European Journal of Immunology

Letter to the Editor

[DOI: 10.1002/eji.202149222]

Phenotypic comparison of human alveolar macrophages before and after in vivo rhinovirus 16 challenge

In the lung, alveolar macrophages serve as the first line of cellular defense against inhaled particles and respiratory pathogens. As any macrophage, alveolar macrophages are remarkably plastic cells and known to adapt their phenotype, driven by signals from the microenvironment. This ensures correct responses necessary for the elimination of threats, damage repair, and restoration of homeostasis. It has been demonstrated that macrophage polarization and function are aberrant in lung disease, including asthma and chronic obstructive pulmonary disease (COPD) [1–6]. This may lead to impaired responses towards infections, resulting in acute worsening of the disease (i.e., exacerbations). Indeed, whereas most respiratory virus infections only provoke a common cold in healthy individuals, they account for the majority of exacerbations in asthma and COPD [7].

Bronchoalveolar lavage (BAL) is commonly applied to retrieve samples from the lower airways. BAL samples mainly comprise alveolar macrophages but also lymphocytes, neutrophils, and eosinophils, which can be further affected by underlying disease. Immunophenotyping of BAL cells is classically performed using flow cytometry, but autofluorescence of (alveolar) macrophages can cause significant problems in data analysis. As such, only little is known about macrophage heterogeneity, especially in the context of inflammatory lung disease, while detailed profiling of cellular responses is crucial for understanding their functional roles.

In the current study, we used two mass cytometry (CyTOF) antibody panels, covering 64 unique surface and intracellular markers (Table S1), for single-cell characterization of human alveolar macrophages in response to an *in vivo* experimental virus infection. The gating strategy for CyTOF analysis is shown in Figure S1. BAL samples were obtained before and two days after rhinovirus 16 (RV16) challenge in two explorative clinical studies; the RILCA study, including patients with mild to moderate asthma and age-matched healthy controls, and the RILCO study, including patients with COPD GOLD stage I-II and age-matched healthy controls. Further details can be found in the Supporting Information.

Baseline characteristics of the diverse group of subjects included in this analysis are presented in Table S2. Initial differential cell counts revealed alveolar macrophages as the predominant cell type in BAL fluid, with no differences before and after RV16 challenge (Table S3). Overlaid t-distributed stochastic neighbor embedding (t-SNE) dot plots of all BAL samples are shown in Figure 1A, with similar clustering before and after the virus. Indeed, the major population was HLA-DR$^+$ and considered alveolar macrophages. This population was further characterized by the expression of other key macrophage markers, including CD206, CD68, CD169, and CD64. Strikingly, we also observed the presence of distinct HLA-DR$^+$CD16$^+$ and HLA-DR$^+$CD16$^-$ subsets, which appeared not to be related to health, disease, or virus infection (not shown). This potentially interesting dichotomy based on the expression of CD16 (FcγRIII) deserves closer investigation in future studies to unravel its potential clinical relevance.

Multivariate partial least squares-discriminant analysis (PLS-DA) using the mean signal intensity of all markers showed separate clustering of alveolar macrophages collected before *versus* after RV16 challenge, with major determinants being CD61, CD116, TIM4, CD206, and TGFβ (Fig. 1B and Figure S2). From the top 15 expressed markers, only CD206 was significantly upregulated after the virus challenge (Fig. 2). Other significantly enhanced markers post-challenge were MRP14 (87.76 ± 12.78 *versus* 166.15 ± 36.59; $P = 0.045$) and TGFβ (2.72 ± 0.57 *versus* 3.49 ± 0.76; $P = 0.005$), whereas the expression levels of CD56 decreased (1.93 ± 0.39 *versus* 1.24 ± 0.15; $P = 0.025$). Virus infection did not affect the mean expression levels of any of the other markers. In addition, the biological relevance of CD56 and TGF-β is questioned due to low expression levels. Of note, PLS-DA also revealed that alveolar macrophages from asthma, COPD, and healthy subjects can be clustered separately, mainly based on the contribution of CD45, ABCA7, CD33, CD64, and CD206 (Figure S3).

