have high predictive value and discard those that do not. It could be argued that such classification methods are of limited use, as they are based on empirical or phenomenological observations rather than physiological understanding of the underlying pathology. However, they are valuable for hypothesis generation and should always be affirmed by clinical intuition and practicability. More relevant to the present context, identification of highly ranked variables derived from complexity analyses would allow us to deduce whether or not controllability or adaptability are important features in respiratory disease. This would provide further validation for the clinical utility of the systems approach. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Comment on Expression of Concern: TLR4 Is Necessary for Hyaluronan-mediated Airway Hyperresponsiveness after Ozone Inhalation

To the Editor:

We would like to comment on the concerns about our article that was originally published in the April 1, 2010, issue of the *American Journal of Respiratory and Critical Care Medicine* (1).

In that article, we provided immunological, histological, and physiological evidence that Toll-like receptor 4 (TLR4) is necessary for hyaluronan-mediated development of inflammation and airway hyperresponsiveness after ozone inhalation. After the publication of the article, we became aware that data provided by the animal pulmonary physiology laboratory at Duke University, including the airway physiology measurements and inflammatory cytokine assays reported in Figures 1, 3, and 8B of the original article, may have been unreliable. This led to the publication of an Expression of Concern in the *Journal* (2).

We therefore had two separate laboratories, an independent laboratory at Duke University, under the supervision of Herman Staats, Ph.D., and one at the National Institute of Environmental Health Sciences, under the supervision of Stavros Garantziotis, M.D., repeat the experiments in question. These laboratories have replicated the key observation that TLR4-deficient mice have decreased airway hyperresponsiveness after ozone or short-fragment hyaluronan exposure. We observed statistically significant differences (by analysis of variance followed by all pairwise multiple comparison procedures, Holm-Sidak method) in airway resistance measured by flexiVent after intravenous methacholine (significant differences at 200 and 400 $\mu\text{g/kg}$; $P < 0.05$; $n = 6$ –10 per group) as well as after nebulized methacholine in a separate experiment (significant differences at 50 and 100 mg/ml ; $P < 0.05$; $n = 4$ per group). The measured values were similar to those reported in the original article. Furthermore, we repeated cytokine analysis in mouse lung tissue after 2-ppm ozone exposure and found significant reduction in TLR4-deficient mice for CXCL1/KC, IL-1 β , IL-6, CCL2/MCP1, and TNF- α ($P < 0.05$ for all, Student's t test). Further, we evaluated the effects of TLR4 on short-fragment hyaluronan-induced airway hyperresponsiveness and confirmed that TLR4-deficient mice had decreased airway resistance (by flexiVent; $P < 0.001$ at 50 mg/ml of nebulized methacholine dose). Finally, we evaluated the effects of TLR4 deficiency on short-fragment hyaluronan-induced cytokine release by bone marrow-derived macrophages *in vitro*. We confirmed that TLR4-deficient macrophages have reduced expression of CXCL1/KC, CCL2/MCP1, and TNF- α after exposure to either 50 or 200 $\mu\text{g/ml}$ of hyaluronan. Thus, these findings validate the originally reported findings in the article.

Taking into consideration the other data presented in the manuscript, we believe the data are valid and confirm the original conclusions of the article. ■

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