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REPLY TO DUTTA ET AL.:

Understanding scRNA-seq data in the context of the tissue microenvironment requires clinical relevance

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Although we agree with Dutta et al. (1) that our conclusion could be more comprehensive, we disagree that our study design and interpretations are biased (2). Neutrophils in asthma are linked to worsening symptoms, but their role as key determinant cells remains obscure in the pathogenesis (3). Reducing neutrophilic inflammation in asthma does not yield any meaningful outcome in clinical trials and animal models (4, 5). Studies of multiple species have demonstrated that a healthy lung maintains a large pool of neutrophils that predominantly marginate within lung tissue (6). This is critical for rapid host defense. Importantly, dexamethasone (DEX) is known to promote the survival of neutrophils (7). Our endpoint was 3 d after low-dose lipopolysaccharide (LPS) (50 ng) installation, which was during inflammation resolution (2). Furthermore, lung cells were resuspended in 1 mL phosphate-buffered saline and BALF cells in 100 μL in the current study (2). We apologize if this causes confusion regarding the units used in figure 3C of ref. 2. These factors may contribute to more neutrophils in lung (figure 3C of ref. 2) but not in BALF (figures 6B and S1C of ref. 2).

Annotation of single-cell RNA deep sequencing (scRNA-seq) based on classical ontologies of cell labels is often not reproducible across different experiments and research groups. Therefore, we employed an automatic identifier, SingleR, that is a highly cited deep-learning algorithm (8). SingleR annotates the scRNA dataset with a mouse reference altas, ImmGen, that includes a complete compendium of genome-wide datasets of ex vivo immune cells and minimizes the biases of established cells or cytokine-dependent primary culture (8). ImmGen only provides reference for Treg and Tγδ and 77 $CD4^+$ T cell populations, not for traditional T helper (Th)1/Th2/Th17 cell subsets. In this regard, we agree with Dutta et al. (1) that enhanced algorithms or databases for the annotation of Th cells are needed for future studies.

Airway epithelial cells (AECs) preform alarmin proteins including interleukin (IL)-25 and IL-33 in cytoplasm and quickly release them upon exposure to pathogens/environmental stimulants during acute host defense responses (9). By contrast, we investigated the persistent AHR and airway inflammation 3 d after LPS installation (i.e., the exacerbation phase), which is the reason we excluded AECs for analysis. As IL-25, IL-33, and TSLP can also be produced by immune cells and are important for type 2 immunity, we examined their expression in our model. Currently, single-cell transcriptomic analysis is still largely a putative analysis predominantly based on optimized algorithms. As enormous clinical and animal studies have demonstrated a key role of IL-13 in pathogenesis, this cytokine serves as an important and valid target to verify our findings. Although we agree with Dutta et al. (1) that more comprehensive studies are needed to fully understand asthma exacerbation, this is beyond the scope of current study.

The dosage of DEX we used (1 mg/kg) is highly clinically relevant. The current recommended dose for asthma exacerbation is 1 mg/kg systemic corticosteroids (10). Additionally, it has been long recognized that there is no significant benefit of a high dose of systemic corticosteroids over a low dose when treating severe acute asthma (11).

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The authors declare no competing interest.

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