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Asthma is characterized by more IRF5+ M1 and CD206+ M2 macrophages and less IL10+ M2-like macrophages around airways compared to healthy airways

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Capsule summary (35 words)

In asthma, macrophage polarization is altered from a state associated with anti-inflammatory responses to a state associated with inflammation as compared to control. Macrophage polarization states is linked to disease severity, sex and ICS treatment.

Keywords

asthma; macrophages; mannose receptor; interferon regulatory factor 5; interleukin 10; polarization

To the Editor:

A number of recent reports have shown that lung macrophages are important in maintaining lung homeostasis, yet they can also significantly contribute to development of asthma^{1,2}. This contradiction in functions can be explained by their plasticity³. Three main polarization states of macrophages are recognized; two types associated with inflammatory responses, called M1 and M2, and one with anti-inflammatory responses, called M2-like. M1 macrophages are associated with Th1 inflammation and are characterized by interferon regulatory factor 5 (IRF5) expression. M2 macrophages are associated with Th2 inflammation and are characterized by increased expression of CD206 (mannose receptor). M2-like macrophages are associated with downregulation of inflammation and characterized by production of high levels of interleukin (IL) 10⁴.

As CD206+ M2 macrophages are induced by the archetypical asthma cytokines IL4/IL13, their numbers and function have been studied more extensively in asthma than IRF5+ M1 and IL10+ M2-like macrophages. Previously, we reported higher numbers of CD206+ M2 in lungs of asthmatics compared to healthy controls, though in relatively small sample size cohort. Moreover, we showed that higher CD206+ M2 numbers are associated with more severe asthma⁵ and that these CD206+ M2 contribute to asthma development in mouse models of allergic lung inflammation¹. No reports exist of the numerical presence of IRF5+ M1 or IL10+ M2-like in human asthma.

To investigate different macrophage polarization states in asthma patients versus healthy controls and how they relate to lung function, sex and inhaled corticosteroid (ICS) use, we used bronchial biopsies from 138 well-characterized asthma patients and 50 matched healthy controls and stained these for total macrophages using CD68 and doublestained them for the three polarization states using IRF5, CD206+, or IL10+ (for details see Material and Methods in the Online Repository). Lung function was assessed and biopsy specimens were taken as described previously⁵. The experimental protocols were approved by the Medical Ethical Committee of the UMCG (METc 2007/007,

clinicaltrials.gov NCT00848406) and all subjects gave written informed consent. **Table I** summarizes the clinical characteristics of all subjects.

We found no differences in the total number of macrophages between healthy males and females and asthmatic males and females (Figure 1A). Asthma patients had higher numbers of IRF5+ M1 and CD206+ M2 in bronchial biopsies than healthy individuals with males having more IRF5+ M1 and females more CD206+ M2 (Figure 1B and 1C, see Figure S1 in the Online Repository for representative photos of the stainings). The reason for this sex difference is unknown, but may relate to different levels of sex hormones males and females are exposed to. Sexually disparate polarization of macrophages has also been shown in a model of myocarditis⁶. In the latter study macrophage polarization was influenced by sex hormone-driven IFN γ or IL4 production by NK cells: testosterone was found to favor IFN γ -production by NK cells, whereas estrogen favored IL4 production.

These data confirm our findings on CD206+ M2 macrophages in asthma and show, in a bigger cohort, that macrophages are differentially polarized in asthma. Although asthma is dominated by the presence of IL4 and IL13 that can induce CD206+ M2, previous indirect evidence suggested that IRF5+ M1 may also be present in asthma⁷. We have now provided direct evidence that IRF5+ M1 are indeed also higher in asthma. Murine studies suggest that IRF5+ M1 can aggravate established asthma⁸ and this is supported by our results since higher numbers of IRF5+ M1 in airway wall biopsies were associated with slightly lower FEV₁/FVC values (Figure 1E).

Since male asthmatics had a significantly lower lung function than female asthmatics (table 1), the above reported sex differences in IRF5+ M1 and CD206+ M2 may be influenced by the difference in lung function. Therefore, a distinction was made between male and female asthmatics with lower lung function (FEV₁ <85% predicted) and with better lung function (FEV₁ > 85% predicted). Asthmatic males with both FEV₁ <85% and FEV₁ > 85% predicted had higher numbers of IRF5+ M1 than similar asthmatic females (Figure 1G and 1H), whereas females with both FEV₁ <85% and FEV₁ > 85%

predicted had higher numbers of CD206+ M2 than males (Figure 1I and 1J). Therefore it is unlikely that the differences found between males and females were caused by differences in lung function.

