

Sputum and serum immunoglobulins in adult asthmatics with recurrent respiratory tract infections

To the Editor

The majority of asthmatics have type-2 inflammation, but nontype-2 inflammation, characterized by airway neutrophilia, can be dominant in the airways in up to 20% of patients.¹ These patients often have lower lung function and a higher likelihood of severe disease.² Molecular sequencing of their respiratory samples suggests that the observed neutrophilia is related to respiratory tract infection (RTI).³ Our understanding of recurrent infection in severe asthma is incomplete, though there is evidence to suggest that humoral deficiency, which is relatively common in asthma, plays a role. Immunoglobulins (Ig) are crucial to mucosal immunity and can suppress bacterial and viral infection through direct neutralization, activation of complement, and opsonization of pathogens. Hypogammaglobulinemia is associated with recurrent respiratory tract infection,⁴ and when present with asthma, Ig replacement reduces exacerbations.⁵ However, recurrent infection in severe asthma often does not have an identifiable cause. We hypothesized that a deficiency of local airway Igs could be contributing to this phenotype. To explore this further, we set three objectives: (a) To measure Igs in sputum and to test the correlation between serum and sputum Ig levels in asthmatics and healthy controls; (b) to compare Ig levels in serum and sputum from asthmatics with recurrent neutrophilic bronchitis and healthy controls; and (c) to compare the effects of subcutaneous (SCIg) vs. intravenous Ig (IVIg) therapy on replacement of Ig levels in both the serum and sputum, and the subsequent clinical outcomes.

To this end, we measured induced sputum (cell-free supernatant) and serum Ig levels (all types and isotypes) in 31 asthmatics with recurrent RTI, 10 asthmatics with a history of eosinophilic bronchitis (sputum eosinophils >3%) but without recurrent RTI, and 12 healthy controls. All samples were collected during a clinically stable period, where the patient had not had an exacerbation during the preceding 8 weeks. To be classified as having recurrent RTI, it was required that a patient on ≥ 2 separate occasions, within the last 5 years, had an infective exacerbation. Infective exacerbations were defined by the growth of a pathogen in sputum culture, or sputum neutrophilia (defined by sputum total cell count $\geq 20 \times 10^6$ cells/gram and sputum neutrophils $\geq 65\%$).⁶ All assays were performed by Eve Technologies, Calgary, Alberta. Among asthmatics, we included individuals without Ig replacement ($n = 11$), and those who were established on SCIG ($n = 10$) or IVIg ($n = 10$) for recurrent RTI by their immunologist. Those on SCIG had their initial sample collected prior to their weekly

SCIG dose and their second sample collected 1-month after the initial sample and after their weekly SCIG dose. Those on IVIg had their initial sample collected prior to their monthly IVIg dose, and their second sample after their monthly IVIg dose (within 48 hours). Those with a known immunodeficiency disorder or radiographic bronchiectasis of greater than mild severity (as defined by the BRICS tool⁷) were excluded. We chose to include patients with mild bronchiectasis due to the relatively frequent occurrence of overlap in airway diseases. Informed consent was obtained from all subjects. The study was approved by the Hamilton Integrated Research Ethics Board.

Subject baseline characteristics and the available immunodeficiency results are summarized in Tables S1 and S2, respectively. A summary of serum and sputum Ig levels in all asthmatic groups are available in Table S3. In asthmatics with recurrent RTI and healthy controls, there was a significant correlation only for IgG₄ ($r = .44$, $P = .045$ and $r = .59$, $P = .0004$; Figures S1A-B). Healthy controls demonstrated negative correlations for IgM ($r = -.48$, $P = .03$) and IgG₁ ($r = -.44$, $P = .04$; Figures S1C-D). Analyzed together, both serum and sputum IgG₂ ($r = .35$, $P = .02$) and IgG₄ ($r = .62$, $P < .0001$) were significantly correlated. For the second objective, we found between group differences for sputum IgM ($P = .0005$), IgE ($P = .003$), and IgG₄ ($P < .0001$), with IgA trending toward significance ($P = .08$; Figures S2A-D, ANOVA). Interestingly, sputum IgM was lowest in eosinophilic asthmatics (mean 52.7 ± 42.8 ng/mL) relative to healthy controls (173.0 ± 102.2 ng/mL, $P = .01$) and asthmatics with recurrent RTI (348.7 ± 282.2 ng/mL, $P = .0004$, Figure 2A, Tukey's multiple comparisons test). IgE in the sputum was higher in eosinophilic asthmatics than healthy controls (6.2 ± 5.4 vs 1.3 ± 0.9 ng/mL, $P = .002$). Finally, asthmatics with recurrent RTI had higher sputum IgG₄ (96.1 ± 47.6 ng/mL) compared to both healthy controls (17.4 ± 15.4 ng/mL, $P = .0009$) and eosinophilic asthmatics (13.3 ± 15.0 ng/mL, $P = .0002$, Tukey's multiple comparisons test). This was associated with a relative reduction in sputum IgA (IgA/Total IgG) in eosinophilic asthmatics compared to healthy controls (median 0.13 [0.09,0.25] vs 0.61 [0.32,0.90], $P = .0007$; Figure 1), with this ratio being similar between asthmatic groups. For serum Igs, asthmatics with recurrent RTI had higher IgG₁ ($2.7 \pm 0.49 \times 10^6$ vs $2.0 \pm 0.32 \times 10^6$ ng/mL, $P = .02$, unpaired t test), total IgG ($9.0 \pm 2.3 \times 10^6$ vs $6.1 \pm 1.2 \times 10^6$ ng/mL, $P = .02$, unpaired t test), and IgM ($1.3 \pm 0.53 \times 10^6$ vs $0.74 \pm 0.19 \times 10^6$ ng/mL, $P = .04$, unpaired t test) compared to healthy controls. For the third objective, serial

