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Allergen-driven HLA-G expression and secretion in peripheral blood mononuclear cells from allergic rhinitis patients

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ABSTRACT

Background

It has been reported that soluble HLA-G serum levels are increased in patients with pollen-induced allergic rhinitis and decrease after immunotherapy. However, no functional study has been conducted so far. The aim of this study was to evaluate the membrane expression and secretion of HLA-G molecules in peripheral blood mononuclear cells from allergic rhinitis patients after *in vitro* incubation with the causal allergen.

Methods and results

Twenty-two allergic rhinitis patients and ten healthy subjects were enrolled. Membrane HLA-G expression was determined by flow cytometry and soluble HLA-G in culture supernatant was determined by immunoenzymatic assay. HLA-G expression was detected in CD4+ (T-helper-2) cells and monocytes after *in vitro* stimulation with the causal allergen but not with non specific stimuli and non causal allergens. Accordingly, the release of soluble HLA-G in culture supernatant occurred only after the stimulation with the causal allergen. Collectively, these results were confirmed by Western blot analysis.

Conclusions

The present study provides the first *in vitro* evidence that in allergic patients HLA-G expression and secretion is specifically induced by the causal allergen. These data may add new insights into the pathogenetic mechanisms underlying allergic inflammation and allergen specific immunotherapy.

1. Introduction

HLA-G is a human non-classical major histocompatibility complex (MHC) molecule mainly expressed in membrane-bound form at the fetal-maternal interface on the extravillous cytotrofoblast [1] and in placental tissue [2] where it contributes to the development of maternal tolerance to the semi-allogeneic fetus [3]. It is also physiologically expressed in certain adult tissues such as thymus [4], cornea [5], pancreas [6] and bronchial epithelial cells [7] as well as in different cells types such as activated monocytes [8] and erythroid and endothelial precursors [9]. The expression of HLA-G antigens has been recently reported in some solid tumours, transplanted organs, cutaneous inflammatory diseases and on virally infected cells in several pathological conditions [10,11]. HLA-G is also detectable as a soluble form (sHLA-G) in several body fluids and derives from the shedding of proteolytically cleaved surface isoforms (sHLA-G1) and/or the secretion of soluble isoforms (HLA-G5, -G6 and -G7) [12–14]. Recently it has been demonstrated that a significant proportion of sHLA-G molecules is contained in microsomes or exosomes released from cells [15–17]. Elevated levels of sHLA-G molecules have been detected in plasma of patients affected by various pathological conditions [18–23] and in cerebrospinal fluid of patients with multiple sclerosis [24].

Several immune regulatory functions have been attributed to both membrane-bound and soluble HLA-G molecules [25,26]. Most of them are immunosuppressive as they inhibit the cytolytic function of NK cells and CD8+ T lymphocytes [27,28], the alloproliferative response of CD4+ T cells [29], the maturation of dendritic cells [30] and the activation of B cells [31]. In addition, HLA-G molecules are able to trigger apoptosis in antigen-specific CD8+ T lymphocytes [28,32,33]. HLA-G seems to be involved also in the tuning of immune responses: *in vitro* studies indicate that incubation of peripheral blood mononuclear cells (PBMC) with HLA-G expressing cells favors a shift towards a T helper (Th)-2 cytokine profile, while incubation with sHLA-G protein induces an *anti*-inflammatory environment due to the release of inteleukin-10 [34,35] Finally, HLA-G-positive $CD4^+$ $CD25^-$ $FoxP3^+$ regulatory T cells have been detected in peripheral blood and in inflamed tissues [36–39].

