

ORIGINAL ARTICLE

Soluble human leucocyte antigen-G and interleukin-10 levels in isocyanate-induced asthma

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Summary

Background We previously reported that in moderate-to-severe asthma there is a deficit of IL-10 secretion that could prevent the production of soluble HLA-G (sHLA-G), a non-classical human leucocyte antigen class I molecule with tissue-protective properties in inflammatory responses.

Objective Our objective was to investigate the production of sHLA-G and the secretion of IL-10 by peripheral blood mononuclear cells (PBMCs) in asthma induced by isocyanates and to compare the results with those obtained in non-occupational allergic asthma.

Method sHLA-G and IL-10 were measured by ELISA in the culture supernatants of unstimulated or lipopolysaccharide (LPS)-stimulated PBMCs obtained from 20 subjects with isocyanate asthma, 16 asymptomatic subjects exposed to isocyanates, 18 subjects with non-occupational allergic asthma, and 26 healthy control subjects.

Results Occupational exposure to isocyanates was associated with high baseline levels of secretion of IL-10 by PBMCs, whether or not the exposed subjects had asthmatic symptoms. However, spontaneous production of sHLA-G by PBMC was significantly higher in subjects with isocyanate asthma compared with asymptomatic-exposed controls. In contrast, PBMCs from subjects with non-occupational allergic asthma produced sHLA-G only after LPS stimulation.

Conclusions sHLA-G production and IL-10 secretion are influenced by workplace exposure to isocyanates and by development of asthma. The different behaviour of both sHLA-G and IL-10 in asthma induced by isocyanates compared with non-occupational allergic asthma suggests a heterogeneous biological role for HLA-G molecules and for IL-10, a key cytokine of immune and inflammatory responses.

Keywords cell culture, cytokines, MHC molecules, occupational asthma

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Introduction

Isocyanates, common causal agents of occupational asthma (OA), are low-molecular-weight agents (LMWA) used world-wide in a number of important industries, such as the production of flexible and rigid foams, paints, adhesives, sealants, insulation products, medical devices, and laminated wood products [1]. Studies on isocyanate-induced asthma indicate that both T-helper type 2 (Th2)- and Th1-type cytokines are involved in this type of OA [2–5]. The pathogenesis of isocyanate-induced asthma is

still controversial, because the disease has both differences from and similarities to non-occupational allergic asthma. In contrast to non-occupational allergic asthma, IgE antibodies are present in a minority of subjects with asthma induced by isocyanates, suggesting that, for these LMWA, there may be mechanisms of sensitization and of development of disease that are independent of IgE antibodies [6].

HLA-G antigens are non-classical HLA class I molecules characterized by a limited tissue distribution, a low allelic polymorphism, and the presence of membrane-bound and

soluble isoforms [7]. Both the isoforms exhibit tolerogenic and anti-inflammatory functions [8, 9]. Several cells, including T and B cells, together with monocytes, produce HLA-G in peripheral blood, but there is evidence that the major source of HLA-G in peripheral blood mononuclear cell (PBMC) culture are monocytes [10]. Cytokines such as IFN- γ and IL-10 trigger the expression of HLA-G by mononuclear cells [11, 12].

The role of HLA-G molecules has been examined in several conditions including embryo implantation, organ transplantation, cancer, viral infections, and inflammatory diseases [13]. In asthma, Nicolae *et al.* [14] have shown that the HLA-G, encoded in the HLA region on chromosome 6p21, is a novel asthma and airway hyperresponsiveness gene. Furthermore, expression of sHLA-G has been detected in bronchial epithelial cells in two asthmatics, suggesting that the expression of this gene may be up-regulated in asthmatic lungs [15].

Using PBMCs from healthy subjects and patients with moderate-to-severe asthma, stimulated by lipopolysaccharide (LPS) in an *in vitro* system, we have shown that asthmatics exhibit a specific deficit of IL-10 secretion and a consequent reduced production of sHLA-G molecules [16].

