

Subtle Immunodeficiency in Severe Asthma: IgA and IgG₂ Correlate with Lung Function and Symptoms

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Key Words

Asthma · Carbohydrate antigens · IgA · IgG₂ · Mucosal protection

Abstract

Background: Atopy, increased serum IgE and eosinophilic airway inflammation are common in asthma and may indicate aberrant immune responses, but the cause(s) are unknown. It was hypothesized that differences in serum immunoglobulins, immunoglobulin free light chains (FLC) and secretory IgA (sIgA) would exist between subjects with asthma of varying severity and normal subjects, and the levels would correlate with lung function, symptoms and airway inflammation. **Methods:** Serum IgG, IgA, IgE and IgM, IgG subclasses and FLC, and bronchoalveolar lavage sIgA were evaluated from 15 normal subjects, 9 mild and 22 severe asthmatics with similar atopic status. Asthma symptoms were obtained by questionnaire, and airway inflammation was assessed by immunostaining for five inflammatory cell types. **Results:** Immunoglobulin levels in all groups were generally within the normal range. However, IgA and IgG were lower in severe asthmatics than normal subjects (overall $p = 0.006$ and 0.02 , respectively). IgA, but not IgG, correlated with lung function and asthma symptoms (r -values >0.58 ; p -values <0.009). Although similar

among the groups, higher sIgA and IgG₂ also positively correlated with lung function and negatively with asthma symptoms (r -values >0.63 ; p -values <0.009). IgA and IgG/IgG₁ positively correlated with tissue mast cells. **Conclusions:** Subtle alterations in IgA- and IgG₂-mediated responses in asthma may be disease-related. As their levels are generally normal, it is possible that the quality/repertoire of immune protection provided by these isotypes, perhaps against carbohydrate epitopes, may be altered in asthma.

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Introduction

Aberrant immune responses may underlie inflammatory processes in asthma. Indeed, increases in serum IgE, allergic reactions to environmental antigens and diminished responses to pneumococcal polysaccharides, all of which have been reported in asthma, are consistent with an abnormal immune response [1, 2]. However, the mechanisms behind this abnormal immune response remain uncertain. While IgE levels can be extremely elevated in asthma, the majority of subjects with asthma has serum immunoglobulin levels (including IgE) within the normal range and lacks other symptoms consistent with immunodeficiency [1]. Also, immunoglobulin levels in bronchoal-

veolar lavage (BAL) or airway lining fluid have been reported to be increased, as would be expected for an ongoing airway inflammatory process [3, 4]. Only a fraction of severe asthmatics have serum immunoglobulins (most often IgG) below normal levels, and this reduction has generally been attributed to steroid therapy [5, 6]. However, an increase in allergic disorders and asthma has been reported in patients with known IgA deficiency [7, 8]. Interestingly, while some IgA-deficient subjects may present with allergy/asthma, others may suffer from recurrent respiratory infections, bronchiectasis or autoimmune disorders. As the clinical syndromes in IgA deficiency are likely to be related to impaired IgA-dependent protection at mucosal surfaces, it is conceivable that in asthmatics without detectable IgA (or other) immunodeficiency, subtle changes in the mucosal immune protection could underlie the pathogenesis of the disease. Therefore, it may not be surprising that supplemental intravenous immunoglobulin therapy even in normogammaglobulinemic severe asthmatics has been reported to decrease symptoms, hospitalization and steroid use [9, 10]. However, there are also studies reporting no significant effect of intravenous immunoglobulin therapy [11–13].

We hypothesized that subtle differences in immunoglobulin classes and subclasses may exist in subjects with asthma of varying severity as compared to normal subjects and that these differences would be related to airway inflammation, lung function and/or symptoms. Four immunoglobulin classes, IgG subclasses and immunoglobulin free light chains (FLC) were determined in serum, and secretory IgA was measured in BAL fluid (BALF) from normal subjects, mild and severe asthmatics. The FLC were included in the analyses following recent reports that FLC can sensitize mast cells and trigger their activation upon contact with an appropriate antigen, which may be important in asthma [14]. All these parameters were correlated with airway tissue inflammatory cell counts, lung function and asthma symptom scores.

