

Future treatment in patients with chronic obstructive pulmonary disease: To reverse or not to reverse steroid resistance—that is the question

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Key words: Roflumilast, neutrophils, chronic obstructive pulmonary disease, steroids, steroid resistance, oxidative stress

Chronic obstructive pulmonary disease (COPD) is a smoking-induced chronic inflammatory disease that affects mainly the lungs. In susceptible subjects airways inflammation becomes autonomous in part through pathogen-associated molecular patterns and tissue-derived damage-associated molecular patterns activating mainly Toll-like receptors expressed by multiple inflammatory cells.¹ The current consensus is that the chronic inflammatory process in patients with COPD requires treatment to stop the ongoing cycle of infection, inflammation, and host tissue damage.²

The inflammatory response in patients with COPD is dominated by neutrophils,³ which circulate in greater numbers in patients with COPD. Moreover, there are now compelling data to indicate that these cells play a key pathogenic role in this condition and represent an important therapeutic target. Critically, the neutrophils in patients with COPD are different compared to cells isolated from healthy subjects. In particular, they display (1) aberrant chemotactic responses, (2) enhanced protease content, (3) intrinsic “priming” leading to greater agonist-induced reactive oxygen species generation, (4) enhanced IL-8 and leukotriene B₄-driven migration to the airways and lung parenchyma,³ and (5) a seemingly acquired resistance to the normal anti-inflammatory effects of corticosteroids.⁴ As a consequence, it is generally agreed that inhibition or “normalization” of neutrophil function in patients with COPD might represent an important therapeutic strategy.²

Compared with other chronic inflammatory diseases, COPD is characterized by a further and possibly unique feature: oxidative stress, which originates from the inhalation of smoke and cellular sources, such as the phagocyte nicotinamide adenine dinucleotide phosphate oxidase-2 (NOX2).⁵ This oxidative environment is thought to have a negative effect on myeloid cell function (including the induction of corticosteroid resistance). However, it is important to note that steroid resistance is also observed in

other chronic inflammatory settings and hence is not specific to COPD.

Milara et al⁶ have performed an extensive study into the putative mechanisms of steroid resistance in circulating neutrophils from patients with COPD and how these can be countered. They conclude that roflumilast N-oxide (RNO), the active metabolite of the phosphodiesterase IV inhibitor roflumilast, in addition to having direct (likely cyclic AMP mediated) anti-inflammatory and bronchodilator effects, also reverses the steroid insensitivity of neutrophils in patients with COPD. With certain caveats, their experiments support the hypothesis that oxidative stress downregulates steroid sensitivity in neutrophils and that this is almost completely reversible by cotreatment with RNO. Although these findings are of interest and align with certain clinical studies relating to the efficacy of these and other nonselective phosphodiesterase inhibitors in smokers with asthma,⁷ the article still lacks a clear hypothesis regarding how glucocorticosteroid (GCS) resistance evolves and the precise mechanism whereby RNO restores the sensitivity these cells to corticosteroids.

A number of mechanisms have been proposed to explain the resistance of immune cells and lung epithelial cells of patients with COPD to steroids, including the following:

1. familial GCS resistance (eg, through inactivating mutations in the glucocorticoid receptor [GR] α or functional single nucleotide polymorphisms, which affect GR transcription)⁸;
2. reduced expression or enhanced degradation of GR α (eg, through the actions of IL-6, IL-8, TNF- α , and nitric oxide)⁹;
3. impaired GR α activity (eg, through GR α phosphorylation induced by IL-2-, IL-4-, or IL-13-mediated p38 mitogen-activated protein kinase activation) or impaired GR α nuclear translocation (eg, through increased expression of the chaperone protein FKBP51)⁹;
4. enhanced GR β expression, which acts as a natural antagonist for GR α (eg, IL-17)¹⁰;
5. inhibition of GR α transrepressive activity through impaired histone deacetylase 2 activity, which reduces histone acetylation (eg, through the effects of oxidative stress, nitric oxide, and hypoxia)⁹;
6. enhanced GCS efflux from cells (eg, because of increased activity of the drug efflux pump P-glycoprotein 170)⁹; and
7. the presence of parallel competing pathways, in which the intensity of the inflammatory response (eg, nuclear factor κ B, activator protein 1, c-Jun N-terminal kinase 1, signal transducer and activator of transcription 5, and Janus kinase 3) saturates out any beneficial effect of GCS.⁹

The study by Milara et al⁶ adds new information to this debate by showing that there is clear synergism between the anti-inflammatory effects of low concentrations of GCSs and RNO

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Disclosure of potential conflict of interest: E. R. Chilvers has received consultancy fees from Cambridge Clinical Research, GlaxoSmithKline, Novartis, Roche, and Med-Immune and has received research support from Roche, GlaxoSmithKline, and Med-Immune. L. Koenderman declares that he has no relevant conflicts of interest.