IL-10 is a key regulator of immune and inflammatory responses [17]. It is secreted by monocytes, macrophages, mast cells, T and B lymphocytes, T regulatory cells, and dendritic cells [17]. In our *in vitro* system, monocytes and lymphocytes are likely the cells producing IL-10 [16]. A number of functions have been attributed to this cytokine including inhibition of eosinophilia, suppression of IL-5 and granulocyte macrophage-colony stimulating factor (GM-CSF), and suppression of nitric oxide (NO) production [18]. Moreover, both prostaglandins and endogenous leukotrienes, important mediators of inflammatory responses, exert their effects via regulation of IL-10 production [19]. It has been shown that IL-10 synthesis is attenuated in persistent asthma compared with mild asthma [20].

Isocyanate-induced asthma is an enigmatic condition that has similarities to and differences from non-occupational allergic asthma. Because we have previously studied the role of the sHLA-G molecule in moderate/severe non-occupational asthma [16], in the present study, we wished to extend our observations to mild/moderate non-occupational allergic asthma and OA induced by exposure to isocyanates, likely due to an IgE-independent mechanism. Therefore, we investigated the role of sHLA-G molecules and IL-10 in four groups: (i) subjects with non-occupational allergic asthma, (ii) subjects with isocyanate-induced asthma, (iii) asymptomatic subjects exposed to isocyanates, and (iv) healthy control subjects.

The production of sHLA-G molecules and the secretion of IL-10 were quantified using a specific ELISA in the culture supernatants of PBMC at baseline and after LPS stimulation, when monocytes are the main HLA-G-secreting cells.

Methods

Four groups of subjects were recruited: 20 subjects with isocyanate-induced asthma, 16 asymptomatic subjects exposed to isocyanates at work, 18 subjects with non-occupational allergic asthma, and 26 healthy control subjects.

The diagnosis of OA was based on the following: exposure to isocyanates, asthma symptoms, airway hyperresponsiveness to methacholine inhalation challenge, and an asthmatic reaction [a decrease in forced expiratory volume in 1 s (FEV₁) of $\geq 20\%$] after exposure to isocyanates in the laboratory. Of the 20 subjects with isocyanate-induced asthma, 14 were occupationally exposed to toluene diisocyanate (TDI), two to hexamethylene diisocyanate, and four to diphenylmethane diisocyanate. Five of the subjects with isocyanate-induced asthma used inhaled corticosteroids.

Of the 16 exposed asymptomatic subjects, 11 were exposed to both TDI and diphenylmethane diisocyanate, and five were exposed to diphenylmethane diisocyanate only.

The subjects with non-occupational allergic asthma had a typical history of bronchial asthma for at least 1 year and airway hyperresponsiveness to methacholine. The severity of the disease, similar to that of isocyanate-induced asthma, was mild persistent according to Global Initiative for Asthma Management and Prevention guidelines [21]. Ten of the 18 patients with non-occupational asthma used inhaled corticosteroids.

All subjects had been free of respiratory infections for at least 4 weeks, and all gave informed consent to participate in the study. The protocol was approved by the ethics committees of the University of Padova and the University of Ferrara.

Pulmonary function tests

Lung function was measured with a dry spirometer (PFT 922; Sensor Medics, Anaheim, CA, USA). Pulmonary function tests included measurements of baseline FEV₁ and forced vital capacity. Values are expressed as a percentage of predicted values [22].

Isocyanate and methacholine inhalation testing

Isocyanate and methacholine inhalation challenges were performed as described previously [23]. An asthmatic reaction occurred when FEV₁ had decreased by $\geq 20\%$ after exposure to isocyanates in the laboratory. Methacholine inhalation challenge was stopped when FEV₁ had decreased by $\geq 20\%$ compared with baseline levels.

Skin prick tests

Atopy was defined by the presence of a personal history of allergies, seasonal rhinitis, eczema, or allergic

conjunctivitis and positive SPT responses (skin reaction with a mean weal diameter of ≥ 3 mm larger than that produced with a saline control) to a panel of common aeroallergens, including grass and tree pollens, animal danders, moulds, house dust mites, and storage mites (Lofarma, Milan, Italy).

