

they had essentially normal levels of Ig and plasma cells. Since in the adult cohort^{1,2} monocytopenia could also be attributed to an acquired phenotype, the absence of monocytes as early as 16 months of life indicates a primary defect in our patient. This case might be a severe early-onset variant of the same disease or a distinct syndrome characterized by a complete absence of B cells, immunoglobulins, and plasma cells, similar to that described in another single adult patient.¹⁰ Interestingly, in our patient also, the presence of monocytes/macrophages in the BM and a few CD68⁺ cells in the lung and skin, as in patients recently described,^{1,2} suggests alternative mechanisms of differentiation either from local precursors or from circulating progenitors of different origin, as proposed by a revised hematopoietic model.⁸

The pattern of infections in this patient is different from that reported in adult patients¹ who showed predominant virus, fungi, and mycobacteria but few bacterial infections. This was somewhat surprising considering the critical role of monocytes against mycobacterial infections, but the young age could have contributed to this. The patient did not develop cytomegalovirus and Epstein-Barr virus infections; the latter was likely due to the absence of target B cells essential for latent and replicative Epstein-Barr virus infection. Given the deterioration of general conditions and worsening of immunodeficiency, at the age of 8 years the patient underwent HSCT from a 10/10 allele-matched unrelated donor following a reduced-intensity conditioning regimen consisting of thiotepa (8 mg/kg), fludarabine (160 mg/m²), treosulfan (42 g/m²), and ATG (30 mg/kg) (Fresenius SE & Co KGaA, Bad Homburg, Hesse, Germany). She received 10.7×10^6 CD34⁺ cells/kg and 10.7×10^8 nuclear cells/kg. Cyclosporine and mycophenolate mofetil were administered as graft-versus host disease (GVHD) prophylaxis. Neutrophil and platelet engraftment was obtained on day 14 and day 16 posttransplantation, respectively, and no serious adverse events occurred. Rapid emergence of CD14⁺, CD16⁺CD56⁺, and CD19⁺ cells in PB (Table I; Fig E1) and a normal distribution of all CD19⁺, CD14⁺, and CD16⁺ precursors in the BM were observed. Eighteen months after HSCT, the patient is in good general conditions with an improvement in growth (weight and length at the 75th and the 25th percentile, respectively) and in pulmonary radiological picture. Chimerism was full donor on monocytes and T, B, and NK cells, with normalization of proliferative responses and immunoglobulin levels. Dendritic cells developed normally (Fig 1, A).

In conclusion, we describe the first early-onset case of agammaglobulinemia with the complete absence of B, NK, monocyte, and dendritic cell precursors. The successful treatment by HSCT, leading to multilineage restoration, confirms our hypothesis that an intrinsic defect in hematopoietic precursors is the basis of this immunodeficiency. The severity of the prognosis in our child and the success of transplantation suggest a potential benefit of early HSCT in severely affected patients, even in the absence of myelodysplasia.

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Sputum inflammatory cells from patients with allergic rhinitis and asthma have decreased inflammasome gene expression

To the Editor:

There are numerous reports supporting the hypothesis that decreased exposure to microbes, as well as genetic factors that attenuate innate immune responsiveness, increase the risk of

TABLE I. Demographic data of subjects and sputum cell composition

Subjects		Physiology				Cell numbers (×1000)				
Type	No.	FEV ₁ (%)	Methacholine challenge PD ₂₀ (mg)	Medications	Allergies	PMN	Mac	Lym	Eos	BEC
Healthy volunteers	9	85-105	Negative	None	None	79 ± 33	150 ± 30	2.0 ± 0.5	1.3 ± 0.9	6.5 ± 2.9
Asthmatic patients	7	83-124	1.0-10.0	Albuterol	Cat, cockroach, dust, grass, mite, shellfish, tree, weed	160 ± 110	250 ± 90	4.9 ± 1.9	5.8 ± 5.6	14 ± 8
Patients with AR	9	100-120	Negative	None, OCP, aldactone	Cockroach, grass, mite, rodent, tree, weed	85 ± 28	350 ± 220	2.8 ± 1.6	2.0 ± 1.6	11 ± 5

BEC, Basal endothelial cells; Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; OCP, over-the-counter prescription; PMN, neutrophils.