As far as allergic diseases are concerned, which are presently considered as disorders characterized by a dysfunction of immunoregulation leading to an amplified Th-2 response [40,41], it has been re-

Abbreviations: sHLA-G, soluble HLA-G; Th, T helper; PBMC, peripheral blood mononuclear cells; AR, allergic rhinitis; mAb, monoclonal antibody; MFI, mean fluorescence intensity; Tregs, regulatory T cells

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ported that elevated levels of sHLA-G molecules are detected in plasma and bronchoalveolar lavage fluid of atopic asthmatics [42–45], that the production of sHLA-G in unstimulated PBMC cultures is significantly higher in subjects with occupational isocyanate-induced asthma as compared with asymptomatic-exposed controls [46] and that the amount of sHLA-G detected in the medium from PBMC stimulated with allergen *in vitro* is reduced after specific immunotherapy [47]. Moreover, previous studies in allergic rhinitis (AR) patients indicated that sHLA-G serum levels were: i) significantly increased in comparison with normal subjects; ii) elevated both outside and during the pollen season in patients with seasonal allergy; and iii) correlated with clinical severity, drug use, allergen-specific IgE levels, type of allergy and response to immunotherapy [48–52]. However, to the best of our present knowledge, no study has been performed to investigate the effect of immune cell exposure to allergens *in vitro* on HLA-G molecules in AR patients. Therefore, the aim of the present study was to evaluate the membrane expression and secretion of HLA-G molecules in immune cells from AR patients after *in vitro* incubation with the causal allergen as well as with non-specific stimuli.

2. Materials and methods

2.1. Study population

Twenty-two allergic patients (11 males and 11 females, mean age 36.14 years) were enrolled in the study. Inclusion criteria were: presence of allergic rhinitis, documented by history of typical nasal symptoms, positive skin prick test for the specific allergen performed according to validated criteria and demonstration of a cause/effect relationship between exposure to the sensitizing allergen and the occurrence of nasal symptoms (*post hoc ergo propter hoc*).

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. Eighteen patients were mono-allergic to either Graminaceae (n. 5), Parietaria (n. 7) or Dermatophagoides (n. 6), whereas 4 patients were allergic to both Parietaria and Dermatophagoides. Patients were evaluated during winter, such as outside the pollen season, but when the exposure to mite was still present.

Ten healthy subjects (5 males and 5 females, mean age 38.26 years) were enrolled as controls. The study was conducted with the approval of the local Ethics Committee and after obtaining the written informed consent by all participants.

2.2. Cell cultures

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood samples (10 U/mL heparin) by Ficoll-Hypaque density gradient centrifugation (Lymphoflot, Biotest, Germany). PBMC were then washed with phosphate-buffered saline (PBS) and either utilized for membrane antigens assessment or cultured up to 72 h in 96-well U-bottomed microplates (Becton Dickinson, CA, USA) with RPMI-1640 complete medium (Biochrom AG, Germany) supplemented with 10% autologous plasma at 37 °C in a 5% CO_2 atmosphere in humified air. PBMC were cultured in the absence (controls) or in the presence of the following stimuli or allergens (kindly provided by Anallergo, Florence, Italy): phytohemoagglutinin (PHA, 10 μg/ml, Sigma Aldrich, Italy), lipopolisaccaride (LPS, 20 μg/ml, Sigma Aldrich, Italy), phorbol-myristate-acetate (PMA, 20 μg/ml, Sigma Aldrich, Italy) + ionomycin (1 μM, Sigma Aldrich, Italy), Olea (1 μg/ml), Parietaria (1 μg/ml), Graminaceae (1 μg/ml), and Dermatophagoides (2.5 μg/ml). At the end of the incubation period, supernatants were collected and kept frozen at −30° C until sHLA-G determination and cells were washed with PBS and used for membrane antigens assessment.

2.3. Flow cytometric analysis

Expression of cell membrane antigens was analyzed by direct immunofluorescence incubating PBMC from each individual (1×10^5 cells in 100 μl of PBS) with the fluorochrome-conjugated *anti*-HLA-G monoclonal antibody (mAb) MEM-G9 (Exbio, Czech Republic), which reacts with the native form of HLA-G1, and with the fluorochrome-conjugated *anti*-CD3, -CD4, -CD8, -CD11c, -CD14, -CD16, -CD19, -CD25, -CD27, -CD45, -CD45RA, -CD56, -CD123, -HLA DR (Beckman Coulter Europe) mAbs at 4 °C for 30 min in the dark. For Foxp3 intracellular staining, cells were permeabilized and fixed using FOXP3 Fix/Perm Buffer Set (Biolegend), according to manufacturer's instructions, then incubated with fluorochrome conjugated *anti*-Foxp3 mAb for 30 min at 4 °C in the dark; fluorochrome-conjugated isotype matched Abs were also used as controls. For intracellular staining, after incubation with the primary mAb cells were fixed and permeabilized with IntraPrep (Beckman Coulter Europe), according to the manufacturer's instructions, and blocked with 5% normal goat serum for 20 min on ice to prevent non-specific antibody binding. Then, *anti*-interleukin (IL)4, -IL10, -IL17 or -γ-interferon (IFN) (BD Biosciences) mAb was added at 4 °C for 30 min in the dark. After staining, the analysis was performed by flow cytometry using a FC500 flow cytometer equipped with CXP software (Beckman Coulter Europe).

