To the Editor,

Based on the dominating inflammatory cell population in the airways, asthma patients are currently distinguished into eosinophilic, mixed, neutrophilic and in some cases paucigranulocytic inflammatory phenotypes. Even though some associations to clinical phenotypes have been established over the last years, for example, association of allergic asthma with eosinophil inflammation or an association of neutrophil inflammation with more severe forms of asthma, for other conditions it is still less clear how these inflammatory phenotypes correspond to clinical manifestations. With a few exceptions, the underlying pathomechanisms called endotypes for the development of one or the other inflammatory and/or clinical phenotype are often not yet understood, which limits development of novel stratified treatment concepts for these endo- and phenotypes. The eosinophilic phenotype is so far the best characterized with regard to the underlying endotypes. In contrast, much less is known about the underlying mechanisms of noneosinophilic inflammatory phenotypes; therefore, they are often just described as non-type 2 asthma conditions.

We have recently established mouse models representing different phenotypes of airway inflammation all based on house dust mite (HDM) exposure using different routes, allergen doses, and timing of sensitization (description of all materials and methods). DOI: 10.1111/all.14424

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

ORCID
Dong Keon Yon [https://orcid.org/0000-0003-1628-9948]
Man Young Han [https://orcid.org/0000-0002-9077-5779]

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T cell requirement and phenotype stability of house dust mite–induced neutrophil airway inflammation in mice

Mi-Ae Kim1
Dong Keon Yon2 [https://orcid.org/0000-0003-1628-9948]
Hye Mi Jee2
Ju Hee Kim2
Jisoo Park1
Seung Won Lee3
Myongsun Sung4
Youn Ho Sheen5
Man Young Han2

1Department of Pulmonology, Allergy and Critical Care Medicine, CHA Bundang Medical Center, CHA University, Seongnam, Korea
2Department of Pediatrics, CHA Bundang Medical Center, CHA University School of Medicine, Seongnam, Korea
3Department of Data Science, Sejong University College of Software Convergence, Seoul, South Korea
4Department of Pediatrics, Soonchunyang University Gumi hospital, Gumi, Korea
5Department of Pediatrics, CHA Gangnam Medical Center, CHA University School of Medicine, Seoul, Korea

Correspondence
Man Yong Han, Department of Pediatrics CHA University School of Medicine 351 Yatap-dong, Bundang-gu, Seongnam, Gyeonggi-do, 463-712, Korea.
Email: drmesh@gmail.com
are provided in the Online appendix App S1). Mice exposed to all three protocols shown in Figure S1A-C demonstrated a significant increase in total leukocyte numbers in BAL compared to controls, and lung histology revealed a comparable extent of peribronchial and perivascular inflammation as well as goblet cell hyperplasia (Figure S2A-B). However, different cell populations dominated the inflammatory influx with an almost exclusive recruitment of eosinophils in the eosinophilic model, a concomitant recruitment of eosinophils and neutrophils in the mixed model and a predominant recruitment of neutrophils (and macrophages) in the neutrophilic model. In addition, the eosinophilic model showed the highest proportion of CD4⁺IL-5⁺ TH2 cells in the lungs, whereas CD4⁺IL-17⁺ TH17 cells were dominating in the neutrophilic model. Interestingly, the frequency of CD4⁺IFNγ⁺ T cells did not show any difference between all model conditions (Figure S2C). Although CD4⁺CD25⁺Foxp3⁺ Treg cells occurred in higher frequencies compared to healthy mice, no differences were observed between the different models (data not shown). Further, lung protein array analyses demonstrated a high abundance of proteins related to proinflammatory innate and TH17 associated immune mechanisms in the neutrophilic model (Figure S1C) as shown in Figure S2D. Besides TNFα, IL-6 and several members of the IL-1 as well as...
the IL-17 cytokine families that also included GM-CSF, G-CSF, the chemokines CXCL-1, CXCL-9, CXCL-10, and CXCL-11 as well as CCL-20. These results confirmed our previous transcriptome data that demonstrated increased mRNA levels for the majority of those factors in lungs from mice that underwent the neutrophilic model. Further highly expressed genes in this model were SAA, S100A8/S100A9, TLR2, and CXCR2 which all have previously been shown to be significantly upregulated in steroid-resistant, neutrophilic severe asthma patients. Accordingly, dexamethasone treatment of mice exposed to this model did not reduce neutrophilic inflammation (data not shown).

