymatic method. The study was conducted during the winter, such as outside the pollen season.

Results: Allergic patients had significantly higher levels of both sHLA-G ($P < 0.0001$) and sHLA-A,-B,-C ($P = 0.011$) molecules than normal controls. Moreover, there was a significant relationship between these two soluble molecules ($r = 0.69$) in allergic patients.

Conclusion: The present study provides the first evidence that both sHLA-G and sHLA-A,-B,-C serum levels are significantly increased in AR patients with pollen allergy.

G. Ciprandi¹, B. M. Colombo¹,
P. Contini¹, P. Cagnati¹, A. Pistorio²,
F. Puppo¹, G. Murdaca¹

¹DIMI, University of Genoa, Genoa, Italy;

²Epidemiology and Statistics Unit, IRCCS G. Gaslini, Genoa, Italy

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Giorgio Ciprandi, M.D.,
Ospedale San Martino
Largo R. Benzi 10, 16132 Genoa,
Italy

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Allergic rhinitis (AR) is sustained by a mucosal inflammation, characterized by mast cell and eosinophil activation. This inflammatory reaction is orchestrated by T helper (Th) 2 cells (1). Th2 cells play an important role in the pathogenesis of AR, as IL-4 promotes isotype switching to IgE synthesis, while IL-5 promotes, mostly in the nasal mucosa, eosinophil accumulation and activation (2, 3). In addition, peripheral blood mononuclear cells display a predominant interleukin (IL)-4 production by Th2 cells over IFN- γ expression by Th1, which constitutes 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 been showed by the possibility that they may be involved in inflammatory diseases (7). HLA-G is a human, nonclassical MHC molecule expressed in human-privileged sites and plays an essential role in the development of maternal tolerance to genetically different foetal tissues (8). Seven HLA-G isoforms are generated by alternative splicing of the primary HLA-G transcript. Four of them, HLA-G1, -G2, -G3 and -G4, are bound to the cell surface, while the remaining three, HLA-G5, -G6 and -G7 are soluble and

are the counterparts of HLA-G1, -G2 and -G3, respectively. The structure of the soluble isoforms resembles that of the corresponding membrane bound isoforms in the extracellular part, but differs at the C-terminus. The major isoforms are HLA-G1 and HLA-G that share immunosuppressive activities mediated by their binding to the receptors CD85j, CD85d, CD158d and CD160. 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 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 allergic patients showing that atopic asthmatic children had significantly higher sHLA-G levels than healthy controls (11).

Moreover, it is known that also classical HLA class I molecules which are characterized by a broad tissue expression and by a high degree of polymorphism (12) are found in serum of healthy individuals in 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 play immunoregulatory activities like inhibition of alloreactive cytotoxic T lymphocytes (CTL) activity, apoptosis of CD8⁺ T lymphocytes and NK cells and suppression of allogeneic mixed lymphocyte cultures (15, 16). As for sHLA-G, there is only one study investigating sHLA class I in allergic disorders reporting that sHLA-I heterodimers are elevated in 64% of patients with atopic dermatitis and that elevations persisted after clinically effective therapy (17).

Therefore, the aim of the present study was to evaluate whether patients presenting with rhinitis because of pollen allergy may have increased sHLA-G and/or sHLA-A, -B, -C serum levels.

Methods

Patients

Forty-nine patients (23 males and 26 females) with AR because of pollen allergy were included in the study; 21 of them were allergic to *Betula alba*, 19 to *Parietaria judaica*, and 9 to *Graminaceae*. Their mean age was 39.24 years (SD 13.8). In order to be enrolled in the study, patients were required to have AR because of pollen allergy. Patients were monosensitized to a single pollen, it was documented by 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 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 4 weeks were excluded. Patients with documented allergy to non-pollen allergens were excluded too.

According to validated criteria, in order to make a diagnosis of AR, a history of nasal symptoms during the pollen season and positive results to skin prick test were considered as sufficient (19).

Twenty healthy subjects (10 males and 10 females, mean age years is 49 ± 5.98 SD) were enrolled as control.