Peripheral blood mononuclear cell cultures

PBMCs obtained by Ficoll centrifugation (Cederlane, Hornby, ON, Canada) were resuspended in Iscove medium (Biochrom, Berlin, Germany) plus 10% fetal calf serum (FCS) at a concentration of 1×10^6 /mL and cultured for 48 h. Cultures were stimulated by addition of 10 ng/mL LPS (Calbiochem, La Jolla, CA, USA). LPS was used because it is the main activator of sHLA-G production in monocytes via an IL-10-dependent pathway [16]. Previous time course and dose-dependence experiments showed that an LPS concentration of 10 ng/mL with 48 h of incubation was the optimal stimulus and time-point to detect sHLA-G1/HLA-G5 in PBMC cultures [16]. The cell viability and percentages of CD14⁺ cells were evaluated by cytofluorimetry (FACS Vantage, Becton Dickinson, Milan, Italy), using propidium-iodide staining and an anti-human CD14-fluorescein isothiocyanate-conjugated antibody (Cymbus Biotechnology Ltd, Hants, UK).

Soluble human leucocyte antigen-G

sHLA-G1, obtained by proteolytic cleavage of the membrane-bound HLA-G1, and HLA-G5 were assayed in culture supernatants in triplicate as reported at the Wet Workshop for Quantification of Soluble HLA-G in Essen, Germany [24]. The capture antibody was the monoclonal antibody MEM-G/9 at a concentration of 20 µg/mL (Ex-bio, Praha, Czech Republic), which recognizes the HLA-G molecule in a β_2 -microglobulin-associated form. The detecting antibody was a horseradish peroxidase-conjugated anti- β_2 -microglobulin monoclonal antibody (Dako, Glostrup, Denmark). HeLa cell wild-type culture supernatants were used as a negative control, and transfected HeLa-G5 cells were used as a positive control. HeLa wild-type and HeLa-G5 cells were cultured in CD Hybridoma AGT Medium (GIBCO, Auckland, NZ) supplemented with 1% FCS and antibiotics. Culture supernatants were collected at cell confluence and concentrated by lyophilization. After depletion of albumin (Enchant Albumin Depletion Kit; Pall Corporation, Ann Arbor, MI, USA), purification of the sHLA-G proteins was carried out as reported previously [25]. The sHLA-G molecules obtained were used as a standard at different dilutions.

The intra-assay coefficient of variation was 1.4%, and the inter-assay coefficient of variation was 4%. The limit of sensitivity was 1.0 ng/mL. The samples with an HLA-G level below the detection limit were indicated as 0 ng/mL.

Interleukin-10

IL-10 levels were determined in triplicate in undiluted samples using a human IL-10 immunoassay kit (Bio-Source, Camarillo, CA, USA) with a detection limit of 0.2 pg/mL.

Statistical analysis

Statistical analysis was conducted using StatView, version 5.0.1 (SAS Institute Inc., Cary, NC, USA) [26]. Results are presented as mean $\pm$ standard error of the mean (SEM), geometric mean $\pm$ standard error of the geometric mean (GSEM), or median and interquartile range, where appropriate.

Differences between groups were determined by ANOVA for multiple comparisons. When each group was compared with the others, probability values were corrected for number of comparisons according to Bonferroni's rule. The symbol P_c denoted Bonferroni-corrected probability.

Differences between unstimulated sHLA-G and IL-10 levels and LPS-stimulated sHLA-G and IL-10 levels were analyzed by Wilcoxon signed rank test. The χ^2 test was used to assess between-group homogeneity of subject characteristics for categorical variables. Student's *t*-test for unpaired data was used to assess differences in the length of exposure to isocyanates between subjects with isocyanate-induced asthma and subjects who were exposed to isocyanates but were asymptomatic, and to assess differences in airway responsiveness to methacholine between subjects with isocyanate asthma and those with non-occupational allergic asthma.

Age, sex, atopy, and smoking were entered in a model of linear multiple regression in which the dependent variables were sHLA-G or IL-10 levels at baseline and all subjects were included. In subjects exposed to isocyanates, age, sex, atopy, smoking, duration of exposure to isocyanates, and current or recent exposure to isocyanates were entered in a model of linear regression in which the dependent variables were sHLA-G levels or IL-10 levels at baseline. Regression analysis was performed with a backward stepwise selection procedure to choose the most useful independent variables in explaining the dependent variable. A *P*-value of < 0.05 was considered significant.