Together, although consequences for individual markers seem limited, our

Correspondence: René Lutter
e-mail: r.lutter@amsterdamumc.nl

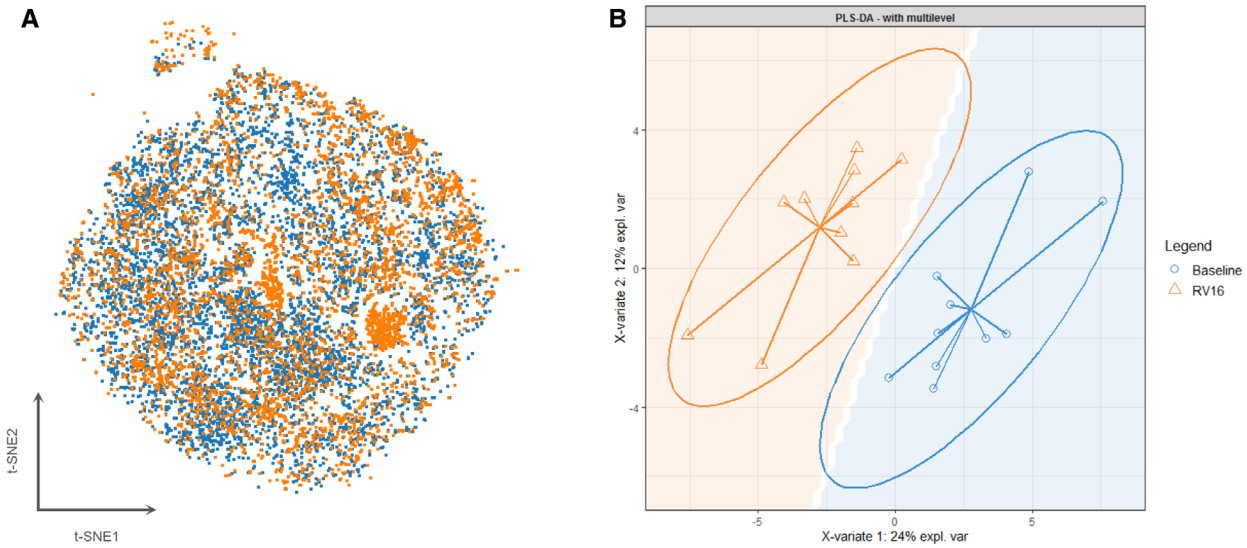

Figure 1. Comparative phenotypic analysis of BAL samples before and after *in vivo* experimental RV16 infection in a heterogeneous group of healthy and asthma/COPD subjects. Data originate from a barcoded and pooled sample, consisting of 10 baseline and 10 RV16 BAL samples, that was split and stained with two antibody panels and acquired on a CyTOF instrument. (A) Overlaid t-SNE projection of multidimensional single-cell phenotypes of BAL samples at baseline (blue) and after challenge with RV16 (orange). All cells are included in the plots and all markers (Table S1 panel B) except CD16 are used for embedding. (B) PLS-DA using the mean signal intensity of all markers (Table S1 panel A and B) for HLA-DR$^+$ alveolar macrophages collected at baseline (blue) and after challenge with RV16 (orange).

current data suggest a modest impact of RV16 on the overall phenotype of human alveolar macrophages already 2 days post-challenge. Besides, the significant effect of RV16 on CD206 expression within this heterogeneous group of subjects is intriguing as it indicates a crucial factor upon virus infection independent of disease. CD206, the mannose receptor, has been recognized as a marker for alternatively activated macrophages with anti-inflammatory and tissue repair functions [8], though classically activating stimuli like IFNγ can further enhance its expression [9]. CD206 was also among the most discriminative or predictive features in our multivariate analysis and has previously been reported to be increased in asthma and COPD [1, 3–5]. Whether the expression of this receptor indeed drives altered responses in the airways remains to be elucidated.