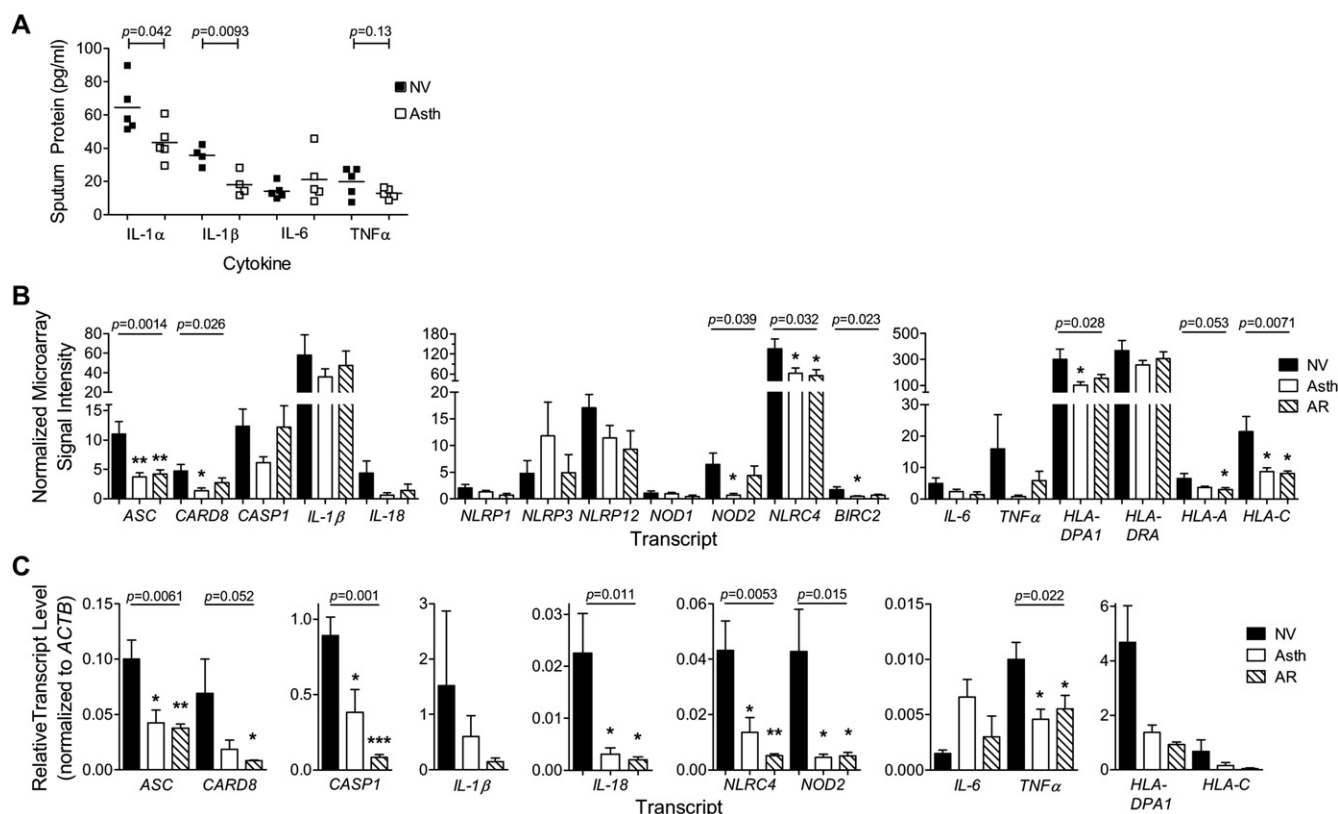


FIG 1. Assessment of innate immune factors in sputum airway cells. **A**, Sputum supernatant protein levels for healthy volunteers and asthmatic patients are analyzed by means of multiplex ELISA, with mean levels indicated by bars. **B** and **C**, Gene expression is assessed by means of oligonucleotide microarray hybridization (Fig 1, B) and PCR (Fig 1, C). Mean normalized microarray signal intensity ratios of experimental to universal reference RNA ± SEM in healthy volunteers, asthmatic patients, and patients with AR are plotted (n = 8-10 per group). Mean PCR-amplified transcripts relative to ACTB ± SEM are plotted (n = 5 per group). Statistical tests include unpaired t tests (Fig 1, A) and 1-way ANOVA (Fig 1, B and C) for 3 groups (with overall P values depicted over the line) with the Dunnett multiple comparison test for healthy volunteers (with P values indicated as follows: *P < .05, **P < .01, and ***P < .001). AR, Patients with AR; Asth, asthmatic patients; NV, healthy volunteers.

asthma and atopy. A growing body of evidence also suggests that adverse health outcomes caused by bacterial infection or microbial colonization in the airways might be increasing in asthmatic patients and other atopic persons. Numerous investigators have shown that asthmatic patients have increased risk of invasive pneumococcal disease.^{1,2} Additionally, persons with poorly controlled asthma have increased bacterial colonization of the airway, as indicated by either increased airway endotoxin levels³ or increased 16S rRNA load in bronchial samples.⁴ Asthmatic

patients also have increased lower respiratory tract symptoms, greater impairment of lung function and bronchial hyperreactivity, diminished circulating CD8⁺ lymphocyte numbers, and deficient IFN-γ and IL-10 responses after experimental rhinoviral infection, which is consistent with other studies that suggest that the severity of viral infection is increased and that viral mechanisms to evade immune response have evolved.⁵ Taken together, these observations suggest that increased disease severity in asthmatic patients might be associated with diminished innate

immunity and increased susceptibility to viral or bacterial airway infection.