The significant activation of innate immune mechanisms in the neutrophilic model raised the question, whether adaptive mechanisms are required at all for the induction of this phenotype. To address this point, we exposed RAG2−/− mice to the neutrophilic phenotype-inducing HDM protocol (Figure S1C). Interestingly, these mice did not develop any inflammation in their lungs (Figure 1A) indicating that innate immune mechanisms alone are not sufficient to induce any kind of inflammation in this model system. Having demonstrated this, we further aimed to identify which T-cell subpopulation is most crucial for the development of the neutrophil inflammatory phenotype and therefore studied mice deficient in functional TH17 (IL-17A/F−/−) or TH1 (Tbet−/−) cells. The lack of IL-17A/F resulted in significantly diminished neutrophilic airway inflammation in the neutrophilic model (Figure S1C) indicating that TH17 cells represent the major driver of this inflammatory phenotype (Figure 1B). In contrast, Tbet−/− mice did not show any change in neutrophil infiltration but rather revealed increased numbers of eosinophils and an even higher level of inflammation. This indicates that TH1 mechanisms suppress TH2 activities in conditions leading to neutrophilic airway inflammation (Figure 1C).
to the protocol shown in Figure S1C. A. Shown are total cell numbers and neutrophil numbers in the BAL, representative histological images of PAS-stained lung sections, T cell-derived IL-17 and IL-10 and proinflammatory cytokines in lung homogenates. B, C. Shown are BAL eosinophil and neutrophil numbers, inflammation scores, and quantification of mucus producing goblet cells. Numerical data are represented as means ± SEM of at least 5 (A) or 8 (B, C) animals per group with significant differences at *P ≤ .05, **P ≤ .01 and ***P ≤ .001 between groups. ns, not significant

It was of further interest whether an established acute inflammatory phenotype remains stable during chronicization of disease under identical or changing exposure conditions. In this line, we established chronic exposure conditions using the mixed phenotype protocol (Figure S1B) resulting in typical features of chronic asthma including airway remodeling as characterized by collagen deposition and smooth muscle hypertrophy coinciding with increased BAL TGFβ1 and allergen-specific IgA levels (Figure S3A-I) at a minimum of 9 weeks of HDM exposure. In a subsequent experiment, we then compared phenotype stability using the mixed and neutrophilic models with steady (Figure 2A) or changing (Figure 2C) exposure conditions. Both phenotypes remained stable under long-term unchanged HDM exposure conditions (Figure 2A-B). Moreover, mice initially exposed to low-dose HDM (and thus representing the mixed AAI) which during the chronic phase received high-dose HDM challenges maintained their mixed inflammatory phenotype pattern. Similarly, mice initially exposed to high-dose HDM and thus showing a neutrophil-dominated inflammation did not show changes in their phenotype when later challenged with low-dose HDM even though a slight (nonsignificant) reduction in the number of both, eosinophils and neutrophils, was observed in these mice (Figure 2C-D). Taken together, this implies that the resulting inflammatory phenotype is mainly determined by the initial sensitization processes since modifications in exposure conditions during the chronicization process did not change the inflammatory phenotype once it is established like also observed in human studies. This should not implicate that phenotypes are stable per se. However, further/stronger triggers, for example, viral and bacterial infections and microbiome composition, various environmental pollutants, or the genetic, epigenetic, or metabolic background might influence phenotype and endotype stability.