2.4. sHLA-G measurements

The determination of sHLA-G molecules was performed by sandwich immunoenzymatic assays. A commercially available assay (Exbio, Czech Republic), which employs the MEM-G9 mAb as capture antibody, was utilized to determine the amount of sHLA-G1/HLA-G5 molecules. In order to specifically determine the amount of sHLA-G1 and HLA-G5 molecules, immunoenzymatic assays were performed utilizing as capture antibodies the 01G and the 5A6G7 mAbs (Exbio, Czech Republic), which recognize sHLA-G1 and HLA-G5, respectively. The HRP-conjugated B2M mAb (Exbio, Czech Republic), which recognizes β2-microglobulin, was employed as detection antibody in all assays. Plates were read with an ELX800 ELISA reader (BIO-TEK Instrument Inc.) and results were expressed as U/ml.

2.5. Western blot analysis

PBMC from one patient allergic to Dermatophagoides were incubated for 72 h in culture medium in absence or in presence of the allergens Olea and Dermatophagoides, as previously described. Monocytes, CD4+ T cells and CD8+ T cells were obtained by positive selection using magnetic beads coated with *anti*-CD14, -CD4 and -CD8 antibodies according to manufacturer's instructions (Dynabeads). The 221 cell line transfected with HLA-G (221-G) was utilized as positive control. Cells were lysed in cold buffer (20 mM HEPES, 150 mM NaCl, 10% [v/v] glycerol, 0.5% [v/v] NP-40, 1 mM EDTA, 2.5 mM DTT, 10 μg/L aprotinin, leupeptin, pepstatin A, 1 mM PMSF, and Na3VO4). Protein concentration was determined with the Bredford protein reagent (BioRad, USA) and 50 μg were resolved by 12% SDS-polyacrylamide electrophoresis. Then, proteins were transferred on nitrocellulose membrane (GE Healthcare, Buckinghamshire, England) at 4 °C for 45 min. After blocking for 1 h in 5% nonfat dry milk and washing with Tris-buffer saline-Tween 20 (10 mM Tris-base pH 7.4, 150 mM NaCl and 0.05% Tween 20), membranes were incu-

bated with appropriate dilutions of MEM-G1 mAb which recognizes the HLA-G heavy chain (Exbio) and *anti*-human actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and finally incubated with *anti*-mouse immunoglobulins horseradish peroxidase conjugated secondary antibody (Santa Cruz Biotechnology). Immunoblots were developed with Immobilon Western chemiluminescent HRP substrate (Millipore Billerica, MA, U.S.A.). Band intensities were determined using IAS 2000 software from Delta Sistemi (Latina, Italy).

2.6. Statistical analysis

Data are reported as mean (±SD). Comparisons between variables were performed by two-tailed Student's *t* test for independent samples. Statistical significance was assumed for $p < 0.05$.

