

RESEARCH LETTER

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Immune cell profiling reveals natural killer and T cell subpopulations to be associated with atopic dermatitis severity

To the Editor,

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by a genetically determined impaired epidermal barrier function and predisposition to develop other atopic comorbidities.¹ The role of adaptive and innate immunity, in particular IL-4 and IL-13 and T lymphocyte populations in AD pathogenesis are established, but the exact cellular mechanisms are not fully understood. At least partially, this is attributed to technical limitations of flow cytometry, that is, limited channels and signal spillover. Mass cytometry overcomes these limitations and allows advanced bioinformatic methods. An unbiased comprehensive analysis of the total peripheral immune cell pool in AD is not available.

Peripheral leucocytes were analysed from 60 well-defined adults (18–55 years) comparable regarding sex and age and recruited at the Charité – Universitätsmedizin Berlin within 5 months. Written informed consent was obtained from all participants. All procedures were approved by the local ethics committee (EA1/080/16) following the Declaration of Helsinki. The AD group was defined by international criteria,² including age-related eczema course, elevated serum IgE (>100 kU/L) and aeroallergen sensitization, absence of systemic treatment. The clinical severity determined by SCORing Atopic Dermatitis (SCORAD) was applied to stratify into two subgroups: moderate (SCORAD 15–40, ADm, $n = 20$, mean 28.8 ± 7.0) and severe: >40, ADs, $n = 20$, mean 56.7 ± 12.5 , intergroup T -test $p < .001$). The reference group with healthy donors (HD) presented without history for atopy (self and family), chronic diseases and the absence of medication intake. The groups were comparable between age (Chi-square Pearson Test $p = .153$) and sex (ANOVA $p = .546$).

The analysis includes 39 surface markers considering all immune cell lineages in the blood. An unbiased, automated cell clustering algorithm (ImmunoClust) identified frequencies of 70 cell clusters and 103 linked populations, of which 44 differed significantly between the groups to be changed in AD samples when compared to healthy donors (HD). In ADs 49 populations and in ADm 20 populations were found ($p < .01$). Most of them can be attributed to the CD4+, CD8+, eosinophil or NK lineage. A principal component analysis (PCA) using frequencies of AD-related immune cell clusters and the SCORAD values revealed a clear separation of HD and ADm from ADs samples (Figure 1A), while HD and ADm samples were overlapping. The candidate populations identified by the unsupervised analysis were

confirmed by manual gating. The frequencies of eosinophils in both AD groups were higher compared to controls with increased frequencies of CD45RO⁺ eosinophils in ADs (Figure 1B), which were described as being less apoptosis-prone compared to other CD45 isoforms.³ The role of eosinophils is currently investigated applying anti-IL-5 receptor alpha antibodies (NCT03563066, NCT04605094). No change in frequencies of Th2 cells (CD3+CD4+CD45RO+CRTh2+) were observed in the ADs and ADm group respectively ($p = .25$ –.66, Figure 1C). Type 2 innate lymphoid cells (ILC2) were present at very low frequencies in the blood limiting a precise quantification, but were rather reduced in AD ($p = .002$, Figure 1C). Our data suggest that Th2 and ILC2 cells exert their functions in the inflamed skin, and are only rarely detectable in the periphery.⁴ Unexpectedly, reduced frequencies of total CD56+ NK cells were identified in AD samples by the unbiased analysis and confirmed by manual gating. These results support an increased NK cell apoptosis in the blood or an increased extravasation in the inflamed skin.⁵ The frequency of a CD38+CD69+ NK cell subset, resembling most probably activated NK cells after tissue egress, was increased in ADm, an effect much more pronounced in AD (Figure 1D) and indicating NK cell activation in the AD skin,⁶ for example, by driving Th2-cytokines in keratinocytes.⁴ Besides Th2 also increased frequencies of activated T helper effector memory cells (CD3+CD4+CD8–CD45RO+CD45RA–CCR7–CD127–CD27+HLADR) and activated Treg's (CD3+CD4+CD25hi CD127–CD38+HLADR) were identified in all AD samples, which could be confirmed by manual gating (Figure 1E), with additional subtle differences on other CD4+ and CD8+ T cell subpopulations. These data underline the important role of T cells in AD, which was also highlighted in a recent study using mass cytometry analysis on CD4+ T helper cells in AD.⁷ Of note, no significant association to ADs or ADm compared to HD were observed in naïve, memory, CD27+/IgD+ B cells or plasmablasts as such or considering activation marker coexpression, for example, CD38 or CD69, myeloid cells, including neutrophils, basophils, CD14+ monocytes stratified into classical and non-classical cells considering CD11c or activation marker coexpression (data not shown).