Numbers of IL10+ M2-like were not significantly different between sexes, but both male and female asthmatics had lower numbers of IL10+ M2-like than healthy individuals (Figure 1D). This finding could explain previous *in vitro* data that macrophages from lavage fluid of asthmatics produce less IL10 than macrophages from healthy subjects⁹. Some studies have also reported higher levels of IL10 in asthmatics as compared to healthy subjects. An explanation for these different findings could be the use of ICS by the majority of the asthmatics in these latter studies, which may have induced IL10 production by macrophages⁹. This is in line with our results showing that ICS treatment was accompanied by higher numbers of IL10+ M2-like (Figure 1N). In addition, we found that a higher number of IL10+ M2-like was associated with a slightly better lung function (Figure 1F). This is of interest because these macrophages may therefore be beneficial in reducing asthma symptoms. In accordance, we found lower numbers of IRF5+ M1 in asthmatics who used ICS than in non-users (Figure 1L), suggesting that these macrophages are susceptible to treatment. CD206+ M2 did not appear to be susceptible to ICS treatment (Figure 1M). However, IL10+ M2-like also express CD206 and after subtraction of the IL10+ subset, we also found lower numbers of CD206+ macrophages in ICS-users suggesting that this polarization state may also be susceptible to ICS inhibition (data not shown). This highlights a limitation of subdividing macrophages into three polarization states while *in vivo* they appear as a continuous spectrum rather than discrete subsets³. The total number of CD68+ macrophages is lower than the sum of the three polarizations states, indicating that there is indeed overlap in markers. We also checked if the ratio of CD206+ M2 to IRF5+ M1 macrophages was in any way correlated to lung function parameters since both these macrophages are found to be higher in asthma, but no significant correlations were found (data not shown).

In conclusion, human asthma is characterized by higher numbers of M1 and M2 and lower numbers of M2-like macrophages. Strategies to boost numbers of IL10+ M2-like may be a good avenue to

112 explore for novel asthma treatments^{11, 12 of Online Repository}. For a number of asthmatics this can be done
113 through ICS treatment, but for those patients not responding to ICS, novel macrophage-polarizing
114 therapies may be an interesting option, as these patients are generally difficult to treat.

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References

1. Melgert BN, Oriss TB, Qi Z, 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.
2. Bourdonnay E, Zasłona Z, Penke LRK, Speth JM, Schneider DJ, Przybranowski S, et al. Transcellular delivery of vesicular SOCS proteins from macrophages to epithelial cells blunts inflammatory signaling. *J Exp Med* 2015;212:729–42.
3. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdts S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 2014;41:14–20.
4. Draijer C, Robbe P, Boorsma CE, Hylkema MN, Melgert BN. Characterization of macrophage phenotypes in three murine models of house-dust-mite-induced asthma. *Mediators Inflamm* 2013;2013:632049.
5. Melgert BN, ten Hacken NH, Rutgers B, Timens W, Postma DS, Hylkema MN. More alternative activation of macrophages in lungs of asthmatic patients. *J Allergy Clin Immunol* 2011;127:831–3.
6. Liu L, Yue Y, Xiong S. NK-derived IFN- γ /IL-4 triggers the sexually disparate polarization of macrophages in CVB3-induced myocarditis. *J Mol Cell Cardiol* 2014;76:15–25.
7. Kim Y-K, Oh S-Y, Jeon SG, Park H-W, Lee S-Y, Chun E-Y, et al. Airway exposure levels of lipopolysaccharide determine type 1 versus type 2 experimental asthma. *J Immunol* 2007;178:5375–82.
8. Meyts I, Hellings PW, Hens G, Vanaudenaerde BM, Verbinnen B, Heremans H, et al. IL-12 contributes to allergen-induced airway inflammation in experimental asthma. *J Immunol* 2006;177:6460–70.
9. John M, Lim S, Seybold J, Jose P, Robichaud A, O'Connor B, et al. Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1 α , granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. *Am J Respir Crit Care Med* 1998;157:256–62.

Table I. Patient characteristics (medians+ranges). Most patients showed mild to moderate asthma symptoms.