3. Results

3.1. Membrane HLA-G expression in in vitro cultured PBMC

In order to analyze the kinetics of HLA-G membrane expression, preliminary experiments were performed on PBMC from 3 patients allergic to Parietaria and Dermatophagoides after incubation with aspecific stimuli and allergens. A progressive increase in the percentage of HLA-G-positive cells was observed in CD4+ T lymphocytes (up to 6%) and in CD14+ monocytes (up to 25%) during 72 h incubation with the causal allergen (Parietaria and Dermatophagoides) but not with non causal allergens (Olea and Graminaceae) or aspecific stimuli (PHA, PMA + ionomycin and LPS) (Fig. 1). Of interest, a high percentage (ranging from 34% to 70%) of CD4+HLA-G+T lymphocytes showed a strong cytoplasmic positivity for IL4 and IL10, but not for γIFN and IL17, after incubation with the causal allergen suggesting that these cells should belong to the Th2 lineage (Fig. 2). By contrast, CD8+ T lymphocytes, CD19+ B lymphocytes, CD56+ NK cells,

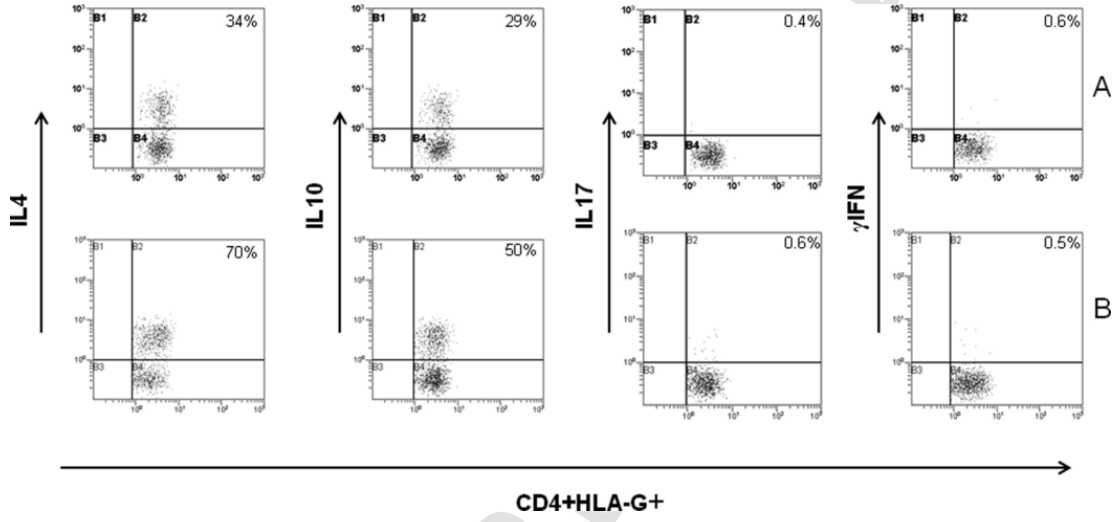

Fig. 1. Representative example of membrane HLA-G expression in CD4+ T lymphocytes (A) and CD14+ monocytes (B) from a patient allergic to Parietaria and Dermatophagoides after 72 h. incubation with culture medium, phytohemoagglutinin (PHA), phorbol-myristate-acetate + ionomycin (PMA+ IONO), lipopolisaccaride (LPS), Olea, Graminaceae (Gram), Parietaria (Par) and Dermatophagoides (Derm).