Next, we aimed to correlate the most prominent cell subpopulations identified in this study, including eosinophils, NKT cells, activated CD8+ or CD4+ T memory cells and activated Treg, but not NK cells with severity determined by SCORAD. In order to avoid a bias by the HD group, we determined with predicted disease severity

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(pEASI, calculated by the serum concentrations of soluble CD25, IL-22 and C-C-chemokine 17 [CCL17, TARC]) from all the sera (Figure 2 and data not shown). These data indicate that the cytokine serum levels as components of the pEASI might be associated with the cell populations identified and support a potential regulatory role of these cell types in AD. In conclusion, mass cytometry-based immune cell profiling revealed the presence of immune cell subsets of different lineages including eosinophils, NK, NKT and activated T cell populations, which may contribute to AD pathophysiology. A better understanding of the

Key messages

- Applying the CyTOF technology on AD blood cells identified mainly NK and T cell subpopulations.
- The identified eosinophil, natural killer and T cell subpopulations show correlation with disease severity.
- Our data highlight the pathogenetic role of NK cells and T lymphocytes in atopic dermatitis.

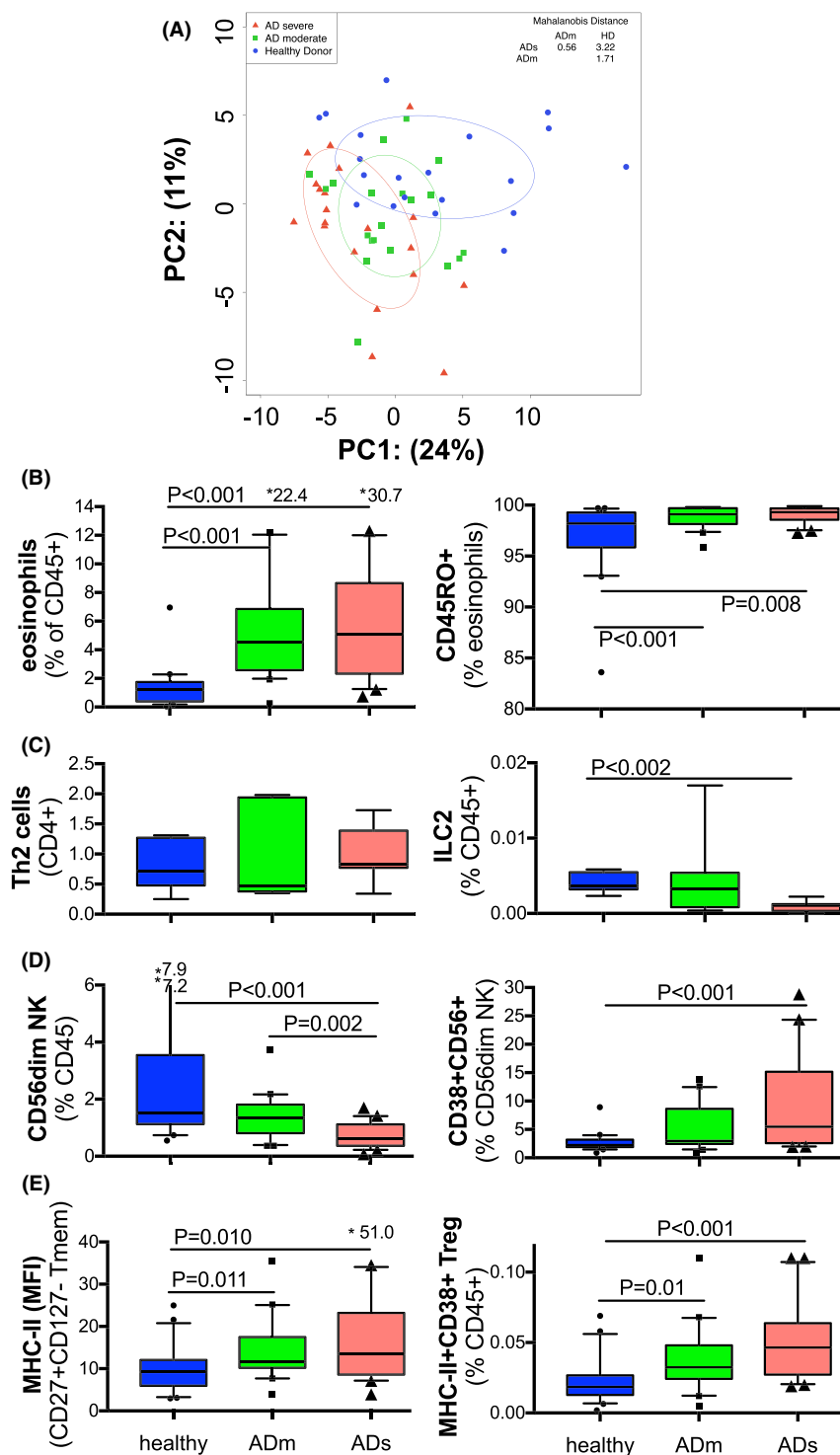
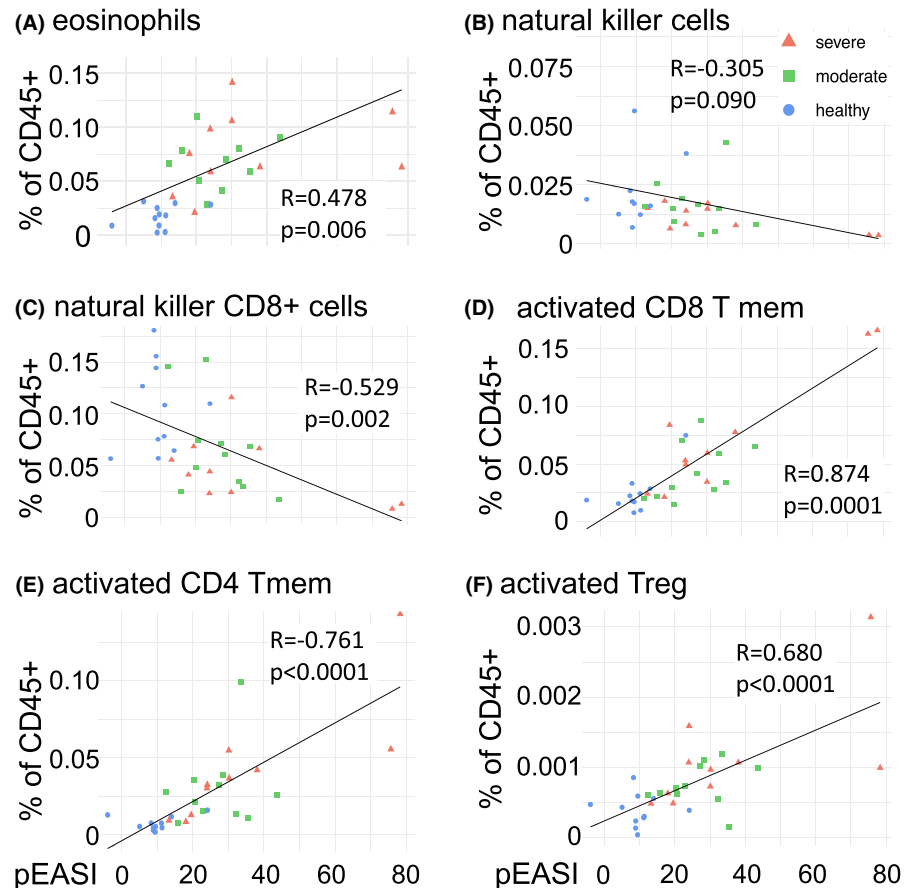


