Drugging the Mighty Neutrophil in Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD), a lung disease characterized by irreversible airflow limitation and varying degrees of neutrophilic inflammation, imposes a tremendous global health burden. Although COPD therapy has been largely monolithic to date, with very few drug classes approved, growing evidence supports the existence of heterogeneous COPD endotypes that may warrant more targeted strategies (1). Reexamining and deconvoluting the effects of established therapies at both the cellular and patient level is a promising approach for revealing new potential drug targets and advancing personalized medicine for COPD.

The neutrophil is necessary and likely sufficient for COPD pathogenesis, with neutrophil elastase recapitulating several features of the disease (2). It has been known for >30 years that neutrophil chemotaxis is elevated in COPD (3). Remarkably, COPD neutrophils also display increased oxidant production, cytokine release, degranulation, endothelial interaction, transendothelial migration, and glucocorticoid resistance (2, 4, 5). Many of these effects are presumed to reflect *in vivo* "priming" by the cytokine milieu. In the case of neutrophil migration, recent studies have clarified that COPD neutrophils actually have elevated velocity but impaired directionality, and that this may arise from increased activity of PI3K (2, 4, 6). Until recently, however, no effective therapies targeting neutrophilic inflammation in COPD have been available.

In 2011, the Food and Drug Administration approved roflumilast, an oral phosphodiesterase type 4 inhibitor that increases intracellular cAMP, for use in COPD. In patients with severe bronchitis and a history of frequent exacerbations, roflumilast improves forced expiratory volume in 1 second and reduces exacerbation frequency (7). Roflumilast reduces sputum neutrophilia (8) and may do so through inhibitory effects on multiple neutrophil functions, including adhesion, degranulation, oxidant release, and chemotaxis (9). COPD neutrophils have elevated expression of phosphodiesterase types 4B and 4D (4), perhaps suggesting increased susceptibility to the drug, but many basic questions about roflumilast's effects on the COPD neutrophil remain unanswered.

In this issue of the *Journal*, Dunne and colleagues (pp. 445–453) demonstrate for the first time a direct inhibitory effect of roflumilast on neutrophil migration in COPD and begin to uncover some of the underlying mechanisms (10). The investigators found that, compared with neutrophils from nonsmokers and smokers without COPD, neutrophils from patients with COPD had higher chemotactic responses to both CXCL1 and leukotriene B4. Extending previous work, they show that *ex vivo* treatment with the active metabolite roflumilast-*N*-oxide inhibited chemotaxis in a concentration-dependent manner, with no difference between subject groups, although COPD neutrophils tended to have a higher half-maximal effective concentration (EC_{50}). The mean EC_{50} for COPD neutrophil chemotaxis to CXCL1 was 4.8 nM, a concentration close to that achievable in the plasma of patients with COPD (9). Of interest, the EC_{50} for inhibition of CXCL1- and leukotriene B4–induced calcium flux, shape change, and CD11b display in neutrophils was found to be ⩾1 order of magnitude higher than that observed for chemotaxis inhibition, suggesting that the mechanism of chemotaxis inhibition may be distinct. Indeed, using cAMP analogs and inhibitors to selectively activate the cAMP effectors PKA, EPAC1 (exchange protein directly activated by cAMP 1), and EPAC2, the authors obtained evidence that roflumilast inhibits neutrophil chemotaxis via activation of EPAC1. Because EPAC1 is a cAMP-activated guanine nucleotide exchange factor for Rap1, a Ras-like GTPase that mediates chemotaxis and other functions of neutrophils (11), they propose that roflumilast operates via actions on neutrophil Rap1.

Strengths of the report by Dunne and colleagues include its meticulous comparative concentration-dependent profiling of roflumilast's effects in COPD and non-COPD neutrophils. The *ex vivo* study of purified neutrophils confirms direct drug effects on the neutrophil. The authors' dissociation of the drug's effect on calcium flux from its effect on chemotaxis importantly points out the pitfalls of using calcium flux as a screen for a drug's effects on downstream functions triggered by G protein–coupled receptors. Finally, the authors' specific implication of EPAC1 and Rap1 takes us a step forward in considering novel drug targets, including some that may potentially avoid the untoward side effects of nonselective cAMP-elevating therapies, including roflumilast itself. Selective downstream pathway intervention may be important given that cAMP elevation by β2-adrenergic receptor agonists reportedly impairs neutrophil phagocytosis and bacterial killing via PKA activation, whereas selective EPAC activation rescues these untoward effects (12). It is noteworthy in this regard that roflumilast has been reported to increase pathogen burden and mortality during bacterial pneumonia in mice (13).

Limitations of the current report should also be noted. *Ex vivo* treatment of neutrophils cannot recapitulate possible *in vivo* drug effects on neutrophil development and maturation. The agonists and inhibitors used by the authors, although well established, may have off-target effects (14). It would have been interesting to distinguish between roflumilast's effects on neutrophil velocity and directionality, and to evaluate possible indirect interactions of the drug with PI3K. Although the authors speculate about Rap1 as an ultimate target of roflumilast, they neither demonstrate Rap1 activation nor inhibit Rap1 in their system, leaving open the possibility of other EPAC effectors (14). Indeed, given that Rap1 plays a role in leading-edge adhesion during neutrophil chemotaxis (11), further work will be required to define the potential duality of Rap1 in neutrophil migration, including the possibility that

indiscriminate Rap1 antagonism and agonism may both derange cell migration by disturbing physiologic nucleotide cycling. Moreover, the roles of specific effectors downstream of Rap1 itself may have to be distinguished, including the possibility that roflumilast inhibits chemotaxis via Rap1-dependent inhibitory effects on Rho GTPases.