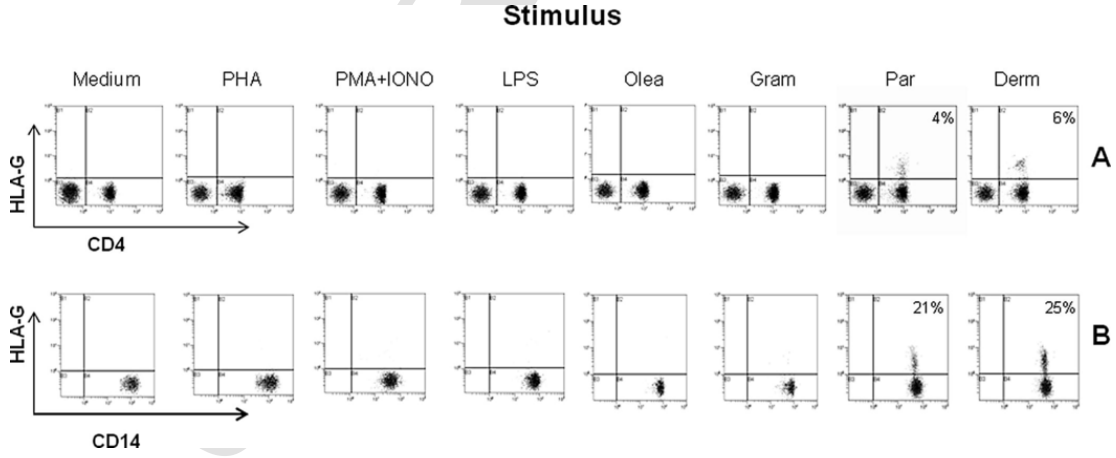

Fig. 2. Cytoplasmic IL4, IL10, IL17 and γIFN expression in CD4+HLA-G+T lymphocytes from a patient allergic to Parietaria and Dermatophagoides after 72 h incubation with the causal allergen. (A: Parietaria; B: Dermatophagoides).

CD4+ T regulatory cells (Tregs) including CD4+CD25+FoxP3+, CD4+CD45RA+CD27+ naïve, CD4+CD45RA$^-$CD27+ central memory and CD4+CD45RA$^-$CD27$^-$ effector memory cells did not show any detectable HLA-G expression after incubation with aspecific stimuli and allergens (data not shown).

Additional experiments indicated that the percentage of HLA-G positive cells in PBMC from patients allergic to Parietaria and Dermatophagoides was significantly higher ($p < 0.0001$) after 72 h incubation with the causal allergen than after incubation with aspecific stimuli or non causal allergens, the highest HLA-G expression being observed in monocytes from subjects allergic to Dermatophagoides (Fig. 3). This latter finding was confirmed by measuring the mean fluorescence intensity (MFI) values of HLA-G-positive cells (Table 1). By contrast, PBMC from patients allergic to Graminaceae showed only a slight increase in the percentage of HLA-G-positive cells after incubation with the causal allergen (data not shown).

3.2. Membrane HLA-G expression in circulating PBMC

HLA-G expression was detected in a small number of circulating CD4+ T cells (about 0.5%) and, among them, in a very low percentage (ranging from 0.1 to 0.3%) of CD4+CD45RA+CD27+ or CD4+CD45RA$^-$CD27+ Tregs. HLA-G expression was not detectable in circulating CD8+, CD19+, CD56+ cells, in CD4+CD25+FoxP3+Tregs and in

