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LETTER TO THE EDITOR

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C3a signaling is not involved in eosinophil migration during experimental allergic lung inflammation in mice

To the Editor,

Eosinophilia of the lungs and airways is a hallmark feature in allergic asthma. Eosinophils can exert proinflammatory and epithelial-damaging effects.[1] New therapies in the field of asthma targeting eosinophils have shown benefit in patients with high blood and sputum eosinophilia, including fewer exacerbation and improved lung function,[2] thereby pointing to the potential importance of therapeutics that interfere with eosinophil recruitment in the treatment of asthma. In recent years, data from preclinical studies unveiled an important role for anaphylatoxins, components of complement system activation, in the pathogenesis of allergic airway disease.[3] Signaling of the anaphylatoxin C3a through its receptor (C3aR) was documented to promote the onset of Th2 responses in different allergen-induced asthma models. Deficiency in or pharmacological blocking of either C3a or C3aR attenuated the allergen-induced Th2 response, which included a marked reduction of the eosinophilia in lung and airways.[3] While many studies focused on the interaction between C3a signaling and cells of the adaptive immune system during allergic inflammation,[4] only few investigated a potential direct effect of C3a signaling on eosinophil function. Infusion of C3a induced eosinophil adherence to postcapillary venules of IL-1β-stimulated mesenteric blood vessels of rabbits, but did not influence subsequent transmigration of eosinophils, suggesting a selective effect of C3a on eosinophil adhesion.[5] It remains elusive if C3a signaling in eosinophils affects their migration to lungs during an allergic response. This study aimed to investigate the role of C3a signaling in eosinophils in their recruitment to the airways during allergic lung inflammation.

We used an established mouse model of airway sensitization and challenge with the clinically relevant allergen house dust mite (HDM) (for details see Figure S1 and Appendix S1). HDM challenge elicited similar increases (P = .94) in C3a concentrations in bronchoalveolar lavage (BAL) fluid from HDM-sensitized WT and eosinophil lineage-deficient ΔdblGATA KO mice (Figure 1A). As expected, HDM challenge induced eosinophilia in the lungs and airways of WT mice whereas ΔdblGATA KO mice showed a complete deficit in eosinophils in both lung tissue and BALF (Figure 1B). Next, we harvested bone marrow cells from wild-type (WT) and C3aR knockout (KO) mice and differentiated these into mature bone marrow-derived eosinophils (bmEos) ex vivo (Figure S2A) as described in detail in the Appendix S1. We confirmed that C3aR mRNA was present in WT bmEos but completely abrogated in C3aR KO bmEos after 14 days in culture (Figure S2B). Total cell numbers grew similarly from WT and C3aR KO bone marrow to approximately 30 × 10^6 cells on day 14 (P = .91)(Figure S2C). Over a time span of 14 days, a high purity of eosinophils, defined as CCR3 and Siglec-F double-positive cells, was achieved from WT and C3aR KO mice (>95% at day 14, P = .92; Figure 1C). Giemsa staining showed the stereotypical bilobed nucleus and eosinophilic granules in eosinophils from both WT and C3aR KO (Figure S2D) cultures.[6] Moreover, circular nuclear morphologies, previously described in mouse eosinophils from peripheral blood were also observed.[6] Together, these data indicate that signaling through C3aR is not essential for the proliferation and differentiation of eosinophils.

Eosinophils are not required for the generation of memory T cells during the sensitization phase.[7] Thus, we adoptively transferred 5 × 10^6 WT or C3aR KO bmEos (intravenously) 24 hours after the first challenge (on day 15) into ΔdblGATA KO mice. Previously, bmEos were shown to have a half-life of eight days following chemotaxis into the lung,[8] enabling the investigation of migrated eosinophils after completing the challenge phase in our HDM model. Twenty-four hours after the last challenge (on day 20), saline-challenged mice demonstrated a lack of bmEos accumulation in both lung tissue and BAL fluid (data not shown). HDM-challenged mice showed similar numbers of WT and C3aR KO bmEos in their lungs (P = .53) (Figure 1D) and BAL fluid (P = .94) (Figure 1E). Together, these data suggest that C3aR-deficient eosinophils are not impaired in their migration toward the lung and airways following HDM challenge in spite of the presence of elevated C3a levels in the airways.

