

Is Asthma Paying the Toll?

Asthma is a heterogeneous, chronic, inflammatory disease that is characterized by airway inflammation, airway hyperresponsiveness (AHR), and airflow obstruction, which clinically translates into episodes of shortness of breath, wheezing, relentless cough, and chest tightness (1). According to a report by the U.S. Environmental Protection Agency in 2016 (<https://www.epa.gov/asthma>), an estimated 24 million people, including more than 6 million children, suffer from asthma in the United States. In fact, asthma accounts for about 14 million physician office visits, 439,000 discharges from hospital inpatient care, and almost 2 million emergency-room visits each year. The annual economic cost of asthma (including direct and indirect costs) amounts to more than 56 billion dollars. Thus, there is an urgent need to develop novel therapeutics for the management of asthma—especially severe asthma.

One of the obstacles in developing new therapies for the treatment of asthma is the difference in disease biology and phenotypes between animal models of asthma (primarily mice) and the disease in humans (2). Asthma is an extremely complex disease with multiple endotypes. Significant heterogeneity exists among asthmatic patients with regard to various parameters, including the triggers that induce an asthma attack (e.g., exercise, allergens, and irritants), the severity of airway obstruction, and the extent of improvement in response to common medications such as inhaled corticosteroids (3). Although there are profound similarities between mouse models of asthma and the disease in humans, including a hyper T-helper cell type 2 (Th2) response, increased eosinophilia, elevated IgE levels, allergen-induced AHR, epithelial hyperplasia, and goblet cell metaplasia, there are also important differences between mouse models of asthma and asthma in humans (4). Such distinctions include differences in the tracheobronchial structure, cellular composition of the airways, and eosinophil distribution, the lack of eosinophil degranulation and intrinsic AHR in mice, and the involvement of exacerbations. Asthma models in nonhuman primates (NHPs) have been developed in an attempt to bridge the gap between mice and humans, and although they are not frequently used, they can provide a powerful tool for preclinical drug development (5). Thus, outcomes from studies that are conducted in NHPs require special attention.

In this issue of the *Journal*, Camateros and colleagues (pp. 55–65) report the use of the Toll-like receptor (TLR) 7/8 agonist S28463 (S28) as a prophylactic treatment in a novel NHP model of *Ascaris suum*-induced allergic asthma (6). Contrary to the conventional *A. suum* model, which uses skin-prick-sensitive, caught-in-the-wild rhesus macaques, this model employs skin-prick-negative NHPs that are subcutaneously sensitized and then chronically challenged with *A. suum*. This approach likely gave the authors greater control over the sensitization process, permitting standardization of the model. Indeed, using this protocol, the authors achieved an allergen-sensitization phase that was maintained for weeks and a strong Th2 response, mainly characterized by type 2 cytokine secretion in the BAL fluid (BALF) after allergen challenge. To assess the safety of S28

treatment along with *A. suum* exposure, they performed blood biochemical and hematologic analyses. The analytics were all in the range of those found in healthy individuals, including alanine transaminase, aspartate transaminase, urea, and creatinine levels. Thus, importantly, S28 prophylactic treatment was shown to be safe.

The authors further report that S28 treatment was capable of reverting *A. suum*-induced IgE production and the major physiological outcome of asthma: increased AHR and airway resistance in response to methacholine exposure. Interestingly, S28 treatment was able to reduce the total number of inflammatory cells that were recruited to the BALF, with the most prominent effects observed on eosinophil counts. The numbers of additional inflammatory cells, including lymphocytes, macrophages, and neutrophils, did not change. An assessment of the cytokine profile in the BALF of *A. suum*-challenged NHPs showed that *A. suum* induced a mixed Th1/Th2 response, and elevated levels of IFN- γ , TNF- α , and IL-12 were reported along with increased IL-4, IL-13, and IL-10 levels. All cytokines, with the exception of IL-2 and IL-5, were reduced to baseline levels after S28 treatment. Although the ability of TLR stimulation (especially using TLR-7/8 agonists) to dampen Th2 responses is not surprising (7), the extensive proteomic and metabolomics analyses of the plasma and BALF of *A. suum* allergen-challenged NHPs with and without S28 treatment in this study produced some interesting results. The proteomic analysis revealed that 177 proteins were altered after *A. suum* allergen treatment. The top five markers that were altered were identified as fatty acid binding protein 5 (FABP5), Rho GDP dissociation inhibitor 2 (ARHGDI2), peptidylprolyl isomerase A (PPIA), aldo-keto reductase family 1 member A1 (AKR1A1), and leucine aminopeptidase 3 (LAP3). Treatment with *A. suum* caused the induction of 70 proteins, 47 of which were unique to the S28-treated group and 23 of which were shared with the *A. suum*-challenged group. Importantly, the 23 shared markers, which were altered after *A. suum* allergen challenge, returned to baseline levels after S28 treatment. Among these were kallikrein (KLKB1), prothrombin (F2), and FABP5. Interestingly, FABPs and especially FABP5 were previously reported to be increased in rodent models of asthma and in asthmatic patients (8, 9). A bioinformatics analysis using Gene Ontology revealed that S28 treatment markedly affected biological pathways involved in “regulation of proteolysis” and “endopeptidase regulator activity.” These data are consistent with the emerging roles of proteolytic pathways in the induction of type 2 allergic responses and especially in the generation of epithelial-derived cytokines such as IL-33, thymic stromal lymphopoietin, and IL-25 (10). They further suggest that S28 treatment may alter these responses. Thus, it will be important to mechanistically assess this notion in future studies.

An additional unique angle that this study provides is a thorough metabolomic analysis of the plasma and BALF of *A. suum* allergen-challenged NHPs with and without S28 treatment. Generating a metabolic signature in asthma and understanding how this signature is altered after treatment could provide new noninvasive biomarkers for diagnosing asthma and predicting treatment success.

Interestingly, the top four metabolites that were differentially expressed in the plasma of *A. suum*-challenged NHPs were phosphocreatine, D-glucuronate, and the dipeptides glycine-phenylalanine and glycine-glycine, whereas the top differentially expressed metabolites in the BALF were glutathione, glutathione disulfide, and kynurenine. Dissecting the data obtained from *A. suum* allergen-challenged NHPs with and without S28 treatment showed that S28 was capable of reverting the metabolomics signature of various metabolites back to baseline levels. For example, phosphocreatine and dipeptide glycine-glycine in plasma were reverted back to baseline after S28 treatment, and glutathione, glutathione disulfide, and kynurenine in BALF were reverted as well. The metabolomics analysis in this study is preliminary and does not entirely correlate with previous analyses (11, 12); however, metabolomic profiles can vary with asthma severity, and thus extensive and cross-species analyses will be needed before we can truly understand the magnitude of these findings. Nonetheless, this initial metabolomics analysis in NHPs will serve as an important reference point for future studies.

Collectively, the findings from Camateros and colleagues establish a new, controlled protocol for the sensitization and allergen challenge of NHPs with *A. suum*. Furthermore, the authors also establish S28463 as a candidate drug for the treatment of severe allergic asthma. The results suggest that future clinical trials of S28 will show the drug to be safe, and highlight several markers that could be used in noninvasive tests of patient plasma to predict treatment success. ■

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