Received for publication April 23, 2014; accepted for publication April 25, 2014.

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J Allergy Clin Immunol 2014;■■■■:■■■■-■■■■.

0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2014.04.030>

and the capacity of RNO to normalize a very diverse array of processes thought to underlie GCS resistance in neutrophils from patients with COPD. These include reversing the increases in phosphoinositide 3-kinase δ , macrophage migration inhibitory factor, and GR β expression at the mRNA level observed in neutrophils from patients with COPD and restoring histone deacetylase activity and MKP-1 expression. Unfortunately, the authors do not integrate these findings into a unifying hypothesis as to how such different processes are controlled by RNO. There are also a number of technical issues regarding this study, including the use of a cohort of patients with COPD with very mild disease (mean FEV₁/forced vital capacity ratio, 69.2% $\pm$ 11%), when a number of studies have suggested that patients with severe disease and frequent exacerbations derive the most clinical benefit from roflumilast.¹¹ All patients were active smokers, and some were studied close to an exacerbation; their *in vitro* assays also relied on the use of pharmacologic concentrations of LPS (1 μ g/mL), which even then induced much lower levels of IL-8 than anticipated.

It is uncertain whether the abnormal neutrophil phenotype observed in patients with COPD is intrinsic or acquired and, if the latter, how and in which compartment this effect occurs. There is a clear imperative to progress these studies to the airway/alveolar neutrophil. The assumption that neutrophils from patients with COPD are intrinsically normal but influenced by local proinflammatory mediators is not necessarily valid because recent research has provided evidence for the existence of multiple neutrophil phenotypes,¹² including myeloid-derived suppressor cells, which might be modulated by smoking.¹³ If an MDSC type of neutrophil is indeed mobilized in patients with COPD, these cells could easily respond differently to RNO, reactive oxygen species, and GCSs. The second proposal is that oxidative stress is the dominant factor in driving the steroid resistance observed in blood neutrophils in patients with COPD and that RNO reverses this effect. Although attractive, the supportive evidence for neutrophil oxidative stress *in vivo* being secondary to a direct effect of cigarette smoking is not definitive. The authors themselves discuss the fact that similar effects on neutrophils have been seen in patients with inflammatory diseases that do not involve cigarette smoke. The alternative hypothesis, that neutrophil priming and the acquisition of steroid resistance occur in the circulation or bone marrow compartments as a result of exposure to inflammatory mediators, such as cytokines, chemokines, and bioactive lipids, seems more attractive.

The importance of targeting the neutrophil for future anti-inflammatory therapy in patients with COPD is clear, and several options now exist, including phosphodiesterase IV, CXCR₂, nuclear erythroid-related factor 2 (Nrf2), phosphoinositide 3-kinase δ , and elastase inhibition.^{14,15} However, it is uncertain at this stage whether inhibition of this cell type alone will be sufficient in a disease that involves multiple cell types and pathologic processes. Likewise, even if it is possible to restore the sensitivity of neutrophils to the anti-inflammatory effects of steroids, it is debatable whether GCS will ever become more attractive in treating

patients with COPD because even in health the effects of steroids on neutrophils are at best modest and in some cases detrimental (eg, increasing the overall number of circulating neutrophils and delaying apoptotic cell death). These concerns are compounded by reports highlighting the effect of inhaled corticosteroids on the incidence of pneumonia. Indeed, given the effect of RNO on intracellular reactive oxygen species generation, the effects of RNO alone or in combination with corticosteroids on bacterial killing and infection risk will need to be carefully assessed.¹⁶

In conclusion, roflumilast shows effectiveness in the treatment of COPD, and the article by Milara et al⁶ suggests a capacity, at least *in vitro*, to reverse the GCS-resistant phenotype of neutrophils from patients with COPD; the mechanism of this effect remains to be defined. This article provides a new impetus to continue to try to target neutrophils in patients with COPD.

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