overall population. In order to reveal a lower, but still significant difference in the cystic fibrosis transmembrane regulator ΔF508 allele prevalence, the number of patients should be increased dramatically. Hopefully, the worldwide existing large collections of DNA specimens from osteoporotic patients will provide an opportunity to enlighten the possible implication of a cystic fibrosis transmembrane regulator mutation in the development of osteoporosis.

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Tolerance to repeat exposure of inhaled endotoxin: an observation in healthy humans

To the Editors:

We read with interest the articles on endotoxin research in the May issue of the *European Respiratory Journal*. The editorial by BALS [1] aptly raised the yet unanswered questions concerning the timing (acute *versus* chronic) and doses of inhaled endotoxin relevant to health and disease, and the questions of whether the outcome of such exposure is always detrimental.

To this end, we wish to add our own preliminary observation of the possibility of tolerance to repeat exposure of inhaled lipopolysaccharide (LPS) in healthy nonatopic humans at 4 weeks. In a double-blind, crossover study, eight healthy human subjects were randomised to receiving either a single inhaled dose of 50 μg salmeterol or placebo prior to being challenged with a 15-μg dose of *Escherichia coli* serotype 026:B6 (Sigma, Poole, UK), in two visits separated by 4 weeks. Using 1 week prior as a baseline, sputum induced at the 6th h after LPS challenge showed no significant differences in the increase of total cell counts in the two treatment periods (mean difference (95% confidence interval) salmeterol *versus* placebo: 10.6×10^6 cells·mL^{-1} (-9.71–30.9); p=0.25) or neutrophils (11.7×10^6 cells·mL^{-1} (-8.33–31.92); p=0.20; unpublished data). The assertion that salmeterol does not protect against airway neutrophilic inflammation was subsequently supported in a more robust study, where subjects were randomised to receiving either daily salmeterol for 3 weeks or placebo, prior to inhaled LPS challenge, in a crossover study [2].

Retrospective power analysis of our results first alerted us to the possibility of intrinsic biological phenomena in a study design of sequential inhaled LPS challenges. Data were then re-analysed with the purpose of looking into the reproducibility of sputum neutrophilia between the two inhaled challenges, treating the effects of the single-dose salmeterol as no more than placebo [2]. Our findings showed that following the first LPS challenge, the mean total sputum cell counts increased by 31.23×10^6 cells·mL^{-1} (95% CI: 13.27–49.20) and the mean sputum neutrophil counts rose by 30.3×10^6 cells·mL^{-1} (12.59–48.11). However, following the second LPS challenge, the mean total sputum cell counts only increased by 11.3×10^6 cells·mL^{-1} (2.14–24.89) and mean sputum neutrophil counts by 10.9×10^6 cells·mL^{-1} (1.02–22.9). The difference between the means was statistically significant (p=0.01; mean difference: 19.8×10^6 cells·mL^{-1} (6.16–33.56) for total sputum cell counts; 19.4×10^6 cells·mL^{-1} (4.73–34.08) for sputum neutrophil counts; fig. 1).

Using such a human experimental model of airway neutrophilia to understand the inhaled effects of endotoxin [3], and to examine for potential anti-inflammatory properties of therapeutic agents [2] appears to be a validated approach. MICHEL *et al.* [3] employed a model of weekly inhaled challenges of incremental LPS doses (0.5 μg, 5 μg and 50 μg) to provide evidence for dose responsiveness of LPS in airway inflammation and systemic effects in healthy human subjects. WALLIN *et al.* [2] tested for possible anti-inflammatory effect of salmeterol *versus* placebo, *via* findings from bronchoscopy, based on a study design of inhaling 50 μg

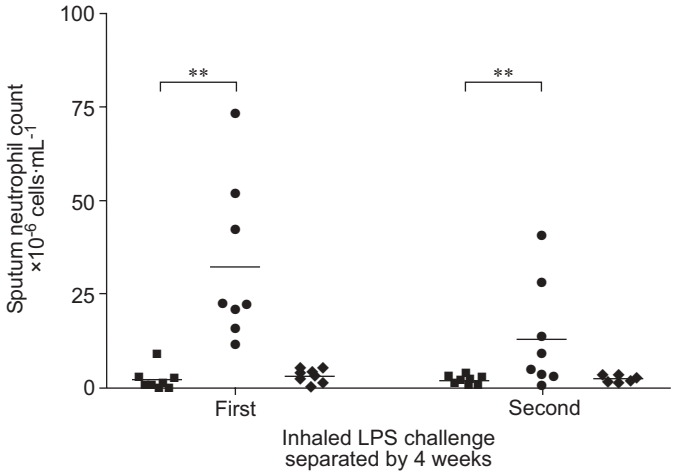

FIGURE 1. Comparison of sputum neutrophilia post-inhaled lipopolysaccharide (LPS; 6th h) between two inhaled LPS challenges separated by 4 weeks. ■: baseline; ●: 6 h post-inhaled LPD; ◆: 1 week post-inhaled LPS. **: denotes p=0.01 between the mean differences.

LPS on two occasions separated by ⩾3 weeks. However, none of these studies had observed tolerance towards subsequent LPS challenge(s) in their healthy human subjects at doses of LPS described that were higher than ours. It is possible that tolerance in healthy nonatopic human subjects only occurs in exposure to lower doses of inhaled endoxin. In fact, existing literature indicates that exposure of 30–40 μg inhaled LPS is probably the clinical threshold to induce symptoms and lung function changes for healthy subjects [4].

More research is required to validate our preliminary observation.

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From the author:

The study that L.C. Loh describes in his letter above adds another interesting aspect that is critical for the response to inhaled endotoxin.

Lipopolysaccharide tolerance is a well-known feature of several host defence cells, although the mechanisms involved are not entirely clear [1]. Tolerance has also been shown to be associated with various cellular processes, such as decreased activity of Gi proteins, protein kinase C, mitogen-activated protein kinase, activator protein-1 and nuclear factor-κB (NF-κB). Inhibitory molecules such as IRAK-M, suppressor of cytokine-signaling-1 and inhibitor-κB are found activated. At the nuclear level, the NF-κB subunit p50 homodimer expression and peroxisome-proliferator-activated receptors-γ are increased. There is evidence from rodent studies that this phenomenon is also relevant for pulmonary innate immunity [2].

The preliminary results described in this letter support this view and it is likely that this mechanism is of biological relevance, because the lung is constantly exposed to small amounts of lipopolysaccharide. The pulmonary exposure with endotoxin probably has many consequences. At this time it is uncertain where lipopolysaccharide tolerance is functionally located in this scenario.

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Pre-analytical conditions for the assessment of circulating MMP-9 and TIMP-1: consideration of pitfalls

To the Editors:

We read with interest the recent article of HIGASHIMOTO *et al*. [1], which reported an increased activity of tissue inhibitor of metalloproteinase (TIMP)-1 in patients with chronic obstructive pulmonary disease (COPD) and asthma. In contrast, the molar ratio between matrix metalloproteinase (MMP)-9 and TIMP-1 was significantly lower in COPD patients than in normal subjects.