**FIGURE 2** Inflammatory phenotypes stability at steady and changing allergen exposure conditions. (A and C) Schematic representation of the experimental conditions of acute (3 wk) and chronic (9 wk) HDM exposure for steady (A) and changing (C) allergen dose exposure conditions. Mice were i.n. sensitized/challenged to low- or high-dose HDM as outlined, and analyses were performed at 72 h after the last challenge. (B and D) Eosinophil and neutrophil cell numbers in the BAL at 3 and 9 wk of allergen exposure under steady (B) and changing (D) HDM dose exposure conditions, respectively (ld: low dose; hd: high dose). Data are presented as mean ± SEM of at least eight animals per group

Altogether, our previous and current observations indicate that the established model of a neutrophil-dominated airway inflammation represents major inflammatory of features of human neutrophil-driven asthma. These established and profoundly characterized model systems provide an excellent tool further identifying similarities and differences in the development and perpetuation of different kinds of allergic/asthmatic airway inflammation phenotypes. These investigations will lead to better understanding of the cellular and molecular mechanisms of the development of different inflammatory phenotypes of asthma, identification of pathomechanistic endotypes associated with these phenotypes and finally to the development of stratified treatment strategies for asthma therapy.

**KEYWORDS** animal models, asthma, endotypes, T cells

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**CONFLICT OF INTEREST**

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Stefanie Hagner¹
Mathilda Keller¹
Hartmann Raifer²
Hern-Tze Tina Tan³,⁴
Cezmi A. Akdis ³,⁴
Thorsten Buch⁵
Milena Sokolowska³,⁴
Holger Garn²

¹Institute of Laboratory Medicine and Pathobiocchemistry, Philipps University of Marburg, Member of the German Center for Lung Research (DZL) and the Universities of Giessen and Marburg Lung School (UGMLC), Marburg, Germany
²Core Facility Flowcytometry, Philipps University of Marburg, Marburg, Germany
³Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland
⁴Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland
Successful oral desensitization and reintroduction in selected glioma patients with procarbazine-mediated hypersensitivity

To the Editor

Oligodendrogliomas are rare, incurable primary brain tumors. Survival can be prolonged with neurosurgery and adjuvant chemoradiotherapy, including the PCV regimen consisting of procarbazine, lomustine, and vincristine. Unfortunately, side effects are common and many patients need dose reduction, therapy postponement, or incomplete cycles of PCV due to cytotoxicity, hepatotoxicity, and cutaneous eruptions.1 Apart from these side effects, drug hypersensitivity reactions (DHR) may occur, particularly for procarbazine. There are no predictors for developing a DHR, but antiepileptic drugs are associated with an increased risk, possibly due to their hepatic enzyme-inducing properties.1 Procarbazine may cause various types of DHR.2,3 Maculopapular eruptions (MPE) are most frequently documented, but fixed drug eruption (FDE), urticaria, and toxic epidermal necrolysis, as well as pneumonitis, have been described.2,4,5 These manifestations suggest that procarbazine-related DHR can be of IgE-mediated, T-cell-mediated, and possibly other mechanisms.

Procarbazine-related DHR is mainly a clinical diagnosis. Intracutaneous testing is not possible due to drug toxicity. Patch tests can be performed for nonimmediate DHR, but their predictive value remains unclear.3 After a procarbazine-related DHR, discontinuation of therapy is advised.2 There are no reports regarding reintroduction of procarbazine after occurrence of a severe DHR. For milder reactions such as MPE, a so-called treating through strategy may be employed, reintroducing procarbazine with concomitant use of antihistamines and corticosteroids.6 While desensitization protocols are available for direct, IgE-mediated DHR against several other chemotherapeutics, no such protocols exist for procarbazine.

We here describe the evaluation of several patients referred for procarbazine-related DHR. Next, we explored the possibility of reintroducing procarbazine using both treating through and desensitization strategies. Between April 2018 and October 2019, six patients with procarbazine-related DHR were referred to our Allergology clinic (Table 1). All suffered from generalized cutaneous reactions without extracutaneous systemic involvement. In patients with a...