Patients and Methods

Subjects

Asthmatics recruited for the study included subjects with severe asthma who had a forced expiratory volume in 1 s (FEV_1) $\leq 70\%$ of predicted on more than one occasion over the previous year, were on 10 mg/day of prednisone or equivalent for at least 50% of the previous year, while using standard asthma medications, and had persistent symptoms, as described previously [15]. Mild asthma subjects were those defined by a $FEV_1 \geq 80\%$ of predicted on treatment with β -agonists alone or with low-dose inhaled corticosteroids ($n = 2$). Normal subjects had no history of any respiratory illness,

although occasional allergic rhinitis symptoms (not formally treated) were allowed. There were no current smokers and no subject smoked >5 pack years. No subject had episodes of any acute illness 4 weeks before blood and tissue sampling. The National Jewish Institutional Review Board approved the study and all subjects gave informed consent.

Asthma Symptoms, Atopic Status and Lung Function Measurements

All asthma subjects completed a questionnaire in which they graded the current frequency/intensity of asthma symptoms (deep chest cough, sputum production, chest tightness, wheezing, shortness of breath and nighttime symptoms) on a scale from 1 to 4 (never, some, most or all of the time). They also underwent allergen skin testing to 10 common aeroallergens and were considered atopic if they reacted to one or more allergens. In addition, FEV_1 , forced vital capacity (FVC) and residual volume (RV; all as % of predicted) and FEV_1/FVC were measured in the Pulmonary Physiology Unit at the National Jewish Medical and Research Center using standard protocols. Methacholine responsiveness (PC_{20} , provocation concentration causing a 20% fall in FEV_1) was measured using the dosimeter method.

Bronchoscopy and Tissue Processing

Serum collection, bronchoscopy with endobronchial biopsy and BAL with 4×60 ml of saline were performed as described previously [15]. Unconcentrated BALF samples and serum samples were stored at -80°C for immunoglobulin measurements. Biopsy tissue was fixed in acetone and embedded in glycol methacrylate resin.

Measurements of Immunoglobulins and Immunoglobulin FLC

Secretory IgA (sIgA) was measured in BALF diluted 1:20 using a commercially available ELISA kit (Alpco Diagnostics, Windham, N.H., USA). Serum immunoglobulin classes and subclasses were measured in the Clinical Laboratory at the National Jewish Medical and Research Center by nephelometry and ELISA, respectively. FLC were measured in the Immunology Laboratory of the Yamasa Corporation (Chiba, Japan) by ELISA, using monoclonal antibodies according to a recently published method [16].

Immunostaining

Tissue sections were immunostained for markers of T lymphocytes (CD3), monocytes/macrophages (CD68), neutrophil elastase, mast cell tryptase (all from Dako, Carpinteria, Calif., USA) and eosinophil major basic protein (clone BMK-13, Accurate Chemical & Scientific, Westbury, N.Y., USA) using Vectastain Elite ABC Kits (Vector Laboratories, Burlingame, Calif., USA). A Scion image analysis program (National Institute of Health, Bethesda, Md., USA) was used to obtain tissue morphometric data and determine cell counts (per mm^2 of submucosa) as described previously [14].

Statistical Analysis

As the data were not normally distributed, non-parametric Kruskal-Wallis tests were used to compare the three groups of subjects for each variable. When an overall significant difference was detected for a particular variable ($p < 0.05$), pairwise group tests were performed using Wilcoxon rank-sum tests. Spearman correlations (r_s) were used to measure the strength of associations between pairs of immunoglobulins and symptom variables, immunoglobulin and cell count variables and immunoglobulin and lung function

Table 1. Subject characteristics

	Gender males/ females	Age (years)	FEV ₁ (% of predicted)	Steroid therapy	
				inhaled	oral
Severe asthmatics (n = 22)	12/10	45 (26–50)	55% ± 4	6	18
Mild asthmatics (n = 9)	4/5	31 (23–41)	86% ± 2	2	N/A
Normal subjects (n = 15)	8/7	32 (24–36)	N/A	N/A	N/A
Overall p value		0.15	<0.0001		
Medians and interquartile ranges (age) and means ± SEM (FEV ₁) are shown.					

variables. Another set of correlations was performed within immunoglobulin variables. The false discovery rate procedure was employed to maintain the false discovery rate at 0.1 within each of these sets of comparisons [17].

Multiple regression analysis was used to determine whether steroid use was a confounding factor in the relationships observed between immunoglobulin variables and lung function, symptoms or cell count variables. First, regression models were fit using lung function parameters, total symptoms (sum of individual symptoms in each asthmatic) or cell counts as the outcome and an immunoglobulin as the sole predictor. These same models were then refit with oral steroid dose and inhaled steroid dose (graded 1, 2 or 3). Asthma severity was controlled for by including it as an additional predictor in these models. All testing was done using JMP or SAS (SAS Institute, Cary, N.C., USA).

