

Letter to the Editor

More alternative activation of macrophages in lungs of asthmatic patients

To the Editor:

The innate immune system is increasingly being associated with asthma induction and progression.^{1,2} Macrophages are an important part of the innate immune system, but their contribution to asthma pathogenesis is not clear.³ Recent articles have associated markers of the alternatively activated phenotype of macrophages (aaMΦs) with asthma.^{4,5} This alternatively activated phenotype develops after exposure to IL-4, IL-13, or both, and it is therefore not surprising that markers of alternative activation, such as AMCase, arginase, and mannose receptors, associate with asthma.^{4,5} Of interest, we recently showed that aaMΦs can contribute to the actual development of asthma as well in a murine model of asthma.⁶ In this model, mice with asthma had higher numbers of aaMΦs in lung tissue than control animals. Furthermore, increasing the number of aaMΦs in lung tissue by means of intratracheal instillation before induction of allergic inflammation amplified the development of airway inflammation even further. Kim et al² recently showed higher numbers of aaMΦs in the bronchoalveolar lavage fluid of a small number of patients with severe asthma compared with numbers seen in healthy control subjects. No reports exist of an actual increase in aaMΦ numbers in lung tissue of asthmatic patients.

To investigate whether patients with asthma have more aaMΦs in airway wall tissue, we recruited 16 asthmatic patients aged of 18 to 45 years (Table I). All had a history consistent with asthma, atopy (positive intracutaneous test results against house dust mite or 2 other aeroallergens), an FEV₁ of greater than 1.5 L and greater than 60% of predicted value, a methacholine PC₂₀ of 9.8 mg/mL or less, an AMP PC₂₀ of 80 mg/mL or less, and no use of oral corticosteroids within 2 months before the study.

Healthy volunteers (n = 9) had no history of lung disease, an FEV₁ of greater than 1.5 L and greater than 85% of predicted value, no atopy, and no airway hyperresponsiveness for methacholine or AMP.

Subjects who smoked during the past 2 years or with a respiratory tract infection during the past 4 weeks were excluded. All subjects provided written informed consent. The medical ethics committee of the University Medical Center Groningen approved this investigation.

Lung function was assessed and biopsy specimens were taken as described previously.⁷ Biopsy specimens were taken from the subcarinae of the right or left lower lobe and snap-frozen and stored at -80°C. Serial sections of 4 μm were cut from frozen biopsy specimens and stained for macrophages in general (anti-CD68 from DAKO, Glostrup, Denmark) and the alternatively activated subset (anti-stabilin-1 from Atlas, Stockholm Sweden, and anti-CD206 from Biolegend, Malden The Netherlands) by using standard immunohistochemical procedures. All antibodies were visualized with 3-amino-9-ethylcarbazole (Sigma, Zwijndrecht, The Netherlands), and positive cells were quantified by means of manual counting (magnification ×200) by a blinded observer (B. R.). Positive cells were counted in 10 randomly assigned representative areas, each consisting of 100 μm of intact basement membrane and extending 100 μm into the intact submucosa, excluding vessels and smooth muscle. Data were

TABLE I. Characteristics of participating subjects

Parameter	Healthy control subjects	Asthmatic patients
No. (male/female sex)	9 (3/6)	16 (10/6)
Age (y)	33 (20-44)	33 (19-44)
Eosinophils (× 10 ⁶ /mL peripheral blood)	0.09 (0.03-0.20)	0.28 (0.08-0.54)*
Total IgE (IU)	61 (2-308)	238 (49->2,000)*
FEV ₁ (% predicted)	110 (85-121)	94 (65-114)*
Reversibility (% predicted)	1.8 (-3.0 to 4.0)	12 (-1 to 36)*
PEF variation (%)	7.6 (4.4-13.4)	13.3 (4.9-36.8)†
PC ₂₀ MCh (mg/mL)	>9.8	0.27 (0.08-6.6)*
PC ₂₀ AMP (mg/mL)	>80	2.0 (0.18-19)*

MCh, Methacholine; PEF, peak expiratory flow.

Values are expressed as medians (minimums-maximums). The Student *t* test for unpaired data was used to compare these clinical variables between groups, including logarithmically transformed measurements of methacholine and AMP PC₂₀, peripheral blood eosinophil numbers, and total serum IgE levels. *P* values of less than .05 were considered statistically significant.

**P* < .01 versus healthy control subjects.

†*P* < .05 versus healthy control subjects.

