RESEARCH LETTER

The GPR84 antagonist, GLPG1205, reduces features of disease in experimental severe asthma

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To the Editor:

Recent evidence has highlighted the detrimental effects of fatty acid receptor dysfunction in the lung, with a major focus on the roles of short and long chain fatty acid receptors and targeting these receptors with specific compounds. Interestingly, medium chain fatty acids (MCFAs) and their receptor (GPR84) have also been identified in the lung but their roles, and the effects of targeting with specific compounds, in homeostasis and disease, are poorly understood (1). Whilst there is evidence that dietary MCFAs may be protective against obesity and infection (2) and that Gpr84 plays important roles in mitochondrial metabolism in skeletal muscle (3), there is increasing evidence that GPR84 is a pro-inflammatory receptor (1) and its activation can induce pro-inflammatory responses, including *Il12b* (4), *Tnf* (1), and *Il8* (1) induced through $G_{i/o}$ (4), Lyn, Akt, Erk1/2, and NF-κB (5, 6) signaling pathways. Importantly, recent studies have fully characterized the structure and ligand binding to GPR84 with the goal of facilitating drug discovery against inflammation and metabolism (7, 8). Mice deficient in *Gpr84* have reduced proinflammatory cytokine responses in models of traumatic nerve injury (9), colitis (10), and acute lung injury (6). Furthermore, Gpr84 has recently been implicated in the regulation of brown adipose tissue responses, including lipid responses, thermogenic gene regulation and oxygen consumption, and this study also demonstrated Gpr84 activation increased intracellular calcium responses and altered mitochondrial respiration (11). A different study has recently shown that targeting Gpr84 with an agonist 6-OAU in combination with CD47 blockade on cancer cells increases tumor-associated macrophage phagocytosis (8). Notably, in two recent reviews, the exciting possibility of targeting GPR84 in diseases have been discussed (12, 13). Collectively, these studies show that Gpr84 activation can exert pleiotropic effects unique to different organs and cells, exposure scenarios, and that differ between homeostatic and disease conditions. However, despite many lung diseases having features of increased inflammation and altered cellular metabolism, no studies to date have explored the role/s of GPR84 in chronic inflammatory diseases of the lung.

GPR84 is present on macrophages and neutrophils (14, 15). We, and others, have shown that these cell types play important disease-causing roles in severe asthma pathogenesis (16, 17). Severe asthma is a chronic lung disease underpinned by robust pro-inflammatory responses and altered metabolism. However, the roles and potential for therapeutic targeting of GPR84 in severe asthma are unexplored.

In this study, we first assessed *GPR84* expression in human lung single cell RNA sequencing data from a publicly available database (gene name: G protein-coupled receptor 84 [*GPR84*]) (18) and confirmed that *GPR84* is present in multiple lung cell types (*e.g.* neutrophils, smooth muscle cells, dendritic cells) in healthy lungs (Figure E1A-D) and patients with asthma (Figure 1A and 1B, and Figure E1E-H). To confirm this data, we also mined an additional publicly available human single cell dataset and found GPR84 expressed in 17,024 of 3,059,466 lung cells analyzed, including in neutrophils, macrophages, and smooth muscle cells (19). There is also some evidence that *GPR84* is expressed in luminal macrophages from healthy lung samples, as well as in patients with asthma (Figures E1A, C, E and G), and this is supported by another study that identified *GPR84* as a gene of interest involved in the response to LPS- or IFN-γ stimulation in human lung macrophages (20, 21).

We next assessed whether Gpr84 is present in mouse tissues by performing immunohistochemical staining for Gpr84 on lung tissue collected from our established murine model of *Chlamydia muridarum* (Cmu) respiratory infection-induced severe asthma, which produces disease features that are highly representative of severe asthma in humans (16, 17, 22). Using this model, we previously showed that Cmu infection of allergic mice drives steroid-insensitive disease features, including neutrophilic airway inflammation, through both excessive NLRP3-mediated IL-1β responses, and a miR-21/PI3K/HDAC2 axis (16, 17). We now demonstrate that Gpr84 is present in mouse lungs in experimental severe asthma (Figure 1C, Figure E2, Figure E3A). Together these data provide strong evidence that GPR84 is indeed present in the lung and in asthma and is amenable to interrogation in mouse models and tissues.

GLPG1205 is a selective GPR84 antagonist, which has been shown to improve lung function in patients with idiopathic pulmonary fibrosis (IPF) (23), and the administration of GLPG1205 in mice with experimental colitis (23) and IPF (24) reduced key disease features. However, the effects of treatment with GLPG1205 in severe asthma are unknown and warrant exploration. Using our Cmu-induced murine model of experimental severe asthma in female mice (16, 17, 22), we examined the effects of treatment with GLPG1205 to determine its potential therapeutic utility (Figure 1D). Mice were administered ovalbumin (Ova) i.p. (day 0; or saline control), followed by intranasal Ova (days 12-13) to induce experimental asthma. Some mice were then infected with Cmu (day 14; or sucrose-phosphate-glutamate [SPG] control) to induce experimental severe asthma. Mice were then re-challenged with Ova

(days 33-34) to model an allergen-induced exacerbation of established disease. Separate groups of mice were treated (days 32-34) with dexamethasone (DEX; 2mg/kg) to model inhaled steroid use and/or GLPG1205 (10mg/kg). Endpoint analyses (day 35) included *in vivo* invasive plethysmography to measure methacholine-induced airway hyperresponsiveness and bronchoalveolar lavage to assess airway inflammation.