This explorative study is unique in using CyTOF for the phenotyping of BAL macrophages before and after experimental RV16 infection in healthy controls and patients with asthma and COPD. However, we do need to address its shortcomings. The number of subjects included is small with no power to actually detect RV16 responses related to disease, leaving the influence of asthma and COPD to be determined. On the other hand, combining all subjects may have biased the results and hence these findings should be interpreted with caution. Moreover, sampling 2 days post-challenge corresponds with the early phase of the immune response, which is also illustrated at the clinical level, as subjects did not yet experience increased symptoms nor a deteriorating lung function (forced expiratory volume in 1 second [FEV_1] percent predicted 96.2 ± 13.1 *versus* 95.4 ± 13.6; $P = 0.57$ and post-bronchodilator FEV_1 percent predicted 103.7 ± 11.0 *versus* 103.4 ± 11.6; $P = 0.86$). Yet, patients did report increased asthma or COPD symptoms at later time points in the study (peak symptoms were detected 4–5 days after infection), in line with virus-induced loss of disease control (not shown).

In conclusion, CyTOF enables detailed characterization of alveolar macrophages at the single-cell level and our findings indicate that two days after *in vivo* RV16 challenge these cells have already responded by changing their overall phenotype. Although there appear to be differences between alveolar macrophages from healthy controls *versus* patients as well, it is not clear whether these translate to functional differences in the respiratory tract. Future research should explore the effect of virus infections on alveolar macrophage polarization at other time points and in lung disease in general.

Linsey E. S. de Groot[1,2], Paul Brinkman[#1], Yanaika S. Sabogal Piñeros[#1,2], Josef Priller[3], Jörg Hamann[#2], Chotima Böttcher[#3] and René Lutter[#1,2]

[1] Department of Respiratory Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
[2] Department of Experimental Immunology (Amsterdam Infection & Immunity Institute), Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
[3] Department of Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité – Universitätsmedizin Berlin, Berlin, Germany

[#]These authors share second or last authorship

Acknowledgments: The authors would like to thank Peter I. Bonta and Christof J. Majoor for performing the bronchoscopies and Barbara S. Dierdorp and Tamara Dekker for technical support. This work was supported by the Lung Foundation Netherlands (consortium grant 4.1.15.002), the German Research Foundation (SFB TRR167, B05 & B07; J. P. and C. B.), and in part by MedImmune (CW417827).