	Total asthma (n=138)	Healthy (n=50)	Statistical difference	Female asthmatics	Male asthmatics	Statistical difference
Sex, males/females	70/68	29/21	n.s.	0/68	70/0	n.s.
Age, years	49 (17-71)	51 (19-73)	n.s.	45 (17-71)	49 (19-68)	n.s.
Body mass index, kg/m ²	26.7 (19-44.2)	23.4 (19-35.8)	n.s.	26.5 (20-44.2)	26.0 (19-35)	n.s.
Atopy, n (%) (positive skin prick test)	95 (69)	18 (37)	p=0.0001	45 (67)	52 (75)	n.s.
Current smoking, n (%)	30 (22)	17 (34)	n.s.	11 (16)	19 (27)	n.s.
ICS use, yes/no	57/81	0/50	p=0.0001	30/38	27/43	n.s.
β ₂ agonist use, yes/no	71/67	n.d.	-	34/34	36/34	n.s.
IgE	78 (1-1668)	n.d.	-	64 (4-1668)	105 (1-1662)	n.s.
PC ₂₀ AMP, mg/mL	198 (0-640)	640 (66.7-640)	p=0.0001	640 (0-640)	151 (0.02-640)	n.s.
FEV ₁ , % predicted	93 (34-134)	111 (88-139)	p=0.0001	99 (53-134)	88 (34-126)	p=0.0001
FEV ₁ /VC, %	72 (40-96)	78 (68-93)	p=0.0001	75 (53-96)	69 (40-89)	p=0.0001
MEF ₅₀ , % predicted	73 (17-152)	88 (57-136)	p=0.0011	81 (31-152)	65 (17-147)	p=0.0074
Blood eosinophils, x10 ⁹ /L	0.17 (0.02-0.97)	0.13 (0.02-0.36)	p=0.0386	0.15 (0.02-0.90)	0.19 (0.04-0.97)	n.s.
Blood neutrophils, x10 ⁹ /L	3.3 (1.4-66.7)	2.8 (1.3-7.6)	p=0.0058	3.3 (1.4-66.7)	3.4 (1.4-6.08)	n.s.
Lung eosinophils, numbers per 0.1 mm ² tissue area	1.8 (0-40)	n.d.	-	1.4 (0-32)	1.9 (0-40)	n.s.
Lung neutrophils, numbers per 0.1 mm ² tissue area	6.2 (0-46)	n.d.	-	6.3 (0-29)	5.5 (0-46)	n.s.
Lung CD3+ T cells, numbers per 0.1 mm ² tissue area	67.6 (4.2-294)	n.d.	-	76.3 (23-216)	58.8 (4.2-294)	n.s.
NO alveolar, ppb	5.3 (0.88-51.7)	3.7 (-0.28-67.8)	p=0.0006	4.8 (0.88-18.34)	5.6 (1.93-51.7)	p=0.045
NO bronchial, nl/s	0.7 (0.08-10.4)	0.6 (-2.67-3.6)	n.s.	0.7 (0.09-4.12)	0.7 (0.08-10.4)	n.s.
BM thickness, μm	5.9 (2.8-12.6)	3.1 (1.6-7.0)	p=0.0001	5.4 (3.1-9.6)	6.3 (2.8-12.6)	p=0.0168
Goblet cells, number per 1 mm BM	36 (0-220)	n.d.	-	36 (0-119)	36 (1.8-220)	n.s.
Collagen III deposition area, %	28 (3-66)	n.d.	-	28 (5.8-59)	28 (3-66)	n.s.

Definition of abbreviations: ICS = inhaled corticosteroid; PC₂₀AMP = provocative concentrations of adenosine

5'-monophosphate causing a 20% fall in FEV₁ ; MEF₅₀ = maximum expiratory flow rate at 50% of vital capacity;

BM= basement membrane; n.s.= not significant; n.a.= not applicable; n.d.= no data.

Figure 1. Aligned dot plots with line shown at median of macrophages in bronchial biopsies of healthy and asthmatic males and females. Number of (A) all CD68+ cells, (B) IRF5+CD68+, (C) CD206+CD68+, and (D) IL10+CD68+ cells. Linear regression of (E) IRF5+CD68+ cells and FEV₁/FVC ratio, and (F) IL10+CD68+ cells and FEV₁/FVC ratio in asthma patients. Number of IRF5+CD68+ cells and CD206+CD68+ cells in male and female asthma patients with a lower FEV₁ than 85 %predicted (G and I) and with a higher FEV₁ than 85 %predicted (H and J). Number of all CD68+ cells (K), IRF5+CD68+ (L), CD206+CD68+ (M), and IL10+CD68+ cells (N) in healthy subjects and asthma patients using ICS and not using ICS. ICS = inhaled corticosteroid.