Results

The characteristics of the subjects in the four groups are reported in Table 1. The majority of subjects exposed to isocyanates, whether asymptomatic or with occupational asthma, were non-atopic, whereas non-occupational asthmatics were all atopic. Asymptomatic exposed subjects were currently exposed to isocyanates. Five OAs were currently exposed or had a recent exposure to isocyanates, and the others had not been exposed for at least 6 months.

Table 1. Characteristics of the study population

	Isocyanate asthma (<i>n</i> = 20)	Asymptomatic-exposed subjects (<i>n</i> = 16)	Allergic asthma* (<i>n</i> = 18)	Healthy controls (<i>n</i> = 26)
Age years	46.3±2.0 [§]	35.2±2.6	40.6±3.5	34.9±1.7
Months of exposure to isocyanates	135.6±24.0 [†]	60.1±14.8		
Sex M/F	15/5	15/1	9/9	9/17
Smoking history %				
Never smoked	30.0	62.6	83.4	57.7
Current smoker	10.0	18.7	11.1	42.3
Ex-smoker	60.0	18.7	5.5	
Atopic/nonatopic	4 ^{††} /16	1/15	18 ^{††} /0	3/23
FEV ₁ (%) pred. [°]	91.4±3.5 ^{&}	100.0±3.1	104.8±3.0	
FEV ₁ /FVC (%)	73.9±1.5	85.3±2.1 [#]	77.3±1.8	
PD ₂₀ FEV ₁ methacholine (mg)				
Gmean and GSEM	506.4 (1.4)		370.0 (1.3)	

*Non-occupational allergic asthma.

[§]Significantly different from asymptomatic-exposed subjects and healthy controls ($P_c < 0.005$ and $P_c < 0.0001$, respectively).

^{||}Significantly different from healthy controls ($P_c < 0.001$).

[†]Significantly different from asymptomatic exposed subjects ($P < 0.05$).

^{††}Significantly different from allergic asthma ($P < 0.0001$).

^{†††}Significantly different from asymptomatic exposed subjects and from healthy controls ($P < 0.0001$).

[°]Predicted values from reference [22].

[&]Significantly different from allergic asthma ($P_c < 0.005$).

[#]Significantly different from isocyanate asthma ($P_c < 0.0001$) and from allergic asthma ($P_c < 0.005$).

Data are presented as mean±SEM unless stated otherwise. FEV₁, forced expiratory volume in 1 s; Gmean and GSEM, geometric mean and standard error of the geometric mean.

Subjects with isocyanate-induced asthma and those with non-occupational allergic asthma were older than healthy control subjects ($P_c < 0.0001$ and $P_c < 0.001$, respectively); subjects with isocyanate-induced asthma were older than asymptomatic-exposed subjects ($P_c < 0.005$). Subjects with OA exhibited lower values of FEV₁ than did subjects with non-occupational allergic asthma ($P_c < 0.005$). Airway responsiveness to methacholine was similar in isocyanate-induced asthma and non-occupational allergic asthma.

Cell viability was 99–100% in each sample, and the percentage of CD14⁺ cells was similar in all the groups examined. Table 2 and Fig. 1a show the levels of sHLA-G1/HLA-G5 in unstimulated and LPS-stimulated PBMC cultures from all three groups of study subjects and healthy controls. Baseline (unstimulated) levels of sHLA-G were significantly higher in cultures from the subjects with isocyanate-induced asthma than in the other groups ($P_c < 0.0001$ as compared with non-occupational allergic asthma and healthy control subjects; $P_c < 0.001$ as compared with asymptomatic-exposed subjects). sHLA-G was detectable in 14 of the 20 PBMC cultures from subjects with isocyanate-induced asthma and in four of the 16 cultures from asymptomatic exposed subjects (χ^2 test: isocyanate-induced asthma vs. asymptomatic-exposed subjects: $P < 0.05$); none of the cultures from subjects in the other groups contained detectable levels of sHLA-G.