What lessons can we derive from roflumilast to apply to more selective therapies in the future? From the clinical information provided, it is difficult to know how many of the patients with COPD studied fell into the bronchitis/frequent exacerbator phenotype that has been linked to roflumilast clinical efficacy. Interestingly, the authors found a very wide (>20-fold) range of EPAC1 expression in neutrophils from different subjects. Unfortunately, the relation of EPAC1 expression to roflumilast EC_{50} was not reported. Should we screen neutrophil chemotactic function or EPAC1/Rap1 activity when selecting patients for future, more targeted therapies? Or is this too specific and reductionist a test, especially given that roflumilast had roughly equivalent efficacy in COPD and non-COPD neutrophils in the present report? Roflumilast has widespread beneficial effects *in vivo*, some of which are believed to derive from other cell types, and potentially from cAMP effectors other than EPAC1/Rap1. Conversely, preclinical reports have identified possible untoward effects of EPAC1 agonism, such as cardiomyocyte hypertrophy (15). As we do the critical work of zooming in on specific cell types and molecules in COPD in an attempt to uncover new druggable targets and mechanisms, it will thus be equally important to zoom back out to maintain the larger, integrated *in vivo* picture. ■

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References

1. Russell DW, Wells JM, Blalock JE. Disease phenotyping in chronic obstructive pulmonary disease: the neutrophilic endotype. *Curr Opin Pulm Med* 2016;22:91–99.
2. Stockley JA, Walton GM, Lord JM, Sapey E. Aberrant neutrophil functions in stable chronic obstructive pulmonary disease: the neutrophil as an immunotherapeutic target. *Int Immunopharmacol* 2013;17:1211–1217.
3. Burnett D, Chamba A, Hill SL, Stockley RA. Neutrophils from subjects with chronic obstructive lung disease show enhanced chemotaxis and extracellular proteolysis. *Lancet* 1987;2:1043–1046.
4. Milara J, Lluch J, Almudever P, Freire J, Xiaozhong Q, Cortijo J. Roflumilast N-oxide reverses corticosteroid resistance in neutrophils from patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2014;134:314–322.
5. Woolhouse IS, Bayley DL, Lalor P, Adams DH, Stockley RA. Endothelial interactions of neutrophils under flow in chronic obstructive pulmonary disease. *Eur Respir J* 2005;25:612–617.
6. Sapey E, Stockley JA, Greenwood H, Ahmad A, Bayley D, Lord JM, *et al*. Behavioral and structural differences in migrating peripheral neutrophils from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011;183:1176–1186.
7. Wedzicha JA, Calverley PM, Rabe KF. Roflumilast: a review of its use in the treatment of COPD. *Int J Chron Obstruct Pulmon Dis* 2016;11:81–90.
8. Grootendorst DC, Gauw SA, Verhoosel RM, Sterk PJ, Hospers JJ, Bredenbröker D, *et al*. Reduction in sputum neutrophil and eosinophil numbers by the PDE4 inhibitor roflumilast in patients with COPD. *Thorax* 2007;62:1081–1087.
9. Hatzelmann A, Morcillo EJ, Lungarella G, Adnot S, Sanjar S, Beume R, *et al*. The preclinical pharmacology of roflumilast–a selective, oral phosphodiesterase 4 inhibitor in development for chronic obstructive pulmonary disease. *Pulm Pharmacol Ther* 2010;23:235–256.
10. Dunne AE, Kawamatawong T, Fenwick PS, Davies CM, Tullett H, Barnes PJ, *et al*. Direct inhibitory effect of the PDE4 inhibitor roflumilast on neutrophil migration in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2019;60:445–453.
11. He Y, Kapoor A, Cook S, Liu S, Xiang Y, Rao CV, *et al*. The non-receptor tyrosine kinase Lyn controls neutrophil adhesion by recruiting the CrkL-C3G complex and activating Rap1 at the leading edge. *J Cell Sci* 2011;124:2153–2164.
12. Scott J, Harris GJ, Pinder EM, Macfarlane JG, Hellyer TP, Rostron AJ, *et al*. Exchange protein directly activated by cyclic AMP (EPAC) activation reverses neutrophil dysfunction induced by β2-agonists, corticosteroids, and critical illness. *J Allergy Clin Immunol* 2016;137:535–544.
13. Kasetty G, Papareddy P, Bhongir RK, Egesten A. Roflumilast increases bacterial load and dissemination in a model of *Pseudomonas aeruginosa* airway infection. *J Pharmacol Exp Ther* 2016;357:66–72.
14. Lezoualc'h F, Fazal L, Laudette M, Conte C. Cyclic AMP sensor EPAC proteins and their role in cardiovascular function and disease. *Circ Res* 2016;118:881–897.
15. Métrich M, Lucas A, Gastineau M, Samuel JL, Heymes C, Morel E, *et al*. Epac mediates beta-adrenergic receptor-induced cardiomyocyte hypertrophy. *Circ Res* 2008;102:959–965.