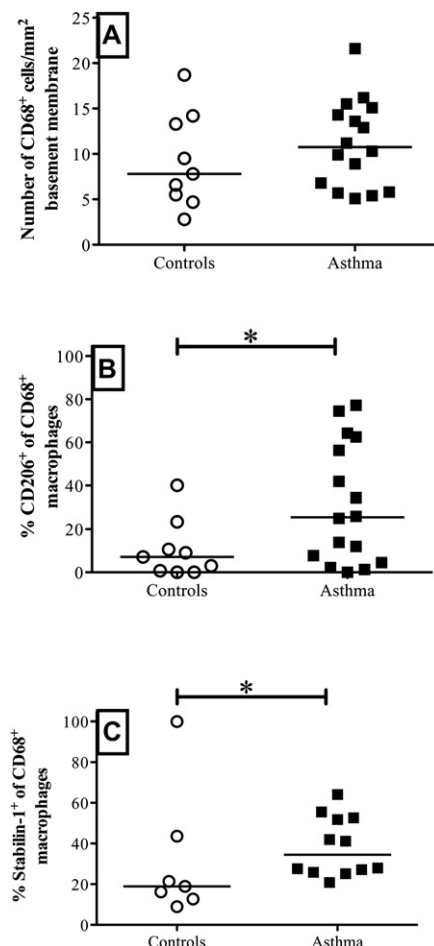


FIG 1. A, Asthmatic patients and healthy control subjects have similar numbers of macrophages (CD68⁺ cells) in airway wall tissue. B, Asthmatic patients have a significantly larger proportion of CD206⁺ macrophages (aaMΦs) in lung tissue than healthy control subjects. **P* < .05, Mann-Whitney *U* test. C, Asthmatic patients have a significantly larger proportion of stabilin-1⁺ macrophages (aaMΦs) in lung tissue than healthy control subjects. **P* < .05, Mann-Whitney *U* test.

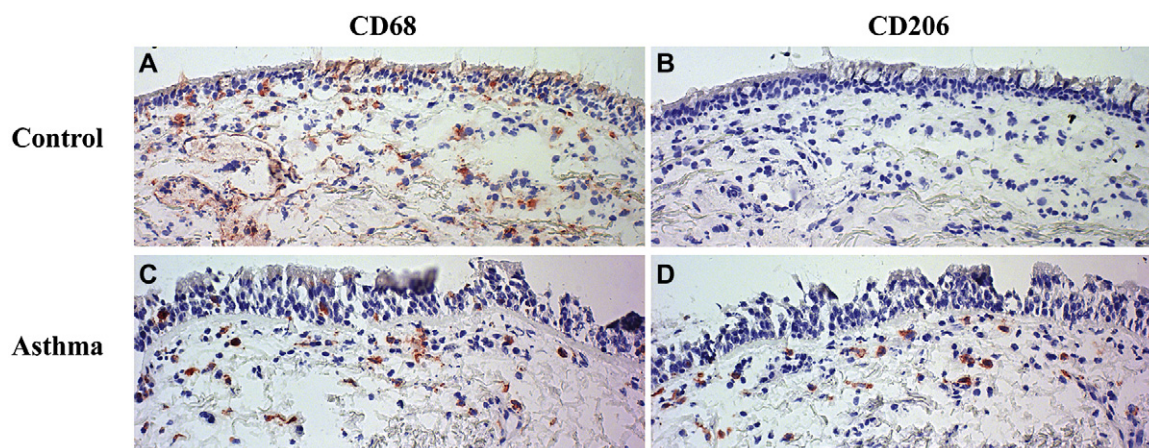


FIG 2. Representative photographs of serial bronchial biopsy sections from a healthy control subject (**A** and **B**) and an asthmatic patient (**C** and **D**) stained for CD68 (*left panels*) and CD206 (*right panels*). Red staining denotes positive cells (magnification $\times 200$).

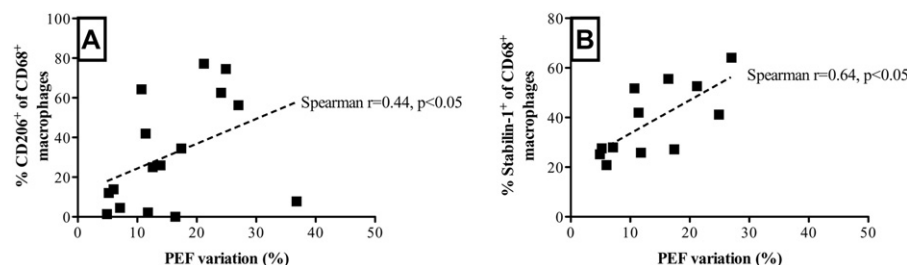


FIG 3. **A**, Asthma severity expressed as the amount of peak expiratory flow (PEF) variation correlates positively with the percentage of CD206⁺ cells present in the lung tissue of asthmatic patients. **B**, Asthma severity expressed as the amount of PEF variation correlates positively with the percentage of stabilin-1-positive cells present in the lung tissue of asthmatic patients. Correlation was analyzed by using the Spearman rank correlation test, and *P* values of less than .05 were considered statistically significant.

expressed as the number of positive cells per square millimeter of tissue ($0.1/\text{mm}^2$ of tissue). Cells positive for both CD68/CD206 or CD68/stabilin-1 on consecutive sections were expressed as the percentage of the total number of single CD68⁺ cells. Because of the limited amount of biopsy tissue available for staining and the loss of sections during the staining procedures, the stabilin-1 staining could not be quantified for all subjects (2 control subjects and 4 asthmatic patients are missing).