Results from a stepwise regression model showed that the only predictor of baseline sHLA-G levels was a

Table 2. sHLA-G levels (ng/mL) in the supernatants of unstimulated and LPS-stimulated PBMC cultures

	Unstimulated	LPS-stimulated [†]
Isocyanate asthma (OA)	2.4 [‡] (0–12.2)	5.9 (3.5–12.6)
Asymptomatic-exposed subjects (AE)	0 (0–1.4)	4.6 (3.5–9.1)
Nonoccupational allergic asthma (AA)	0	10.0 (6.0–12.0)
Healthy controls (C)	0	4.2 (1.8–14.3)

[†]Significantly different from unstimulated cultures in all groups (OA: $P < 0.001$; AE and AA: $P < 0.0005$; C: $P < 0.0001$).

[‡]Significantly different from unstimulated cultures in other groups ($P_c < 0.0001$ vs. AA and C; $P_c < 0.001$ vs. AE).

Data are expressed as median and interquartile range.

sHLA, soluble human leucocyte antigen; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells.

diagnosis of isocyanate-induced asthma ($P < 0.005$), while age, sex, atopy, smoking, duration of exposure to isocyanates, current or recent exposure to isocyanates and baseline IL-10 levels had no effect. After LPS stimulation of PBMC cultures, baseline sHLA-G levels increased significantly in all groups compared with the baseline and did not differ significantly between groups (Table 2).

In subjects with occupational or non-occupational allergic asthma, the levels of sHLA-G were not different in those on inhaled corticosteroids compared with those not on inhaled corticosteroids.

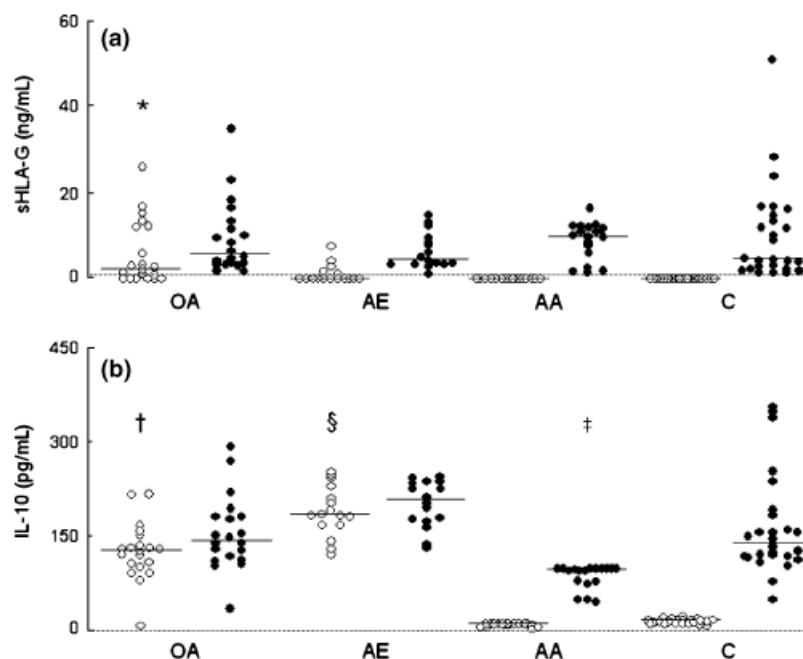


Fig. 1. Individual values of soluble human leucocyte antigen (sHLA)-G levels (ng/mL) (a) and IL-10 levels (pg/mL) (b) in the supernatants of unstimulated (open circles) and LPS-stimulated (black circles) peripheral blood mononuclear cells cultures. OA, isocyanate asthma; AE, asymptomatic subjects exposed to isocyanates; AA, non-occupational allergic asthma; and C, healthy control subjects. Horizontal bars represent medians. Dashed lines indicate ELISA detection limits. *Significantly different from unstimulated cultures of all other groups ($P_c < 0.0001$ vs. AA and C; $P_c < 0.001$ vs. AE). †Significantly different from unstimulated cultures of all other groups ($P_c < 0.0001$). §Significantly different from unstimulated cultures of all other groups ($P_c < 0.0001$). ‡Significantly different from LPS-stimulated cultures of OA ($P_c < 0.001$), AE ($P_c < 0.0001$), and C ($P_c < 0.0001$).