We next examined the potential of C3a as a chemoattractant for bmEos in vitro using a transwell system (for protocol see Appendix S1). The potency to attract bmEos was expressed as chemotactic index (CI) defined as: number of cells migrated in response to chemoattractant/ number of cells migrated in response to vehicle control. As expected, WT bmEos migrated toward hCCL24, a chemoattractant for both human and murine eosinophils,[9] in a dose-dependent fashion with a maximum CI of 11 at 1 µmol/L hCCL24 (Figure 2). C3a-mediated chemotaxis of WT eosinophils exhibited a maximum response of CI = 1.3, indicating that C3a at best is a very weak chemoattractant for eosinophils, thereby corroborating the in vivo

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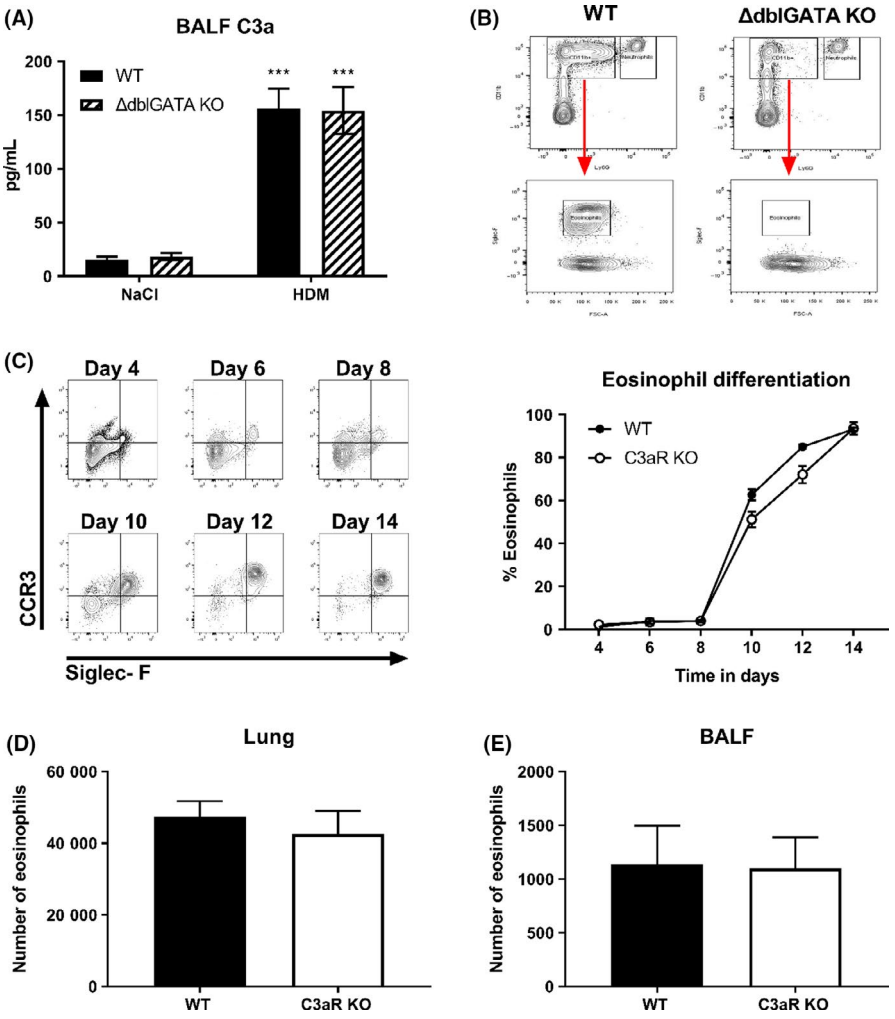

FIGURE 1 Generation of bone marrow-derived eosinophils ex vivo and adoptive transfer in HDM-challenged eosinophil-deficient mice. A, BALF C3a in WT and ΔdblGATA KO mice 24 h after the last saline or HDM challenge. B, Identification of bone marrow-derived eosinophils in the lung by flow cytometry in WT and ΔdblGATA KO mice 24 h following last HDM challenge. C, Identification of eosinophils by double-positive staining for CCR3 and Siglec-F in response to IL-5 on each indicated time point. Percentage of eosinophils following incubation with IL-5 from day 4 till 14. Number of eosinophils in the (D) lung or (E) BALF of recipient (ΔdblGATA KO) mice following adaptive transfer of either WT or C3aR KO bmEos. A parametric *t* test was used for the comparison between groups. Data are representatives of at least two independent experiments and expressed as means ± SEM from 6 to 8 separate cultures or mice per group. ***$P < .001$