Results

Subjects

As shown in table 1, 22 severe asthmatics (60% atopic), 9 mild asthmatics (all atopic) and 15 healthy subjects (73% atopic) were evaluated. Atopic status did not differ significantly among the groups ($p = 0.1$). Subjects were well matched for gender and were of similar age. Steroid therapy in 12 severe asthmatics consisted of both inhaled and oral corticosteroids, 6 were receiving high-dose inhaled corticosteroids only, while 4 were on oral corticosteroids only. The median oral steroid dose was 28 mg (interquartile range 16–40). In the mild asthma group, only 2 subjects were using low- and medium-dose inhaled steroids, respectively.

Immunoglobulins and Immunoglobulin FLC in Serum

Thirteen severe asthmatics, 6 mild asthmatics and 14 normal subjects had complete data for all serum immunoglobulin classes. A subset of those with complete im-

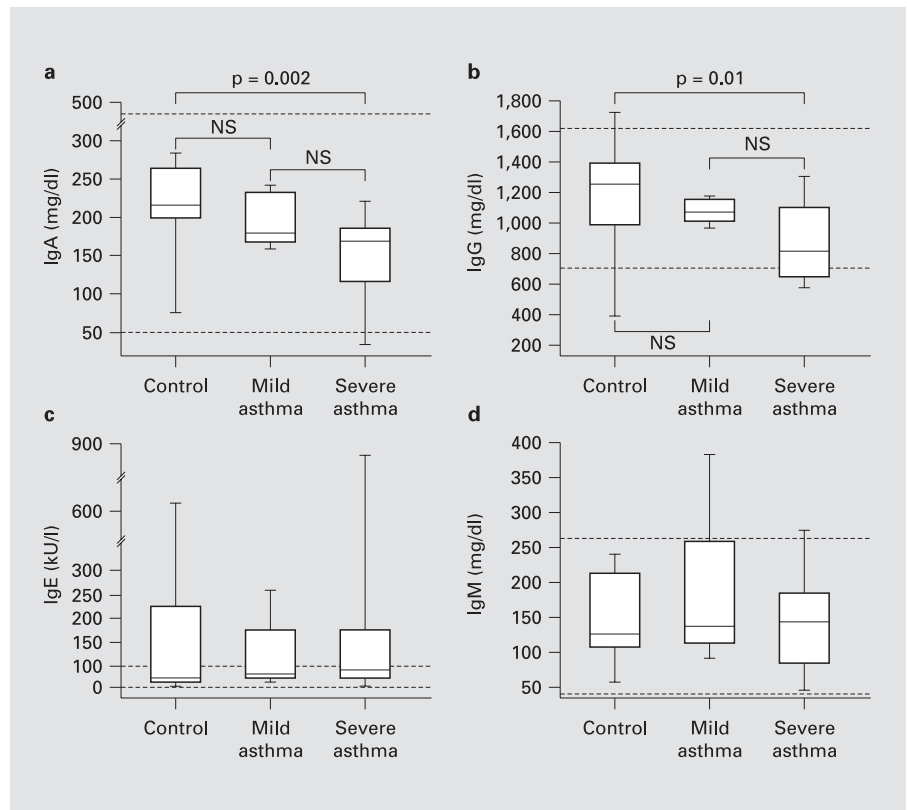
munoglobulin class data (randomly selected 10 severe asthmatics, 6 mild asthmatics and 11 normal subjects were randomly selected) also had immunoglobulin subclasses and FLC determined. Three additional severe asthmatics had incomplete data for immunoglobulin classes.

Only IgA and IgG significantly differed among the groups (fig. 1a, b). IgA and IgG were significantly decreased in severe asthmatics as compared to normal subjects, but were similar to the levels in mild asthmatics. Mild asthmatics did not differ from normal or severe asthma subjects.

IgE and IgM serum concentrations were similar between the groups ($p = 0.95$ and $p = 0.68$, respectively; fig. 1c, d). As shown in figure 1c, median IgE levels in all groups were within the normal range (45, 67 and 85 kU/l in normal subjects, mild and severe asthmatics, respectively; normal range = 0–100 kU/l). IgG subclass levels were also similar among the groups (p values: for IgG₁ = 0.30; IgG₂ = 0.39; IgG₃ = 0.37; IgG₄ = 0.96; data not shown). The serum levels of κ -FLC, λ -FLC and the κ/λ ratios did not vary among the groups (data not shown).