Table 3. IL-10 levels (pg/mL) in the supernatants of unstimulated and LPS-stimulated PBMC cultures

	Unstimulated	LPS-stimulated [†]
Isocyanate asthma (OA)	127.5 (103.6–142.7) [‡]	143.4 (115.0–180.5)
Asymptomatic exposed subjects (AE)	184.5 (167.7–219.6) [§]	207.1 (176.0–235.1)
Nonoccupational allergic asthma (AA)	9.7 (9.4–9.8)	96.0 (78.2–98.2)
Healthy controls (C)	15.6 (11.2–17.7)	139.5 (117.5–182.0)

[†]Significantly different from unstimulated cultures in all groups except AE (OA: $P < 0.05$; AA: $P < 0.0005$; C: $P < 0.0001$).

[‡]Significantly different vs. AE, AA and C ($P_c < 0.0001$).

[§]Significantly different vs. AA and C ($P_c < 0.0001$).

^{||}Significantly different vs. OA, AE and C ($P_c < 0.001$, $P_c < 0.0001$, and $P_c < 0.0001$, respectively).

Data are expressed as median and interquartile range.

LPS, lipopolysaccharide.

Under baseline conditions, IL-10 concentrations were significantly higher in cultures from subjects with isocyanate-induced asthma and asymptomatic-exposed subjects than in the other groups ($P_c < 0.0001$) (Table 3, Fig. 1b).

Results from a stepwise regression model showed that the predictor of baseline IL-10 levels was exposure to isocyanates ($P < 0.001$).

After LPS stimulation of PBMC cultures, IL-10 levels increased significantly compared with baseline in all groups except asymptomatic-exposed subjects (Table 3). IL-10 levels were significantly lower in subjects with non-occupational allergic asthma compared with isocyanate-induced asthma ($P_c < 0.001$), asymptomatic-exposed subjects ($P_c < 0.0001$), and healthy control subjects ($P_c < 0.0001$). In subjects with occupational or non-occupational allergic asthma, sHLA-G and IL-10 levels were not different in subjects on inhaled corticosteroids compared with those not on inhaled corticosteroids. The levels of sHLA-G and IL-10 were not significantly different between OAs currently or recently exposed to isocyanates and those removed from exposure.

Discussion

We showed that exposure to isocyanates, a common cause of OA, was associated with high levels of baseline production of the cytokine IL-10 by PBMCs, irrespective of whether or not the exposed subjects exhibited work-related symptoms. However, the production of sHLA-G in unstimulated PBMC cultures was significantly higher in subjects with isocyanate-induced asthma compared with asymptomatic-exposed controls. Furthermore, we showed that subjects with non-occupational allergic asthma produced sHLA-G only after LPS stimulation. In this group of

subjects, IL-10 concentrations increased after LPS stimulation but did not reach the high levels observed in subjects exposed to isocyanates. To our knowledge, this is the first study to examine the effects of isocyanates with regard to sHLA-G and IL-10 expression by controlling for exposure and disease.

The pathophysiology of OA induced by exposure to isocyanates is controversial [6]. Although the mechanisms involved in isocyanate-induced asthma remain unclear, this common type of OA shares many functional and morphologic features with non-occupational IgE-dependent asthma. The airway inflammatory process is similar in both diseases, and both are characterized by an increased number of activated inflammatory cells and a thickened reticular basement membrane [27]. They also share an increased expression of lymphocyte markers and increased numbers of cells producing pro-inflammatory cytokines [2, 3]. However, no appreciable levels of mRNA for IgE receptors have been found in isocyanate-induced asthma [28].

Our findings suggest further differences in the pathophysiology of OA compared with non-occupational allergic asthma. The common view, even though controversial [20, 29–31], of an association between asthma phenotype and reduced IL-10 production does not seem appropriate in isocyanate-induced asthma, whereas it is supported by the lower concentrations of IL-10 that we observed in subjects with non-occupational allergic asthma compared with healthy control subjects. Because the target of LPS, in our *in vitro* PBMC culture, are CD14-positive cells, i.e. monocytes, and LPS stimulation induces a dose-dependent IL-10 secretion, which precedes the release of sHLA-G, we believe that monocytes are the major cell source of IL-10 and sHLA-G detected in the supernatant of our cultures [16]. However, we cannot exclude that other cells, namely lymphocytes, may be responsible for elevated baseline IL-10 secretion detected in PBMC from subjects exposed to isocyanates. In addition, we cannot tell whether the effect of isocyanate exposure was specific for IL-10 because other cytokines were not measured. Therefore, IL-10 cannot be considered a biomarker of isocyanate exposure.