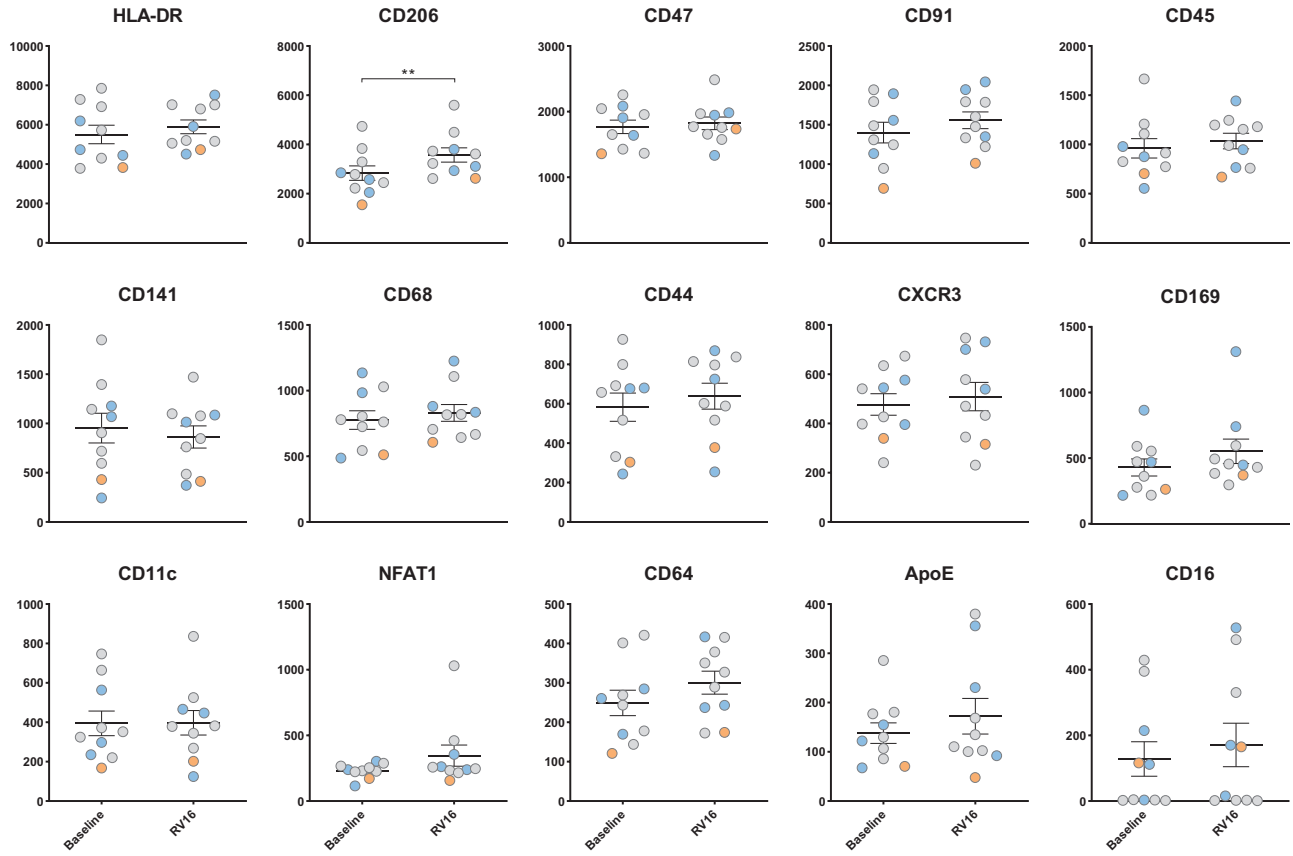

Figure 2. Mean signal intensity of the top 15 expressed markers (Table S1 panel A and B) as determined by CyTOF analysis of HLA-DR$^+$ alveolar macrophages at baseline and after *in vivo* infection with RV16 (both n = 10). Dots represent patient individuals (blue = asthma; orange = COPD; gray = healthy); bars and whiskers represent mean ± SEM. $^{**}P < 0.01$ paired *t* test.

Conflict of interest: R. L. received financial support from MedImmune, GSK, Chiesi, Foresee, and NutriLeads, none of which has influenced any statement in the manuscript. All other authors have no commercial or financial conflict of interest.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202149222.

References

1 **Bazzan, E. et al.**, *Respir Res.* 2017. **18**: 40.

2 **Bewley, M. A. et al.**, *Am. J. Respir. Crit. Care Med.* 2017. **196**: 845–855.

3 **Draijer, C. et al.**, *J. Allergy Clin. Immunol.* 2017. **140**: 280–283.E3.

4 **Girodet, P. O., et al.**, *Am. J. Respir. Cell Mol. Biol.* 2016. **55**: 467–475.

5 **Melgert, B. N., et al.**, *J. Allergy Clin. Immunol.* 2011. **127**: 831–833.

6 **Taylor, A. E. et al.**, *Eur. Respir. J.* 2010. **35**: 1039–1047.

7 **Mallia, P.** and **Johnston, S. L.** *Chest* 2006. **130**: 1203–1210.

8 **Martinez, F. O. et al.**, *Blood* 2013. **121**: e57–e69.

9 **van de Garde, M. D. et al.**, *J. Immunol.* 2014. **192**: 1196–1208.

Abbreviations: **BAL**: bronchoalveolar lavage fluid · **COPD**: chronic obstructive pulmonary disease · **FEV$_1$**: forced expiratory volume in 1 second · **PLS-DA**: partial least squares-discriminant analysis · **RV16**: rhinovirus 16 · **t-SNE**: t-distributed stochastic neighbor embedding

Keywords: asthma • COPD • innate immunity • macrophages • rhinovirus

Full correspondence: : René Lutter, Departments of Respiratory Medicine and Experimental Immunology, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
e-mail: r.lutter@amsterdamumc.nl

Received: 15/2/2021
Revised: 22/6/2021
Accepted: 6/9/2021
Accepted article online: 7/9/2021

Additional supporting information may be found online in the Supporting Information section at the end of the article.