When evaluating individual data, 4 severe asthmatics and 1 normal subject had immunoglobulin readings below the normal limits. In those 4 severe asthmatics, total IgG levels ranged from 581 to 656 mg/dl (normal range = 700–1,620 mg/dl). IgG subclasses were, however, all within the normal range. One of the 4 severe asthmatics also had an abnormally low IgA (27 mg/ml; normal range = 50–462 mg/dl). The 4 severe asthmatics with low IgG were all on oral steroid therapy (15–40 mg). One normal atopic subject had an abnormally low IgG (391 mg/dl), and all IgG subclasses except IgG₃ were also below normal. Low FLC values were also measured in this individual. Despite these abnormalities, there was no clinically apparent immunodeficiency.

Fig. 1. Immunoglobulin classes in serum from normal subjects and patients with mild or severe asthma. Presented are medians and interquartile ranges. Dotted lines mark the normal range for each immunoglobulin class. **a** IgA (normal range 50–462 mg/dl); overall $p = 0.006$. **b** IgG (normal range 700–1,620 mg/dl); overall $p = 0.02$. **c** IgE (normal range 0–100 kU/l); overall $p = 0.95$. **d** IgM (normal range 44–266 mg/dl); overall $p = 0.68$.



Secretory IgA in BALF

Secretory IgA in BALF samples from 16 severe asthmatics, 8 mild asthmatics and 12 normal subjects did not significantly differ between the groups [median (interquartile range) 3.2 $\mu\text{g/ml}$ (2.7–6.1) in normal subjects vs. 4.4 $\mu\text{g/ml}$ (2.5–5.7) in mild asthma and 3.5 $\mu\text{g/ml}$ (2.2–4.8) in severe asthma; $p = 0.43$].

Relationships between Immunoglobulin Classes/Subclasses

As expected, IgG correlated with IgG₁, the predominant IgG subclass ($r_s = 0.88$; $p < 0.0001$). IgG also positively correlated with IgA ($r_s = 0.47$; $p = 0.005$). Secretory IgA correlated with IgG₃ levels, while IgM correlated with IgG₄ ($r_s = 0.50$; $p = 0.009$ for both correlations). No other significant correlations existed after the false discovery rate procedure.

Immunoglobulins and Cellular Inflammation in Airways

When the serum immunoglobulin levels, FLC and sIgA were correlated with airway inflammatory cell counts, serum IgA, IgG and IgG₁ positively correlated

with the number of mast cells in airway tissue (fig. 2c, d; $r_s = 0.53$ – 0.57 ; $p = 0.002$ – 0.004). No other correlations were noted.

Immunoglobulins and Lung Function

Serum/BALF immunoglobulin levels from severe and mild asthmatics were compared with lung function measurements. Serum IgA positively correlated with FVC (% of predicted; fig. 2a; $r_s = 0.65$; $p = 0.002$), while IgG₂ correlated with FEV₁ (% of predicted; fig. 2b; $r_s = 0.66$; $p = 0.007$). These correlations all passed the false discovery rate procedure. No other immunoglobulin classes, IgG subclasses, FLC or sIgA correlated with lung function or airway reactivity (PC₂₀).

Immunoglobulins and Asthma Symptoms

IgA and sIgA inversely correlated with asthma symptoms. Secretory IgA negatively correlated with chest tightness and shortness of breath ($r_s = -0.70$; $p = 0.001$ and $r_s = -0.66$; $p = 0.003$, respectively), while serum IgA negatively correlated with nighttime symptoms and sputum production ($r_s = -0.69$; $p = 0.003$ and $r_s = -0.63$; $p = 0.009$, respectively). Also, λ -FLC inversely correlated with night-