measurement of sputum and serum Ig levels demonstrated that IVIg but not SCIg caused a marked increase in total IgG and all IgG subtypes (all $P < .003$, paired t test). Interestingly, IVIg led to a significant improvement in sputum IgA (2400 ± 860 vs 2100 ± 880 ng/mL, $P = .03$, paired t test; Figure S3A), while SCIg reconstituted sputum IgG₁ (5800 ± 2800 vs 3400 ± 1400 ng/mL, $P = .008$, paired t test), IgG₂ ($0.99 \pm 1.1 \times 10^4$ vs $0.30 \pm 2.4 \times 10^4$ ng/mL, $P = .01$, paired t test), IgG₄ ($79 [31,250]$ vs $75 [20,120]$ ng/mL, $P = .04$, Wilcoxon test), and total IgG ($1.7 \pm 1.5 \times 10^4$ vs $0.68 \pm 0.37 \times 10^4$ ng/mL, $P = .008$, paired t test; Figure S3B). There were fewer infective exacerbations in the 12 months following initiation of Ig replacement compared to the preceding 12 months for IVIg (0.75 ± 0.70 vs 2.9 ± 1.9 exacerbations, $P = .02$, paired t test; Figure 2), but not following SCIg replacement. Furthermore, in the 12-month postinitiation, the IVIg group had fewer infective exacerbations (median 0 [0,1] vs 1.5 [1,3], $P = .01$, Mann-Whitney test; Figure 2) than the SCIg group.

This is the first study to measure a complete sputum Ig profile in an asthmatic population. Ig handling in health and disease is complex, as demonstrated by the limited correlations between serum and sputum Ig levels, and differences between asthmatics and

healthy controls. This could be influenced by abnormal lymphoid follicles and altered luminal transportation from the downregulation of polymeric Ig receptors or neonatal Fc receptors.⁸ Aside from elevated IgG₄, there were no absolute differences in sputum Igs between the recurrent RTI phenotype and healthy controls. When we included eosinophilic asthmatics in our analysis, we found that this subset of patients had the lowest sputum IgM and IgA, while levels were comparable between healthy controls and asthmatics with recurrent RTI. Furthermore, both groups of asthmatics demonstrated a relative deficiency of IgA (lower IgA/IgG). Taken together, this suggests that asthmatics have an abnormality of local humoral immunity and that despite a seemingly appropriate primary immune response to infection (as demonstrated by Ig levels being similar between healthy controls and recurrent RTI), an uncompensated relative deficiency of IgA may predispose to infection. It is not clear if this abnormality is intrinsic to asthma itself, or related to its treatment (eg, corticosteroids), though it could be related to an imbalanced cytokine milieu. Compared to healthy controls, asthmatics with recurrent RTI had elevated sputum B-cell attracting chemokine 1 (IgG class-switching), without differences in a proliferation-inducing ligand, B-cell activating factor, and IL-21 (IgA class-switching⁹). Finally, the administration of SCIg increased sputum IgG and its subtypes, while IVIg increased sputum IgA and total serum IgG, with the latter associated with fewer infective exacerbations than the former. The reason that IVIg could be more effective than SCIg in reducing infective exacerbations is not clear, but could potentially be related to a rise in sputum IgA, which would be particularly important at this mucosal surface. Alternatively, it could be related to immune modulatory effects related to the dosing or route of administration between IVIg and SCIg. Such effects were not specifically examined (as we did not expect IVIg to increase sputum IgA), but could include the induction of class-switching, upregulation of luminal transport (eg, polymeric Ig transporters), or chemokine-induced upregulation of IgA⁺ plasmablasts. With the mechanism of Ig replacement being unclear, we provide observational evidence that it may reduce infective exacerbations for patients where a recurrent RTI phenotype is established by sputum analysis.

