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Levels of soluble human leukocyte antigen-G are increased in asthmatic airways

To the Editors:

Human leukocyte antigen-G (HLA-G) is a non-classical, class Ib, major histocompatibility complex antigen, encoded by a gene on chromosome 6p21 within the HLA complex [1]. HLA-G is constitutively expressed during pregnancy where it has a critical role in maintaining immune tolerance toward the allogenic fetus and placenta [2, 3], but has also been associated with inflammatory diseases such as psoriasis, multiple sclerosis, and ulcerative colitis, and with solid-organ transplantation [3, 4]. We recently reported associations between variation in HLA-G and risk for asthma in Chicago-area asthma families, in multigenerational Dutch asthma families and in a birth cohort at high risk for developing asthma [1, 5]. A role for HLA-G in asthma pathogenesis was further suggested by the demonstration of expression of a soluble isoform of HLA-G, sHLA-G5, in airway epithelial cells [1] and of increased circulating plasma levels of sHLA-G in children with atopic asthma [6]. Because airway inflammation in asthma involves a T-helper cell (Th) type 2-skewing of lymphocytes similar to pregnancy, HLA-G is an attractive candidate molecule for promoting the immune profile characteristic of asthma. Localisation of HLA-G to airway epithelium suggests that its dysregulation could contribute to airway inflammation in chronic asthma. To evaluate this further, we hypothesised that HLA-G abundance would be increased in asthmatic airways.

To test this hypothesis, we measured concentrations of sHLA-G in bronchoalveolar lavage (BAL) fluid obtained from 12 non-asthmatic control subjects and 15 subjects with mild persistent asthma. The use of human subjects was approved by the University of Chicago Institutional Review Board (Chicago, IL, USA). Asthma was diagnosed using National Asthma Education and Prevention Program guidelines. Subjects with a smoking history of $\geqslant 10$ pack·yrs^{-1}, who had used oral corticosteroids within 6 months of study, who had received emergent care or had been hospitalised for asthma within 6 months of study, were excluded. Bronchoscopy was done at a time of stability for each subject.

The demographic, clinical and pulmonary function data for the subjects in our study are presented in table 1. As expected, subjects with asthma had a lower forced expiratory volume in 1 s % predicted ($p=0.01$), more atopy ($p=0.0001$) and more peripheral blood eosinophils ($p=0.02$) compared with control subjects. However, there were no significant differences in cells counts in bronchoalveolar lavage (BAL) fluid between the two groups. Lavage fluid was concentrated approximately 30-fold using Centriprep ultra-filtration chambers (Millipore, Inc., Billerica, MA, USA) with a 3-kD molecular weight cut-off filter. The retentate was analysed for the presence of sHLA-G using an ELISA assay (Exbio, Inc., Vestec, Czech Republic). The capture antibody, MEM-G/9, recognises shed G1 and secreted G5, and the secondary antibody, anti-β2m, ensures measurement of β2m-configured soluble G [7]. The limit of sensitivity was ~0.2 U·mL^{-1}. Values were adjusted for the degree of concentration as noted above and expressed as U·mL^{-1} BAL fluid.

sHLA-G levels were increased in the BAL fluid of 15 asthmatic subjects (median 6.8 (interquartile range, 2.8–7.8) U·mL^{-1}) compared with 12 control subjects (median 1.6 (1.0–3.0) U·mL^{-1}, $p=0.01$ by Mann–Whitney test) (fig. 1). One control value and no asthmatic values were below the limit of detection for the assay. We also examined whether racial background accounted for the observed differences in sHLA-G. There was no significant difference in sHLA-G levels in nine Caucasian asthmatic subjects (median 5.6 (1.5–6.9) U·mL^{-1}) *versus* six African–American asthmatic subjects (median 6.9 (6.2, 8.6) U·mL^{-1}, $p=0.24$ by Mann–Whitney test). There were too few African–American control subjects for analysis in that group.

TABLE 1 Demographic and clinical characteristics of study subjects

	Normal	Asthmatic	p-value#
Subjects n	12	15	
Age yrs	37±3	32±3	0.22
Female	6 (50)	11 (73)	0.26
White	11 (92)	9 (60)	0.09
Smoking history	1 (8)	7 (47)	0.05
History of nocturnal asthma		6 (40)	
History of inhaled corticosteroid use		10 (67)	
Past history of oral corticosteroid use		5 (33)	
Past hospitalisation for asthma		8 (53)	
Past history of emergent care for asthma		10 (67)	
Presence of ⩾1 positive skin prick tests to allergen	1 (8)	14 (93)	0.0001
Peripheral blood eosinophil count cells·µL^{-1}	119±24	218±29	0.02
FVC L	4.54±0.29	4.09±0.30	0.26
FEV_1 L	3.79±0.23	3.15±0.23	0.05
FEV_1 % pred	105±3	91±3	0.01
FEV_1/FVC ratio %	84±1	78±2	0.02
Methacholine PD_{20} mg·mL^{-1}	>10¶	1.35±0.47	

Data are presented as mean±SEM or n (%), unless otherwise stated. FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; pred: predicted; PD_{20}: provactive dose causing a 20% decrease in FEV_1. #: p-values calculated using Fisher's exact 2×2 test. A p-value <0.05 was considered to be significant; ¶: normal subjects with a methacholine PD_{20} <10 mg·mL^{-1} were excluded from the study.