When we counted the number of macrophages in bronchial biopsy specimens, we did not find differences between asthmatic patients and healthy control subjects (Fig 1, A). However, a significantly higher percentage of macrophages with an alternatively activated phenotype (ie, either CD206 [macrophage mannose receptor] or stabilin-1 positive) was present in airway wall tissue of asthmatic patients than in healthy control subjects (for quantification of staining, see Fig 1, B and C, and for representative photographs of CD68 and CD206 staining, see Fig 2). Moreover, we also found that the percentage of aaMΦs correlated significantly with peak expiratory flow variation, a marker of asthma severity and airway inflammation, as reported by us in a previous study.⁷ We here show that peak expiratory flow variation positively correlates with the percentage of CD206⁺ cells (Fig 3, A) and the percentage of stabilin-1-positive cells (Fig 3, B).

These data confirm our findings in mice and Kim et al's findings² in bronchoalveolar lavage fluid showing that inflammation in the lungs of asthmatic patients is accompanied by increased numbers of aaMΦs in airway wall tissue.⁶ We used 2 markers of alternative activation, namely CD206 and stabilin-1, to identify aaMΦs, both of which are considered among the most specific markers for human aaMΦs to date.⁸ The percentage of alternatively activated macrophages is higher in asthmatic patients, and this percentage also increases with increasing asthma severity. Stabilin-1 showed a better correlation with asthma severity than CD206, likely because human lung dendritic cells express CD206 as well,⁹ whereas no such reports exist for stabilin-1. Because dendritic cells can also express low levels of CD68, we might have included some dendritic cells in our counts for CD206, thereby increasing the variability of our CD206 data.

Our data compellingly show that aaMΦs are abundantly present in airway wall tissue of human asthmatic patients. Their exact role needs further clarification. They might act as innocent bystanders because of the high IL-4 and IL-13 levels usually present in patients with allergic inflammation, but they could also play an active role in the induction and progression of allergic airway inflammation. From our data in mice in which we showed that they can aggravate allergic inflammation,⁶ we postulate that

aaMΦs might contribute to allergic inflammation, but unfortunately nothing is known for human aaMΦs. Although human and murine aaMΦs do not share the same markers, they do appear to be functionally similar.⁸ They probably evolved as a defense mechanism against parasites that are too big to be killed by phagocytosis. To do this, they upregulate receptors (eg, mannose and scavenger receptors) that can sample antigens from parasites to be presented further to induce damaging immune responses. These uptake receptors, however, might also be responsible for the uptake and presentation of normally innocuous antigens and turning them into genuine allergens. Whether aaMΦs themselves can subsequently function as antigen-presenting cells inducing allergen-specific T_H2 responses or whether they need the help of dendritic cells for this remain questions for further research. These types of studies are needed to elucidate the behavior of this fascinating cell type in (human) asthma and might lead us to new therapeutic opportunities.

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REFERENCES

- Holt PG, Strickland DH. Interactions between innate and adaptive immunity in asthma pathogenesis: new perspectives from studies on acute exacerbations. *J Allergy Clin Immunol* 2010;125:963-74.
- Kim EY, Battaile JT, Patel AC, You Y, Agapov E, Grayson MH, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nat Med* 2008;14:633-40.
- Peters-Golden M. The alveolar macrophage: the forgotten cell in asthma. *Am J Respir Cell Mol Biol* 2004;31:3-7.
- Vercelli D. Arginase: marker, effector, or candidate gene for asthma? *J Clin Invest* 2003;111:1815-7.
- Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, et al. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 2004;304:1678-82.
- Melgert BN, Oriss TB, Qi ZB, Dixon-McCarthy B, Geerlings M, Hylkema MN, et al. Macrophages: regulators of sex differences in asthma? *Am J Respir Cell Mol Biol* 2010;42:595-603.
- ten Hacken NH, Timens W, Smith M, Drok G, Kraan J, Postma DS. Increased peak expiratory flow variation in asthma: severe persistent increase but not nocturnal worsening of airway inflammation. *Eur Respir J* 1998;12:546-50.
- Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol* 2009;27:451-83.
- Cochand L, Isler P, Songeon F, Nicod LP. Human lung dendritic cells have an immature phenotype with efficient mannose receptors. *Am J Respir Cell Mol Biol* 1999;21:547-54.

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