The major findings from this study were that treatment with GLPG1205 had no effect on lung Gpr84 levels (Figure E3A) but reduced steroid-insensitive airway inflammation (Figure 1E), including macrophages (Figure 1F), neutrophils (Figure 1G), eosinophils (Figure 1H), lymphocytes (Figure 1I), as well as steroid-insensitive airway hyperresponsiveness (Figure 1J and Figure E3B and E3C). Our observations are supported by findings from a previous lipopolysaccharide-induced model of acute lung injury, in which genetic deletion of *Gpr84*, the equivalent to the use of an antagonist in our study, reduced lung inflammation, and in separate *in vitro* experiments antagonism of GPR84 reduced neutrophil chemotaxis, ROS production, and degranulation (6). In addition, we have previously shown evidence for potential crosstalk between neutrophilic and macrophage inflammation in severe asthma using this model (17). In that study, we showed that anti-Ly6G treatment (which targets neutrophils for depletion) in mice reduced IL-1β-induced macrophage inflammation. We propose that GLPG1205 is likely exerting some of its beneficial effects on neutrophils to reduce neutrophil numbers in the first instance, and then this causes a concomitant decrease in macrophage numbers in the airways owing to crosstalk between these two cell types. Further studies assessing neutrophil chemotactic markers, and the crosstalk between neutrophils and macrophages in severe asthma are warranted. In addition, as lymphocyte numbers are also reduced with GLPG1205 treatment, assessing lymphocyte chemotactic markers would also be informative.

Therefore, these data suggest that the beneficial effects of GLPG1205 treatment may occur through the inhibition of neutrophil recruitment and subsequent activation, and highlight the potential application of using this compound in several inflammatory diseases. To explore the possibility of targeting Gpr84 after the allergen-induced exacerbation is established, future studies assessing GLPG1205 administration after day 35 in the protocol (Figure 1D) would be informative.

A large body of evidence demonstrates that airway contraction and hyperresponsiveness can exist in the absence of inflammation, highlighting the possibility that treatment with GLPG1205 also exerted effects on these pathophysiological features in our model independent of its effects on airway inflammation. In this study, whilst neutrophil numbers were robustly reduced in the GLPG1205+DEX group, this combination of treatments resulted in reduced, but variable, AHR. Thus, we sought to delineate the specific effects of GLPG1205 on airway smooth muscle function, without the confounding effects of the immune system and remodeling. To test whether GPR84 responses are important in airway smooth muscle contraction, precision cut lung slices (PCLS) from naïve male mice were exposed to increasing doses of methacholine in the absence, or presence, of GLPG1205. Interestingly, the potency of, but not maximum contraction induced by, methacholine was reduced in the presence of GLPG1205 compared to vehicle (3% DMSO; Figure 1K). To the best of our knowledge, no studies have shown that GLPG1205 can bind directly to muscarinic receptors, however, downstream signaling from both muscarinic receptor and GPR84 activation is known to involve the regulation of calcium. Therefore, our data suggest that GPR84 responses may regulate calcium signaling pathways in airway smooth muscle and future studies assessing the effects of GLPG1205 treatment on calcium oscillations and myosin cycling are warranted.

There are limited studies to date that have assessed Gpr84 in the lung. Our experimental findings, and those available through publicly available datasets, have demonstrated that Gpr84 gene expression is present in multiple cell types in the lung, including neutrophils, luminal macrophages, airway smooth muscle cells, and protein levels are present in neutrophils and macrophages. Further characterization of Gpr84 protein levels in mouse and human neutrophils and other cell types is also warranted. Taken together with other studies that have also assessed Gpr84, this opens the exciting possibility of targeting Gpr84 which may have pleotropic effects depending on the specific organ, tissue, or cell type involved.

In conclusion, our data provide important and novel pre-clinical evidence for targeting the pro-inflammatory receptor, GPR84, in the lung to beneficially modify airway smooth muscle function and reduce airway inflammation in severe asthma (Figure E4).

Figure 1. Lung single cell RNA sequencing data (tSNE plots) from (*A*) patients with asthma and (*B*) *GPR84* expression. Data are publicly available at: https://asthma.cellgeni.sanger.ac.uk. (*C*) Representative images of $GPR84^+$ lung tissue (DAB; brown) and hematoxylin counterstain (blue), scale bar = 50 μm. Female WT BALB/c mice were sensitized to Ova (day 0, i.p.) and challenged with Ova (days 12-13) followed by re-challenge (days 33-34). Non-allergic controls were sham-sensitized with saline (Sal). Some groups were inoculated i.n. with 100 inclusion-forming units (IFU) of *Chlamydia muridarum* (Cmu; day 14) to induce experimental severe asthma. Controls were sham-infected with sucrose phosphate glutamate (SPG) buffer. The response to steroid treatment was assessed by i.n. administration of dexamethasone (DEX; days 32-34). GPR84 responses were assessed by i.n. administration of GLPG1205 (days 32-34) in the absence, or presence, of DEX. Endpoints were assessed on day 35. (*D*) Schematic of the study protocol. I Total leukocytes, (*F*) macrophages, (*G*) neutrophils, (*H*) eosinophils, and (*I*) lymphocytes were enumerated in bronchoalveolar lavage fluid (BALF) on d35. (*J*) Airway resistance (Rn) in response to increasing doses of nebulized methacholine (MCh), and at 10 mg/mL of MCh (statistics at maximal dose from airway hyperresponsiveness curves). *E-J* are from one experiment, n = 6-8/group. Data are presented as means ± SEM. $*P < 0.05$; $**P < 0.01$; $****P < 0.0001$. PCLS were prepared from male WT BALB/c mice. (*K*) Average contraction over the last minute of perfusion of each concentration of MCh in the presence of vehicle (3% DMSO) or GLPG1205 (100 μM) and pEC_{50} values obtained from individual fitted curves. *K* is from one experiment, n = 5/group (same mice). Data are presented as means ± SEM. $***P < 0.001$.

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