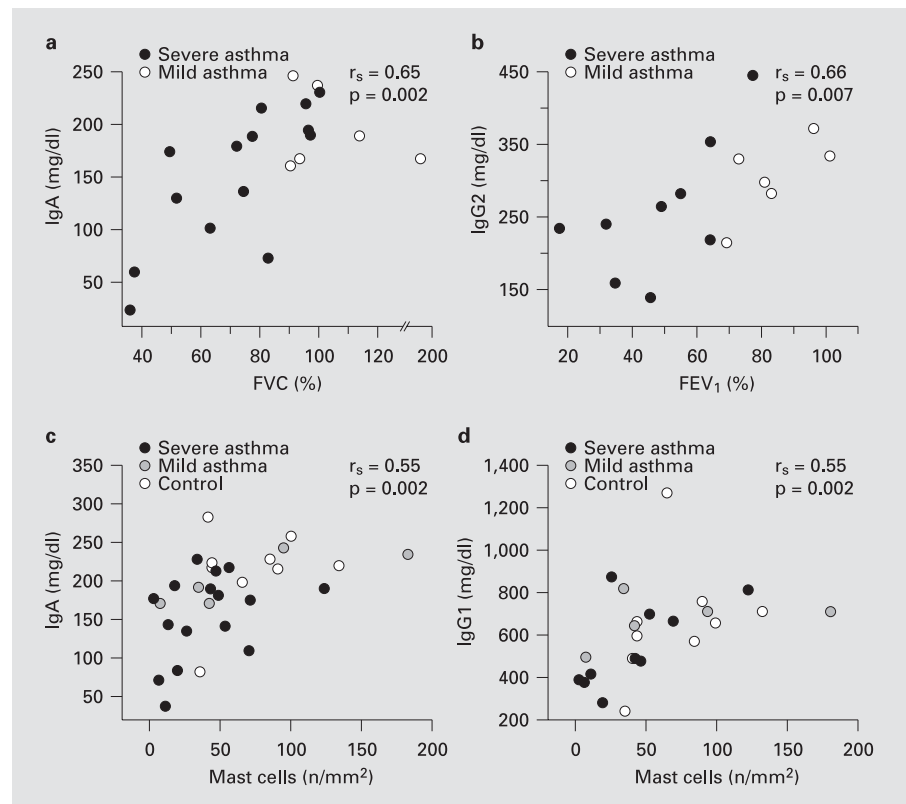


Fig. 2. Correlations between lung function measures and serum IgA (a) and IgG₂ (b) and between the tissue mast cell counts and serum IgA (c) and IgG₁ (d).

time symptoms ($r_s = -0.71$; $p = 0.007$). No other immunoglobulin classes, IgG subclasses, κ -FLC or κ/λ ratio correlated with asthma symptoms.

Potential Confounding Effects of Steroids

There was no evidence of confounding by steroid use on immunoglobulin and lung function variables, total symptoms or mast cell counts in the regression models that included subjects with asthma. Oral and inhaled steroid doses were not significant predictors ($p > 0.05$ in all cases, $p > 0.15$ in the majority of cases) in those models. More importantly, either minimal or no changes in the magnitude of effect or its significance were observed for the immunoglobulin variables after the inclusion of oral and inhaled steroids, with or without asthma severity included.

Discussion

This study suggests that IgA- and IgG₂-dependent immune responses and/or mechanisms may be altered in asthma and that subtle alterations in the levels of those

immunoglobulins correlate with elemental clinical asthma outcomes, such as lung function and asthma symptoms. Strong correlations of IgA, IgG₂ and sIgA with lung function and symptoms were detected in subjects having asthma of varying severity. These relationships persisted after application of the stringent false discovery rate procedure, which eliminates results with more than a 10% likelihood of being falsely positive. Perhaps more interestingly, these correlations existed in the absence of abnormally low immunoglobulin levels.

In analyses of individual immunoglobulin classes/subclasses, the subject groups differed only in the levels of IgA and total IgG. Although both medians were within normal ranges, IgA and IgG were decreased in patients with severe asthma as compared to normal subjects, while patients with mild asthma were intermediate and not significantly different from either group. Of these two classes, only IgA was consistently associated with lung function and symptoms in asthmatics, suggesting that IgA rather than IgG may be disease-related.

Although there were no differences in IgG₂ levels among the groups, IgG₂ correlated with lung function. IgG₂ is produced primarily in response to polysaccha-