We do not know the fate of inhaled isocyanates in the human body and the nature of the isocyanate antigen that is eventually produced [32]. The demonstration that human monocytes *in vitro* take up diisocyanate–albumin conjugates and undergo activation supports a role for these cells in the immune response to isocyanates [33]. The same investigators found that experimental *in vivo* exposure to isocyanates was associated with a transient increase in peripheral blood monocytes in subjects not sensitized. It may be argued that isocyanate-exposed subjects had a different composition of PBMCs. However, the same number of cells was placed in culture and the proportion of CD14-positive cells in PBMC was compar-

able in all groups of subjects. Therefore, we are confident that the differences in IL-10 and sHLA-G are related to a different behaviour of the cells rather than to differences in their number. Another possible factor of a different production of IL-10 is the effect of age because older subjects have a higher secretion of IL-10 from PBMCs cultured for 24 h [34]. This explanation, however, cannot account for the increased IL-10 levels in the asymptomatic-exposed subjects who were younger than those with isocyanate-induced asthma or non-occupational allergic asthma. Furthermore, regression analysis showed that age was not a predictor for baseline IL-10 levels.

A question not yet answered is the link between sHLA-G and IL-10 expression. While it has been shown that CD4⁺ and CD8⁺ T and B cells together with peripheral blood monocytes are able to secrete HLA-G, monocytes were the main source [10]. The increase of sHLA-G upon LPS stimulation supports the hypothesis that CD14-positive cells are the predominant cells responsible for the release of sHLA-G in our *in vitro* system. Our previous study demonstrated that the defective production of HLA-G in PBMC from moderate-to-severe asthmatics was dependent on a specific deficit of IL-10 production [16]. In fact, the addition of exogenous IL-10 to the cultures restored normal HLA-G levels. The results of the present study are in line with the hypothesis that HLA-G production by PBMC in culture is dependent on IL-10, except that in the group of asymptomatic isocyanate-exposed subjects. Further experiments are necessary to explain why HLA-G production seems to be dissociated from IL-10 levels in these subjects.

Even though the severity of non-occupational allergic asthma was similar to that of isocyanate-induced asthma, the regulation of sHLA-G and IL-10 in the two diseases appears to be different. In non-occupational allergic asthma, there are low levels of IL-10 and no production of sHLA-G under baseline conditions. Compared with the results obtained from healthy control subjects, non-occupational allergic asthmatics exhibit impaired IL-10 secretion after LPS stimulation. This is in line with our previous findings in moderate-to-severe asthma [16] and strengthens the conclusion that the regulation of sHLA-G/IL-10 differs in isocyanate-induced asthma. We had no evidence that the atopy detected in all patients with non-occupational allergic asthma influenced the results, because it was not associated with sHLA-G or IL-10 levels in regression analysis. Moreover, our previous study [16] showed that in moderate-to-severe asthma, sHLA-G produced after LPS stimulation was irrespective of the atopic status.

Although the findings reported in the present study are statistically significant, we acknowledge that they are preliminary because of the small number of subjects. However, our results underline some differences between OA induced by LMWA and IgE-mediated

non-occupational asthma, and suggest that the immune response elicited by isocyanates is different from that induced by high-molecular-weight proteins such as aero-allergens. Our data support the hypothesis that human monocytes had a role in the clinical outcome of isocyanate exposure [33, 35]. However, more investigation is required to understand whether the ability of PBMCs to produce HLA-G is specific for OA caused by diisocyanates or whether similar findings are obtained in workers with OA caused by other respiratory sensitizers or with irritant-induced work-related asthma.

In summary, we have presented evidence of active sHLA-G production by unstimulated PBMCs from subjects with isocyanate-induced asthma that is associated with increased baseline secretion of IL-10. In contrast, PBMCs from non-occupational allergic asthma are not activated under baseline conditions, and their reduced IL-10 production possibly influences sHLA-G secretion. The results suggest that there is a different regulation of IL-10 and sHLA-G in OA induced by isocyanates compared with non-occupational allergic asthma.

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