In summary, an absolute airway Ig deficiency may not contribute to the recurrent RTI phenotype in severe asthmatics. However,

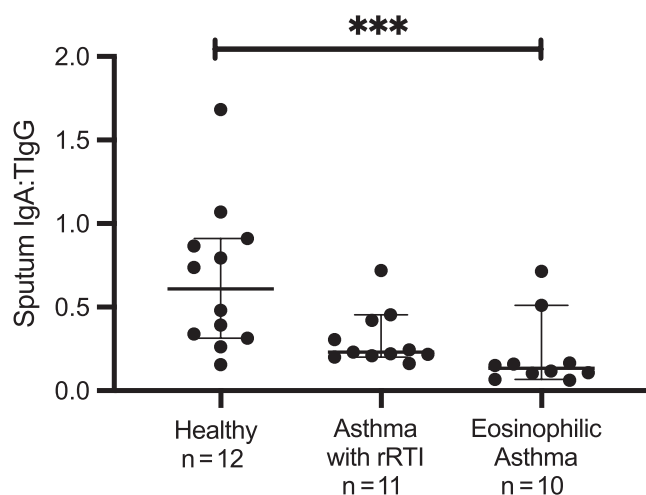


FIGURE 1 Difference in IgA/IgG ratio (median [IQR]) between asthmatics with recurrent respiratory tract infections, eosinophilic asthmatics, and healthy controls. Data are log-transformed. rRTI, recurrent respiratory tract infection. *** $P \leq .001$

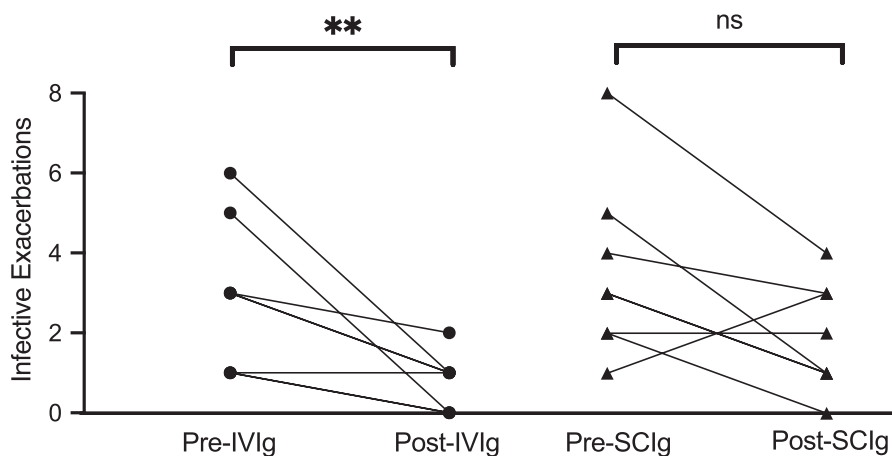


FIGURE 2 Frequency of infective exacerbations in the 12 mo prior to and after initiation of intravenous and subcutaneous immunoglobulin replacement in asthmatics with recurrent respiratory tract infection. Mann-Whitney test. IVIg, intravenous immunoglobulin, SCIg, subcutaneous immunoglobulin. ** $p \leq .01$

Ig replacement is associated with fewer infective exacerbations, which is potentially mediated by improvements in sputum Ig levels, including an unexpected increase in IgA after IVIg. A prospective clinical trial comparing SCLg vs IVIg in these patients is warranted.

KEYWORDS

asthma, airway infections, immunoglobulin, sputum cell counts

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CONFLICT OF INTEREST

Dr Ho, Dr El-Selahi, Dr Mukherjee, Ms Huang, Ms Radford, Ms Kjarsgaard, Ms Lavigne, and Ms Halabecki do not have anything to disclose. Dr Wasserman reports grants from CSL Behring during the conduct of the study; personal fees from CSL Behring, personal fees from Takeda outside the submitted work. Dr Nair reports grants and personal fees from AstraZeneca, grants from Boehringer Ingelheim, grants and personal fees from Novartis, grants and personal fees from Sanofi, grants and personal fees from Teva, personal fees from Theravance, and personal fees from Knopp outside the submitted work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.