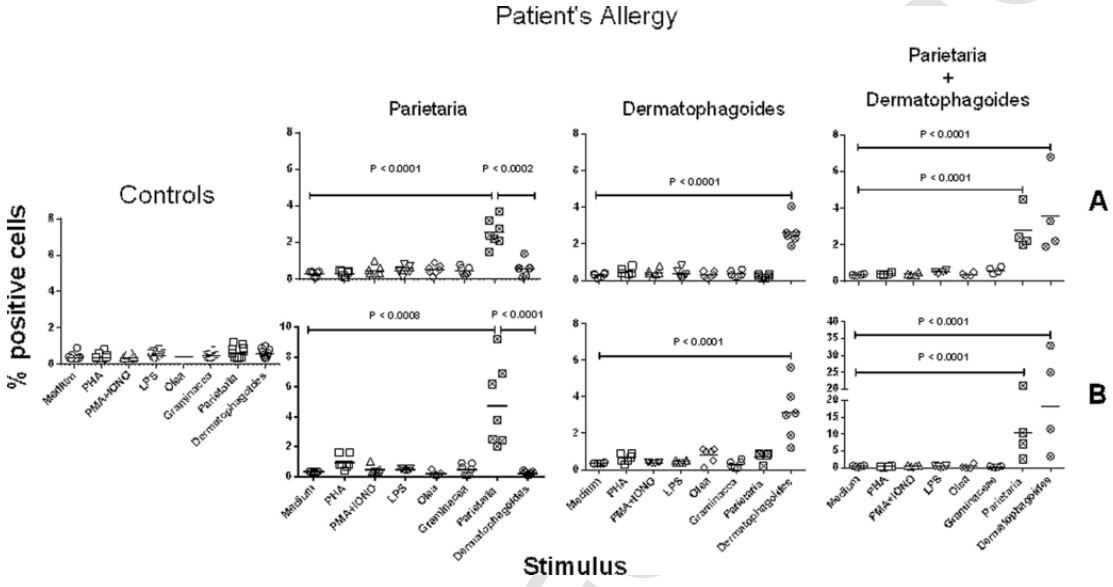

Fig. 3. Percentage of CD4+HLA-G+T lymphocytes in PBMCs (A) and of CD14+HLA-G+ in monocytes (B) from healthy control subjects and patients allergic to Parietaria, Dermatophagoides and Parietaria + Dermatophagoides after 72 h incubation with different stimuli. Horizontal bars represent the mean.

Table 1
HLA-G membrane expression in CD4+ T lymphocytes (A) and in CD14+ monocytes (B).

Stimulus	Controls (n. 6)	Patient's allegy		
		Parietaria (n. 6)	Dermatophagoides (n. 6)	Parietaria and Dermatophagoides (n. 4)
A				
Medium	0.35 ± 0.02[§]	0.36 ± 0.02	0.36 ± 0.02	0.38 ± 0.03
PHA	0.37 ± 0.05	0.34 ± 0.02	0.35 ± 0.03	0.37 ± 0.04
PMA + ionomycin	0.36 ± 0.01	0.37 ± 0.05	0.36 ± 0.02	0.38 ± 0.01
LPS	0.32 ± 0.01	0.33 ± 0.03	0.35 ± 0.04	0.37 ± 0.05
Parietaria	0.35 ± 0.02	2.17 ± 0.53**	0.34 ± 0.01	1.99 ± 0.32**
Olea	0.37 ± 0.03	0.36 ± 0.03	0.32 ± 0.01	0.34 ± 0.05
Graminacea	0.34 ± 0.05	0.33 ± 0.02	0.35 ± 0.03	0.36 ± 0.04
Parietaria	0.37 ± 0.03	0.34 ± 0.01	0.36 ± 0.05	0.33 ± 0.02
Dermatophagoides	0.34 ± 0.01	0.36 ± 0.02	2.74 ± 0.94**	1.72 ± 0.12**
B				
Medium	0.33 ± 0.01	0.35 ± 0.02	0.37 ± 0.02	0.52 ± 0.17
PHA	0.37 ± 0.05	0.36 ± 0.04	0.38 ± 0.06	0.39 ± 0.05
PMA + ionomycin	0.35 ± 0.02	0.54 ± 0.31	0.38 ± 0.02	0.66 ± 0.25
LPS	0.42 ± 0.06	0.40 ± 0.04	0.41 ± 0.07	0.43 ± 0.05
Olea	0.37 ± 0.03	0.37 ± 0.05	0.36 ± 0.04	0.38 ± 0.05
Graminacea	0.33 ± 0.01	0.32 ± 0.01	0.37 ± 0.03	0.34 ± 0.02
Parietaria	0.34 ± 0.01	3.91 ± 2.10*	0.36 ± 0.01	4.81 ± 2.15*
Dermatophagoides	0.34 ± 0.02	0.35 ± 0.01	3.00 ± 0.85**	4.34 ± 2.65*

[§] Values are expressed as mean ± SD of the mean fluorescence intensity (MFI); *$p < 0.001$ and **$p < 0.0001$ as compared to medium, non specific stimuli and non causal allergens.

$CD3^{-}CD19^{-}CD16^{-}CD56^{-}$HLA-DR + CD11c+/CD123+ myeloid or plasmacytoid dendritic cells (data not shown).

3.3. sHLA-G determination in culture supernatants

In order to analyze the kinetics of sHLA-G release in culture supernatant, preliminary experiments were performed culturing PBMC from 3 allergic patients with the causal allergen up to 120 h. Results indicated that a progressive increase of sHLA-G molecules is detectable during the incubation period reaching its maximum after 72 h (data not shown).