Immunoperoxidase labelling of endobronchial biopsies fixed in 10% neutral buffered formalin was done using a primary antibody directed against sHLA-G (clone 2A12; Exbio, Inc.), which targets intron 4 sequences present only in sHLA-G. sHLA-G was present in the epithelium of endobronchial biopsies collected from 6 of 9 control subjects and 9 of 11 subjects with mild asthma that had sufficient epithelium present in the mucosal layer for review. Staining was evident in each major epithelial cell type (data not shown). No visible differences in labelling patterns or intensity were apparent between asthmatic and control subjects, and no other submucosal airway cells and structures were stained. No notable infiltration of inflammatory cells was present in any biopsy as judged by haematoxylin and eosin staining.

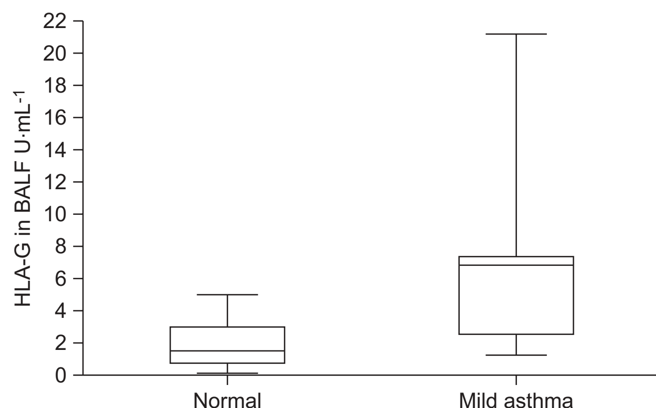

FIGURE 1. Level of soluble human leukocyte antigen (HLA)-G in bronchoalveolar lavage fluid (BALF) of 12 control subjects and 15 subjects with mild, persistent asthma. BALF was concentrated and then analysed by ELISA. Data are presented as a box plot showing the 25th (lower box) and 75th (upper box) percentiles; the bars demonstrate the range. The middle bar represents the median concentration. One control subject had a value below the limit of detection of the assay.

We report, for the first time, increased levels of sHLA-G in BAL fluid from subjects with asthma compared with control subjects, supporting a role for sHLA-G in asthma pathogenesis. Our results are consistent with our previous reports of associations between regulatory polymorphisms in *HLA*-G and asthma [1, 5], and a study showing higher plasma levels of sHLA-G in 27 Turkish school children with atopic asthma compared with either 26 non-atopic, asthmatic children or 16 normal controls [6]. We note that all but one of our subjects with asthma was atopic and that we previously reported an association between *HLA-G* genotype and atopy in Dutch children [1]. Thus, it is possible that HLA-G influences asthma susceptibility through atopic pathways.

The source of the differences in HLA-G concentrations that we observed is the airway epithelium, as there was no detectable HLA-G in other airway structures. We propose that epithelial-derived sHLA-G has a paracrine role in regulating the activity of key inflammatory cells found in asthmatic airways. We note that in other contexts HLA-G has been shown to suppress dendritic cells and T-cells that participate in inflammation [8], and to activate FoxP3+CD4+CD25+ regulatory T-cells that can suppress cells that participate in airway inflammation [9].

Our observation does not provide insights into cause and effect: is HLA-G driving the inflammation in asthmatic airways or is it a reactive attempt to suppress inflammation present in asthmatic airways? In pregnancy, HLA-G is thought to promote the skewing of T-cells toward a Th2 phenotype and to activate T-regulatory cells [2, 3], an immune phenotype which parallels

that seen in asthma. It is tempting to speculate that some individuals are genetically predisposed to over-express HLA-G in response to specific signals. Once secreted, HLA-G could promote a cascade of events that result in worsening inflammation. Several polymorphisms in the promoter region of *HLA-G* coincide with transcription factor binding sites could account for inter-individual differences in expression of HLA-G [10]. We previously identified a polymorphism in the 3′ untranslated region of *HLA-G* which disrupts a microRNA target site and demonstrated allele-specific expression of HLA-G in the presence of microRNAs that bind to that target [5]. Therefore, either lack of suppression or over-expression of HLA-G could explain the association we report here with asthma. We note that the small numbers of subjects in this study precludes more detailed analysis of relationships between genetic variation and HLA-G expression. Futuremore, larger studies are required to clarify the potential modulating role of HLA-G on the clinical manifestations of asthma and the role of genetic variation on expression levels.

In conclusion, sHLA-G is present in greater concentrations in BAL in mild asthma. We suggest that the overexpression or lack of suppression of HLA-G contributes to the disease process and that sHLA-G represents a novel pathway of asthma pathogenesis.

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Pneumocystis pneumonia in an HIV-negative patient with no overt risk factors on presentation

To the Editors:

Pneumocystis pneumonia (PCP) is a potentially life-threatening opportunistic infection that can occur in HIV-positive and HIV-negative individuals. The most significant risk factor for PCP in HIV-negative patients is chemotherapy, with a median time from cancer diagnosis to the first episode of PCP of 2 yrs. We report here a case of PCP in an adult male who had no identifiable risk factors on presentation. 3 weeks after the first signs and symptoms of PCP, he manifested blast crisis of acute myeloid leukaemia. This would be the first case demonstrating PCP as the sole presentation of an underlying occult leukaemia.

Our patient was a 55-yr-old homosexual male nonsmoker who presented with a 7-day history of progressive dyspnoea, dry cough, fever and chills. On examination, the patient's blood pressure was 130/70 mmHg, heart rate was 95 beats·min^{-1}, respiratory rate 25 breaths·min^{-1}, oxygen saturation 95% (3 L nasal oxygen), and temperature 37.9°C (100.2° F). The remainder