Blood samples were collected from patients and controls to determine sHLA-G and sHLA-A, -B, -C molecules serum level.

The study was performed during the winter, such as outside the pollen season, and was conducted with the approval of the local Ethics Committee and after obtaining the written informed consent by all participants.

Soluble HLA molecules immunoassay

sHLA-G molecules were determined by a commercially available immunoenzymatic assay (EXBIO, Praha, Czech Republic), which has been recently validated in a workshop (20) according to the manufacturer's instructions and results were expressed as ng/ml. 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- β 2-microglobulin monoclonal antibody NAMB-1 and results are expressed as ng/ml.

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 percentiles and minimum and maximum values.

Comparisons of quantitative variables between two groups of patients were performed by means of the nonparametric Mann-Whitney *U* test as the considered variables were not normally distributed.

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

Results

The mean sHLA-G serum level was 35.86 ± 2.7 ng/ml in allergic patients and it was 12.79 ± 2.4 ng/ml in normal subjects. Figure 1 shows the levels of sHLA-G in allergic patients and in normal subjects. Patients with AR have significantly higher levels than normal subjects ($P < 0.0001$).

The mean sHLA-A, -B, -C serum level was 1309 ± 73.3 in allergic patients and it was 1001 ± 145.7 in normal subjects. Figure 2 shows the levels of sHLA-A, -B, -C in allergic patients and in normal subjects. Patients with AR have significantly higher levels than normal subjects ($P = 0.011$).

Finally, there is a significant moderate correlation ($r = 0.69$) between sHLA-G and sHLA-A, -B, -C levels in allergic patients as showed in Fig. 3. However, there is no significant correlation in normal subjects ($r = 0.37$).

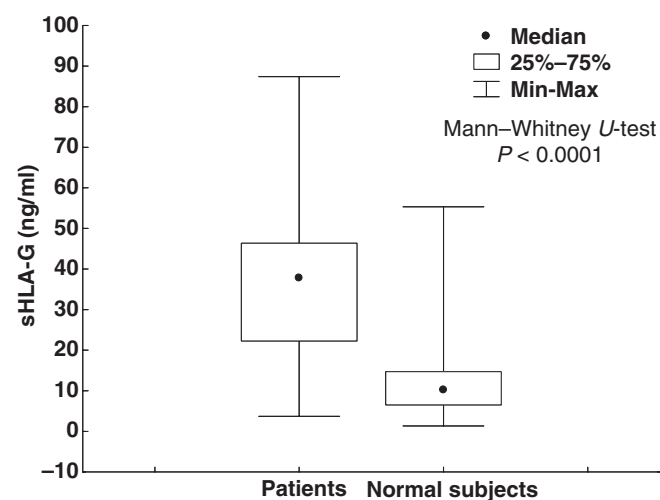


Figure 1. Levels of sHLA-G serum levels in normal subjects and allergic patients. Data are expressed as medians; boxes represent 25th and 75th percentiles; bars represent minimum and maximum values.

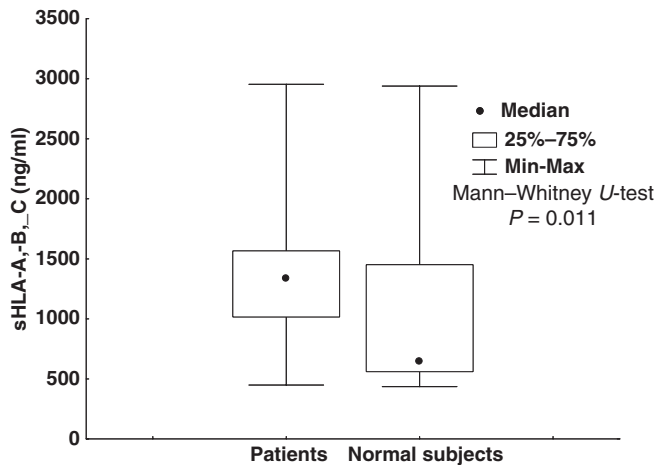


Figure 2. Levels of sHLA-A,-B,-C serum levels in normal subjects and allergic patients. Data are expressed as medians; boxes represent 25th and 75th percentiles; bars represent minimum and maximum values.