Experiments were then performed incubating for 72 h PBMC from AR patients in medium alone and in medium supplemented with the causal allergen or non causal allergen. Results indicated that the amount of sHLA-G1/HLA-G5 molecules was significantly higher in the supernatant of PBMC incubated with the causal allergen (p values ranging from <0.005 to <0.0001) than in the supernatant of PBMC incubated with culture medium or non causal allergen (Table 2). Moreover, these experiments also defined that the supernatant of PBMC cultured with the causal allergen mainly contained HLA-G5 molecules (Table 2).

3.4. HLA-G determination by Western blot analysis

Western blot analysis of monocytes, CD4+ T cells and CD8+ T cells obtained from PBMC of a patient allergic to Dermatophagoides confirmed that HLA-G is detected in the membrane of monocytes and CD4+ T cells after incubation with the causal allergen, but not with non causal allergen (Fig. 4).

4. Discussion

Allergic rhinitis is characterized by a Th-2 polarized inflammation [40,41]. Allergic patients have also a defect of Tregs that may be restored by specific immunotherapy [51]. HLA-G molecules might play an important role in the mechanisms of immune tolerance towards allergens as both membrane bound and soluble forms have immunosuppressive properties. In fact, it has been demonstrated that antigen presenting cells and monocytes expressing HLA-G molecules are able to create a tolerogenic milieu enriched in IL-10 which, in turn, promotes the up-regulation of membrane-bound and soluble HLA-G and induces Tregs [39]. Moreover, it has been reported that sHLA-G molecules inhibit the proliferation of T lymphocytes induced by allogeneic dendritic cells as well as the activity of cytotoxic T lymphocytes and induce apoptosis of T and NK CD8+ cells through CD8 ligation and Fas/sFasL interaction [28–32]. The immunoregulatory properties of HLA-G have prompted a number of studies aimed at evaluating their cell membrane expression and serum levels in patients affected by a variety of disorders [18–23]. When considering allergic diseases, elevated levels of sHLA-G molecules have been detected in plasma and bronchoalveolar lavage fluid of atopic asthmatics [42–45] and in plasma from AR patients, where their levels correlated with clinical severity and response to pharmacological therapy [48,49]. Of interest, specific immunotherapy is able to reduce the sHLA-G antigen levels in serum [50] and in the supernatant from PBMCs stimulated *in vitro* with allergen [47]. More recently, it has been reported that patients with seasonal allergic rhinitis had significantly higher sHLA-G serum levels than patients with perennial allergic rhinitis [52]. Moreover, it has been showed that there was a moderate relationship between sHLA-G and allergen-specific IgE levels in patients with allergic rhinitis and asthma, but there was no difference between patients with rhinitis and asthmatics [51]. On the basis of this evi-

Table 2
sHLA-G level in culture supernatant.

Patient's allergy	Stimulus	sHLA-G1/G5	sHLA-G1	sHLA-G5
Parietaria	Medium	2.03 ± 0.55[§]	1.77 ± 0.53	0.24 ± 0.01
	Parietaria	38.98 ± 18.64**	2.45 ± 0.66	31.41 ± 18.00**
	Dermatophagoides	2.42 ± 0.80	1.69 ± 0.64	0.53 ± 0.47
Dermatophagoides	Medium	2.87 ± 0.91	1.52 ± 0.22	0.25 ± 0.01
	Parietaria	3.84 ± 1.96	1.88 ± 0.29	2.17 ± 2.61
	Dermatophagoides	30.62 ± 10.02**	3.07 ± 0.58	23.21 ± 4.49**
Parietaria and Dermatophagoides	Medium	1.81 ± 0.47	1.40 ± 0.23	0.23 ± 0.02
	Parietaria	52.96 ± 24.32*	3.00 ± 0.88	51.15 ± 23.25*
	Dermatophagoides	41.38 ± 12.37**	2.54 ± 1.47	40.88 ± 13.24**

[§] Values are expressed as mean (±SD) U/ml;* $p < 0.005$ and ** $p < 0.0001$ as compared to medium and/or non causal allergen.