FIGURE 1 Differences in peripheral blood cells correlate with severity of atopic dermatitis and unravel major differences in defined immune cell subpopulations. (A) PCA from AD-associated cell populations obtained by FlowSOM/immunoClust analysis. Confirmation by manual gating of candidate cell populations including (B) eosinophils (C) Th2 (left) and ILC2 (right) (D) NK cells (E) CD4+ T memory cell and Treg subsets. Data represent median and interquartile ranges of blood cell frequencies (each $n = 20$; regarding Th2/ILC2 each $n = 7$). The dots represent outliers, with shown y-value if above the axis limit). p -values were calculated using the Mann-Whitney U-Test

FIGURE 2 Correlation of blood immune cell subsets with AD severity. Cell subpopulation frequencies in the blood of ADs, ADm and healthy donors determined by mass cytometry were correlated to serum predicted EASI (pEASI) for (A) eosinophils (Siglec8+ lineage-/CD45+), (B) NK cells (CD56+ lin-), (C) NK CD8 cells (CD56+CD16+CD8+CD3-HLADR-CD27-CD38-), (D) activated cytotoxic T memory cells (CD8+CD4-CD45RO+CD45RA-CD27+CD56-CD161-CD127-HLADR+), (E) activated memory T helper cells (CD4+RO+RA-CD27-/HLADR+), (F) activated Treg (CD4+CD25+CD127-CD45RO+CD45RA-HLADR+)



inter-cellular interactions of these subsets may improve our understanding of AD pathomechanism and will open new treatment options of AD beyond cytokine blockade or JAK inhibition.

AUTHOR CONTRIBUTIONS

M.W. designed the study, V.G. performed all experiments and analysed the data, S.B. established the antibody panel and CyTOF-measurements, T.S. analysed the data, H.M. analysed the data, A.G. supervised the experimental procedures, G.H. designed the study and analysed the data. All coauthors contributed to writing of the manuscript.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

Additional information about study methods and findings are available in the following repository <https://osf.io/n28qs>.

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REFERENCES

1. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet*. 2020;396(10247):345-360.
2. Darsow U, Wollenberg A, Simon D, et al. ETFAD/EADV eczema task force 2009 position paper on diagnosis and treatment of atopic dermatitis. *J Eur Acad Dermatol Venereol*. 2010;24(3):317-328.
3. Blaylock MG, Sexton DW, Walsh GM. Ligation of CD45 and the isoforms CD45RA and CD45RB accelerates the rate of constitutive apoptosis in human eosinophils. *J Allergy Clin Immunol*. 1999;104(6):1244-1250.
4. Rojahn TB, Vorstandlechner V, Krausgruber T, et al. Single-cell transcriptomics combined with interstitial fluid proteomics defines cell type-specific immune regulation in atopic dermatitis. *J Allergy Clin Immunol*. 2020;146(5):1056-1069.
5. Mack MR, Brestoff JR, Berrien-Elliott MM, et al. Blood natural killer cell deficiency reveals an immunotherapy strategy for atopic dermatitis. *Sci Transl Med*. 2020;12(532). doi:10.1126/scitranslmed.aay1005
6. Mobus L, Rodriguez E, Harder I, et al. Elevated NK-cell transcriptional signature and dysbalance of resting and activated NK cells in atopic dermatitis. *J Allergy Clin Immunol*. 2021;147(5):1959, e1952-1965.
7. Farrera C, Melchiorri R, Petrov N, et al. T-cell phenotyping uncovers systemic features of atopic dermatitis and psoriasis. *J Allergy Clin Immunol*. 2020;145(3):1021, e1015-1025.