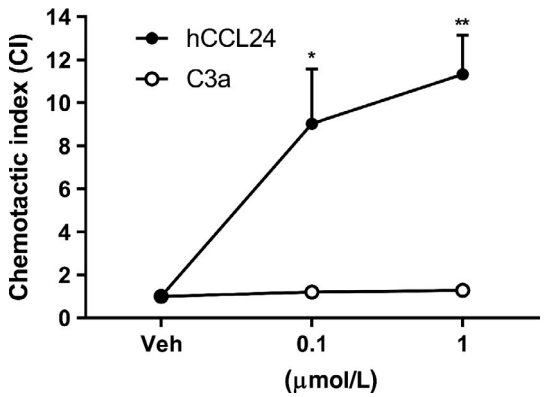

FIGURE 2 C3A is not an important chemoattractant for bone marrow-derived eosinophils ex vivo. Chemotaxis of WT bmEos in response to increasing dose of hCCL24 or C3a. Chemotactic index (CI) is used as measure for the extent of ex vivo chemotaxis. A parametric *t* test was used for the comparison between groups. Data are representatives of at least two independent experiments (n = 6-8 per group) and expressed as means ± SEM. *$P < .05$, **$P < .01$ for comparison between hCCL24 and C3a at the indicated dose

findings. In addition, these in vitro results are consistent with the findings from a previous study.[5]

In conclusion, this study shows that C3a does not exert important chemoattractant activity on bmEos during HDM-induced allergic lung inflammation in mice. As such, this report supports the notion [3] that C3a signaling promotes eosinophilia during allergic inflammation via an altered Th2 response rather than through a direct effect on eosinophils.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

Jack Yang[1]
Cornelis van't Veer[1]

Marieke S. ten Brink[1]
Alex F. de Vos[1]
Tom van der Poll[1,2]

[1]Center of Experimental and Molecular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
[2]Division of Infectious Diseases, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Correspondence
Jack Yang, Amsterdam UMC, Meibergdreef 9, Room G2-130, 1105AZ Amsterdam, The Netherlands.
Email: j.yang@amsterdamumc.nl

ORCID

Jack Yang https://orcid.org/0000-0002-5548-7771

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SUPPORTING INFORMATION

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

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Biologicals in allergic diseases and asthma: Toward personalized medicine and precision health: Highlights of the 3rd EAACI Master Class on Biologicals, San Lorenzo de El Escorial, Madrid, 2019

To the Editor

Biologicals have transformed the way of treatment of many immune-mediated disorders including cancer, autoimmune, and allergic diseases.[1,2] Biologicals and the understanding of their impact on diseases is a rapidly evolving field in which emerging important questions arise. Decisions on when to prescribe biologicals, how to develop clinical tools to elaborate an accurate endotype-based diagnosis and treatment approaches, how to identify and manage adverse and hypersensitivity reactions (HSR), and how to use biologicals in pregnancy, children, or elderly require additional research and evidence-based recommendations. All these aspects and other timely hot topics were addressed in the 3rd Master Class on Biologicals organized by the Biologicals Working Group and the Basic and Clinical Immunology Section of the European Academy of Allergy and Clinical Immunology (EAACI) in May 2019 in San Lorenzo de El Escorial, Spain.

Biologicals are products of high molecular weight that may be produced by living organisms, used to diagnose, prevent, and treat different diseases. Among them, monoclonal antibodies (mAbs) against specific targets are suitable for precision medicine as they bind to specific epitopes with high affinity, thus ensuring safety and efficacy. Biologicals provide therapeutic options when conventional approaches fail and contribute to our knowledge on the molecular mechanisms underlying complex diseases. In the era of precision medicine, personalized treatments are expected to allow a better selection of responders using well-defined biomarkers and might offer the opportunity to stop disease progression.[1,2] Several biologicals are approved or under development for the treatment of different