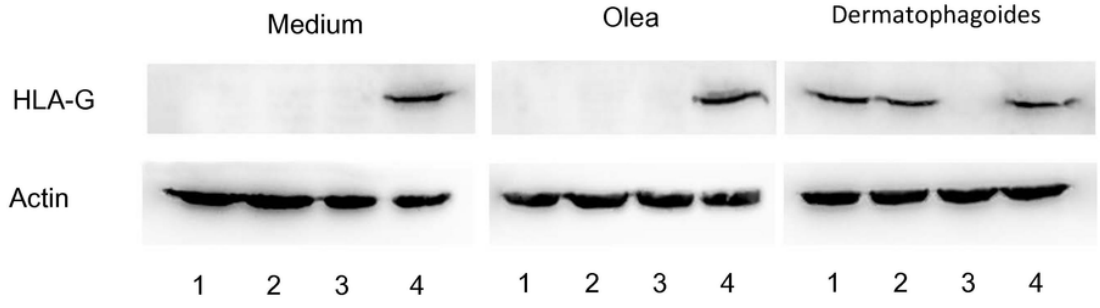

Fig. 4. Western blot analysis of monocytes (lane 1), CD4+ T cells (lane 2) and CD8+ T cells (lane 3) from a Dermatophagoides allergic patient after 72 h of incubation in absence (medium) or in presence of the allergens Olea and Dermatophagoides. 221-G cells are the positive control (lane 4).

dence, it has been suggested that sHLA-G might be considered a biomarker of allergic reaction.

The present study was aimed at evaluating the *in vitro* expression and release of HLA-G molecules by PBMC after incubation with both allergenic and non-allergenic stimuli in AR patients exposed or not to the causal allergen. HLA-G membrane expression was specifically induced by incubation with the causal allergen, but not by incubation with non-causal allergens or non-specific stimuli, underlining the specificity of the immune response to allergens. Of interest, only CD4+ T cells, in particular Th-2 cells, and monocytes expressed HLA-G after allergenic challenge whereas CD8+ T lymphocytes, B lymphocytes, NK cells and Tregs did not show any detectable HLA-G expression after incubation with aspecific stimuli and allergens. Notably, monocytes expressed HLA-G at higher levels than CD4+ T lymphocytes suggesting that these cells could be the most relevant source of HLA-G production. The exposure to the causal allergen seems to be the main factor inducing HLA-G expression. In fact, patients allergic to mites, evaluated during winter when the exposure to mite was still present, showed the more intense membrane HLA-G expression, whereas grass pollen allergic patients, who were evaluated far from the pollen season, showed a very low increase of HLA-G expression.

The measurement of sHLA-G in culture supernatants confirmed the cell membrane results, the highest amount of sHLA-G molecules being found when the causal allergen was used as stimulus. Of note, soluble molecules detected in culture supernatants mainly belong to the HLA-G5 isoform strongly suggesting that they are actively secreted by immune cells after incubation with allergen.

On the basis of this *in vitro* study we could hypothesize that HLA-G molecules might play a role also in allergic reaction *in vivo*. This hypothesis is consistent with a series of *in vivo* studies showing that sHLA-G serum level is increased in allergic patients and correlates with clinical severity, drug use, allergen-specific IgE levels, type of allergy, and response to immunotherapy [48–52].

At present, the role of membrane bound and soluble HLA-G molecules in immunoregulation appears complex and not univocal. In fact, although HLA-G molecules mainly exert immunosuppressive effects [28–33], they may also play an immunoregulatory role [36–39] by tuning immune responses towards either a Th-2 or a Th-1 cytokine profile [34,35]. Accordingly, it has been recently proposed that HLA-G should be no longer qualified as a "shield" to protect tissues and cells from immune destruction but, rather, as an "immune checkpoint" molecule [53]. The results of our *in vitro* experiments may suggest that the expression and secretion of HLA-G molecules may contribute to the Th-2 shift which characterizes the allergic reaction.

In conclusion, the present study provides the first *in vitro* evidence that: i) HLA-G is specifically expressed and released by PBMC after stimulation with the causal allergen; ii) the main source of HLA-G molecules are monocytes and, to a lesser extent, CD4+ Th-2 cells; iii) the natural allergen exposure significantly affects HLA-G expression and release. Further studies are needed to better clarify the functional role of HLA-G in allergic diseases.

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