ride/carbohydrate antigens, including microbial surface antigens such as pneumococcal capsular polysaccharides. Diminished IgG₂ responses to polyvalent pneumococcal vaccine (which contains antigens from 23 *Streptococcus pneumoniae* serotypes) have been reported in subjects with severe asthma, suggesting an abnormal response to carbohydrate antigens may exist in asthma [1, 2]. The types of antigens that elicit IgG₂ and IgA production appear to be similar, as the majority of environmental antigens that induce IgA are glycosylated. It has been reported that subtle alterations in serum levels of IgA and mannan-binding lectin (binds carbohydrates on the surface of pathogens) in early childhood, although within normal ranges, may indicate susceptibility to infections and asthma not detectable by conventional criteria [18]. The increased susceptibility to respiratory infections of IgA-deficient subjects suggests that IgA is important in antimicrobial protection. Deficiency in IgA is not uncommon (between 1 in 400 to 1 in 2,000 people). It can be concomitant with IgG₂ deficiency and blunted responses to pneumococcal vaccine [8]. Thus, both IgA and IgG₂ bind similar class of antigens and may be important for antimicrobial protection and appropriate immune responses at mucosal surfaces. As in this study both IgA and IgG₂ were within normal ranges (although IgA was lower in severe asthmatics than in normal subjects), it is conceivable that the strong correlations with lung function and/or symptoms in asthma could reflect a reduced or altered IgA and IgG₂ repertoire and thus an inadequate host response. A reduced repertoire within IgA/IgG₂ isotypes could result in inappropriate or allergic reactions against common environmental antigens, which are typical for asthma. In addition, a reduced or altered B cell repertoire could have repercussions for overall immune responses. Recent studies suggest that B cells, either as antigen-presenting cells or immunoglobulin producers, may play a crucial role in the regulation of T cell function and immunity [19, 20]. Tsitoura et al. reported that T cell tolerance to aeroallergens could not be established in B cell-deficient mice [21]. In addition, Stäger et al. reported that natural antibody-induced complement activation was essential for the priming of CD8⁺ cells and establishment of a protective adaptive immune response, suggesting that appropriate interaction between humoral and cellular immune responses is required for an effective immune protection [22]. Therefore, B cells and their innate and adaptive repertoire could have a broad and significant impact on overall establishment and regulation of immunity. Consequently, inappropriately developed or deficient immu-

noglobulin repertoire or specificities within inappropriate immunoglobulin isotypes at either mucosal or systemic level could have complex implications, including dysregulation of both humoral and cellular immune responses. Thus, in the context of a dysregulated immune response in asthma, the suggestion that intravenous immunoglobulin therapy alleviates asthma symptoms through an immunoregulatory effect may be justified [10, 23]. Further study in this area would appear to be warranted.

Previous studies have reported low serum IgG in severe asthma, and have suggested these low levels may be related to steroid therapy [1, 5, 6]. Indeed, lower levels of immunoglobulins in severe asthmatics in this study may be in part related to the suppressive effect of steroids. However, multiple regression modeling applied in this study indicated little or no confounding of the steroid dose on the relationship between immunoglobulin levels and lung function, symptoms or cell counts. This finding may indicate that lower immunoglobulin levels perhaps precede and/or underlie the development of severe asthma rather than occurring secondary to steroid therapy. It is also possible that these changes are secondary responses to asthma. More studies are required to address those issues.

There were no significant relationships between most inflammatory cell types and immunoglobulin levels. However, mast cell counts were significantly and positively associated with serum IgG (and IgG₁) and IgA levels. While the importance of this relationship is unclear, it is possible that mast cell-derived cytokines and/or costimulatory signals targeting B cells may play a greater role in immunoglobulin production than previously thought [24, 25]. Additionally, the mast cell proteases, tryptase and chymase, can initiate antigen breakdown, facilitating antigen processing and presentation [26]. Still, the converse is also just as likely, i.e. that mast cell proliferation or survival is enhanced through immunoglobulin-mediated effects. Finally, as mast cells numbers appear to decrease in response to steroids, the relationship between immunoglobulins and mast cells could be through the effects of steroids [27]. When subjects were recruited for this study, it was assumed that atopy would differ among the groups, being lower in normal subjects. However, as normal subjects were not eliminated on the basis of allergic skin responses or minor upper airway/allergic symptoms (not requiring treatment), neither the level of atopy nor IgE differentiated the subject groups. While this could be viewed as a limitation of our comparisons, it could also be viewed as a strength. In the sub-

jects studied, the differences in immunoglobulins between the normal and asthmatic subjects remained, even when matching for atopy and IgE. It could be argued that if a 'pure' normal control group (without detectable atopy) had been evaluated, one would have seen even greater differences in immunoglobulin profiles.

In conclusion, this study suggests that subtle immunodeficiency, perhaps due to a reduced repertoire or inappropriately low level of responses within IgA and IgG₂ isotypes, may contribute to the development of the syndrome of asthma. Possible mechanisms include altered

local responses to the environment, with dysregulation of immune responses and repercussions at a systemic level. More studies are required to explore the proposed mechanisms of asthma pathogenesis.

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