Inflammasomes are newly appreciated innate immune regulatory complexes that process IL-1 β and IL-18 when activated by a number of pathogenic, environmental, or host-derived danger signals. We have made seminal observations demonstrating the important role of inflammasomes in mediating host defense against influenza and bacterial pathogens, as well as modulating responses to alum and contact dermatitis.⁶ The mounting evidence that persons with asthma might have impaired innate immune responses propelled us to hypothesize that inflammasome function might be reduced in asthmatic patients compared with that seen in healthy nonatopic volunteers.

To test this hypothesis, we examined sputum samples in our sample repository from healthy volunteers, asthmatic patients (5 with atopy), and volunteers with allergic rhinitis (AR; all with positive skin test responses) recruited by using protocols approved by the University of North Carolina Institutional Review Board, as previously described,⁷ to assess IL-1 β and inflammasome-regulated mechanisms that alter immune responsiveness. All volunteers had normal lung function, with allergy status confirmed by history and allergen skin test results and asthma status confirmed by clinical history and methacholine challenge (Table I). Sputum cell pellets contained predominantly macrophages and neutrophils, with less than 10% squamous epithelial cells (Table I). Although cell counts were not significantly different between the groups, trends of double neutrophils in asthmatic patients compared with those in the other 2 groups and double macrophages in patients with AR compared with healthy volunteers were evident. The sputum supernatants from asthmatic patients and healthy volunteers were assessed for protein levels of IL-1 α , IL-1 β , IL-6, and TNF- α by using the multiplex immunoassay (RayBiotech, Inc, Norcross, Ga). Asthmatic patients showed decreased levels of IL-1 α and IL-1 β , whereas no significant differences in IL-6 or TNF- α levels were observed between these groups (Fig 1, A).

mRNA from sputum cell pellets was assayed for gene expression of inflammasome-associated genes (*ASC*, *CARD8*, *CASP1*, *NLRP1*, *NLRP3*, and *NLR4*); noninflammasome nucleotide-binding leucine-rich repeat factors (*NLRP12*, *NOD1*, *NOD2*, and *BIRC2*); the cytokines *IL1B*, *IL18*, *IL6*, and *TNFA*; and HLA molecules. Two-color cDNA microarray hybridization was performed on labeled sputum RNA probes (EpiCentre, Madison, Wis) to assess gene expression, with signal intensity ratios normalized to housekeeping genes (β -actin and 60S ribosomal RNA). Second, total sputum cell RNA was transcribed to cDNA by using MMLV reverse transcriptase and amplified by means of quantitative PCR with gene-specific probe sets (Applied Biosystems, Foster City, Calif) to assess gene expression relative to the housekeeping gene β -actin.