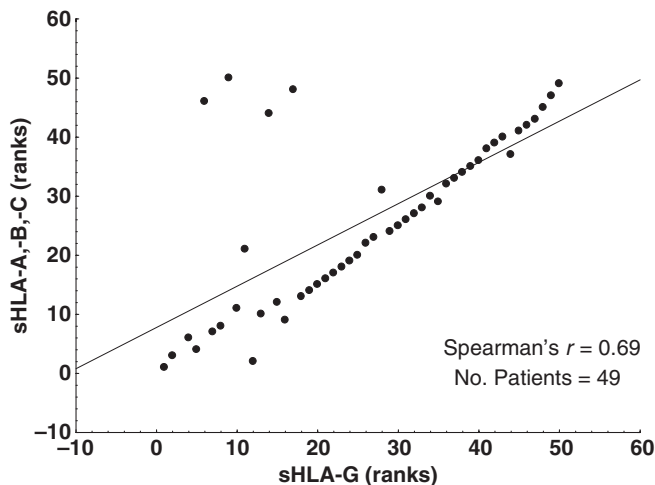


Figure 3. Correlation between the levels of sHLA-G and sHLA-A,-B,-C in allergic patients. Data are expressed as ranks.

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 able to induce, maintain and amplify the allergic inflammation (22). In addition, a defect of 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 towards 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 (23) and, in analogy to sHLA-A, -B, -C molecules, induce

apoptosis of T and NK CD8⁺ cells and inhibit cytotoxic T cell activity through CD8 ligation by Fas/sFasL interaction (24). 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).

A previous study reported that atopic children with asthma have higher sHLA-G levels than normal controls (11). Another study, conducted *in vitro*, showed that there is a defective production of soluble HLA-G molecules by peripheral blood monocytes in patients with asthma (25).

As far as sHLA-A, -B, -C molecules in allergic disorders are concerned, there is only one study that reported their increase in 9 out of 14 children with atopic dermatitis, and that elevations persist after clinically effective therapy (12).

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 PER because of pollen allergy.

Results obtained first show that a significant increase of both sHLA-G and sHLA-A, -B, -C molecules is detectable in patients with AR in comparison with normal subjects and that this increase is more evident for the sHLA-G molecule. Second, a significant relationship exists between the serum levels of these two soluble molecules in AR patients.

These findings confirm previous preliminary reports demonstrating elevated levels of both soluble molecules in other allergic disorders (6, 25).

In addition, *in vitro* studies have shown that sHLA molecules released by cell lines are bound to nano-vesicles termed exosomes. It is thought that exosomes may represent the major reservoir of sMHC class I molecules in biological fluids. However, most studies have been confined to *in vitro* assays performed with cell lines (26, 27). It has been showed that sHLA molecules in the serum or plasma differ from exosome-bound sHLA in different ways. In contrast to exosome-associated sHLA, circulating sHLA is of low density, has a low apparent molecular mass (35–44 kDa) and is not detergent-labile. Moreover, the majority of MHC class I molecules in blood are not physically linked.

It might be hypothesized that sHLA molecules in these patients derive from immune cells involved in the underlying allergic reaction, even though these patients were symptom-free as not exposed to pollen. Functional studies therefore need to clarify the role of sHLA contribution in allergic reaction.

Moreover, it has to be noted that the study model is represented by a cohort of patients with pollen allergy evaluated outside the pollen season, such as without inflammation and symptoms. Therefore, these findings assume a more relevant value as the increase of these soluble molecules reflects the *per se* status of allergy beyond allergen exposure and consequently it may be more meaningful. Indeed, as previously reported (9),

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 a contextual and correlated increase of both

sHLA-G and sHLA class I in patients with rhinitis because of pollen allergy. These preliminary findings need to be confirmed by further studies in other allergic disorders.

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