Gene expression for the MHC molecules *HLA-DPA1*, *HLA-A*, and *HLA-C* and the inflammasome molecules *ASC*, *CARD8*, and *NLR4* was significantly decreased in asthmatic patients and patients with AR compared with that seen in healthy volunteers, with *CASP1* trending toward reduced levels in asthmatic patients compared with healthy volunteers (Fig 1, B). Additionally, *NOD2* and *BIRC2* expression was lower in asthmatic patients compared with that seen in healthy volunteers, whereas expression of *NLRP1*, *NLRP3*, *NLRP12*, and *NOD1* did not vary significantly between groups. Decreased gene expression for *IL1B*, *IL18*, and *TNFA* was evident in asthmatic patients and patients with AR.

In comparison, real-time PCR results showed significantly diminished expression of *ASC*, *CARD8*, *NLR4*, *CASP1*, and *TNFA* in asthmatic patients and patients with AR versus that seen in healthy volunteers, with trends of decreased *IL1B*, *HLA-DPA1*, and *HLA-C* expression (Fig 1, C). Although the results between microarray and PCR analyses mostly align, there are discrepancies that might be attributed to technical issues, such as dissimilar oligonucleotide targets between the microarray and PCR methods used for transcript detection, disparate efficiencies in measuring low-abundant or degraded transcripts, or both. Gene-specific PCR amplification might provide a more accurate representation of sputum cell expression because of sensitive detection.

Our results are consistent with the hypothesis that relative to healthy volunteers, asthmatic patients and patients with AR display reduced innate immune capability. Specifically, we found diminished airway cell expression of the inflammasome components *ASC*, *CARD8*, and *NLR4*, as well as *IL1B*, the key mediator released after inflammasome activation, in both the asthmatic and AR groups. However, we were unable to determine gene expression of specific cell types in sputum or which cell type might account for decreased gene expression in the asthmatic and AR groups. Eosinophils, which are more typical in sputum from atopic persons, have less expression of inflammasome genes than dendritic cells, neutrophils, or macrophages.⁸ Also, we were unable to differentiate M1 from M2 macrophages in this study. Macrophages can be polarized to the M2 phenotype by the T_H2 cytokines IL-4 and IL-13 and have decreased inflammasome activity with decreased IL-1 β secretion compared with M1 macrophages.⁹ Because macrophages were the most abundant cell in sputum from each group and M2 macrophages are more likely to be present in atopic persons, we hypothesize that M2 macrophages in the airways of asthmatic patients and patients with AR might account for the observed diminished sputum cell inflammasome gene expression. Finally, it is not known whether, on appropriate stimulation, inflammasome expression would increase in cells from asthmatic and atopic patients.

In summary, our pilot data support the hypothesis that the development of asthma and atopic diseases is associated with decreased inflammasome function. Future studies will focus on determining inflammasome gene expression and function in isolated sputum macrophages and granulocytes from asthmatic patients and atopic persons and the relationship between T_H2 cytokines, the expression and function of inflammasome complexes, and asthma disease severity.

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Correction

With regard to the July 2011 article entitled “Reductions in claudin-1 may enhance susceptibility to herpes simplex virus 1 infections in atopic dermatitis” (*J Allergy Clin Immunol* 2011;128:242-246.e5.), the authors wish to acknowledge the contributions of Peisong Gao, MD, PhD, Assistant Professor of Medicine from the Johns Hopkins Asthma and Allergy Center. The FLG genotype data used in this paper was originally generated by Dr Gao (Gao et al. *J Allergy Clin Immunol* 2009;124:507-13).