technology, which is able to identify both if the inhaled steroid has been used and if the inhaler has been used correctly. This will allow the test to be administered without a home visit and, thus, much less expensive to deliver. Burgess and colleagues appear to fail to appreciate that one of the utilities of this test is the ability to prevent this population inappropriately progressing to omalizumab and other emergent complex therapies, which is substantially more expensive.

Our data highlight the variation of adherence to different medications, with some participants adherent to oral corticosteroids and nonadherent to inhaled corticosteroids (ICS) (1). These individuals failed to suppress their F_{ENO} after directly observed ICS as they were not steroid naive. For this subset of patients, the test still has clinical utility in discriminating those who are likely to have a significant response by improving ICS adherence. The classification of these patients as nonadherent by the composite adherence measure negatively influenced the performance statistics of the F_{ENO} suppression test, accounting for the majority of patients inaccurately classified.

The recruitment difficulties for this study highlighted by Burgess and colleagues were due to several factors: many patients were taking medication appropriately and not suitable for inclusion, patients with poor adherence are less likely to consent to participate in a clinical trial (as opposed to an intervention in routine clinical practice as presented in the letter by Burgess and colleagues), and recruiting subjects at regional clinics resulted in significant travel issues for the clinical investigator. Many of these issues will no longer be relevant when the test is delivered using remote technology and is part of routine clinical practice.

Burgess and colleagues provide some evidence in support of using an electronic monitoring device in identifying nonadherence in children with poorly controlled asthma. However, it is recognized that monitoring inhaler use in this way has limitations; for example, it gives no information about whether or not doses were actually inhaled or were simply discharged, is also relatively expensive, patients can occasionally use the inhaler without the device attached, and battery failure and data retrieval issues have also been cited as being problems with the devices (2–5). The data from their current practice are somewhat unclear, as they integrate the identification of nonadherence with an adherence improvement strategy, and their pediatric patients differed from our population of adults referred to a tertiary care specialist difficult asthma clinic. It seems highly likely that the reasons for nonadherence and the benefits of an electronic monitoring strategy will be very different in these different populations, not least because of input from parents. However, we agree that Burgess and colleagues seem to provide further support to our previous work, specifically that when nonadherence is identified in the clinic, a concordance interview can result in a change in adherence behavior (6).

Our study enrolled patients in a tertiary referral center, and the F_{ENO} suppression test has been developed for this target population, as stated in our article (1). It specifically assesses adherence in patients with an elevated F_{ENO} and who are likely to be steroid responsive (7). This eliminates nonadherence due to lack of efficacy of treatment (8) and targets patients in whom improving ICS adherence will result in improved asthma control. This test also has the added advantage of identifying those patients who will benefit most from omalizumab, that is, poorly controlled asthma despite adherence to high-dose therapy with an elevated F_{ENO} (9), ensuring that only suitable patients progress to expensive biological therapies will almost certainly offset the cost of the test. We reiterate that the F_{ENO} suppression test is suitable for specialist difficult asthma clinics. It has clinical

utility in identifying nonadherence in the most at-risk patients with asthma and also allows appropriate targeting of omalizumab and other emergent biologic therapies in patients with adherence.

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Moving Forward in Sepsis Research

To the Editor:

We thank Dr. Perlman and colleagues for their timely and thoughtful perspective on the *Proceedings of the National Academy of Sciences of the United States of America* study describing large discrepancies between murine and human leukocyte transcriptional profiles obtained in critical illness settings (1, 2). The gulf between human and mouse in sepsis research was vividly illustrated by the recent negative phase 3 trial of Toll-like receptor 4 (TLR4) blockade (3). Yet, the discovery of TLR4 as the body's sensor for gram-negative endotoxin is rightly considered foundational in immunology and was driven in large part by murine studies recognized

by the 2011 Nobel Prize in Physiology or Medicine (4). How should the scientific community resolve this paradox?

The cross-species comparison of expression arrays by Seok and colleagues has injected new, important data into this ongoing conversation (2). Proving the absence of an association between mouse and human is difficult, as highlighted by the methodological issues raised in Perlman and colleagues' editorial (1). Given the profound implications of Seok and colleagues' article, clinicians and researchers interested in critical illness would have benefited from an even more intense effort by the authors to compare "apples to apples." We propose that two additional experiments would have strengthened their conclusions, particularly regarding the dissimilarity in leukocyte responses to endotoxin.

First, Seok and colleagues showed that the mouse transcriptional response to endotoxin was markedly attenuated immediately downstream of TLR4 (2). Because the murine version of TLR4 is considered the true ortholog of the human gene, this surprising result made us wonder whether an adequate exposure to endotoxin was achieved or whether the transcriptional signature of high-TLR4-expressing cells in mice (e.g., monocytes) was swamped out by lower-expressing cells (e.g., lymphocytes). Had Seok and colleagues been able to adjust endotoxin dose or duration to achieve comparable TLR4 signaling responses in their own readout, the subsequent negative findings would have been greatly bolstered.

Second, Seok and colleagues could have compared transcriptional responses to endotoxin of homologous cell types between species—for example, monocytes isolated from each species stimulated with the same endotoxin serotype analyzed at comparable doses and durations of exposure. Had the *ex vivo* profiles been unexpectedly similar, readers could then have interpreted the *in vivo* dissimilarity more deeply, for example, by raising the possibility that mouse and human responses to endotoxin do not diverge at the initial molecular events, but rather, in their subsequent effects on the intercellular network found within the body.

Overall, we commend Seok and colleagues on their nuanced approach to the mouse-as-sepsis-model question. We believe that ample evidence exists to support use of the mouse as a discovery tool, but that future mechanistic studies in sepsis should emphasize early validation in the human setting (5). Rather than delaying proof-of-concept human studies, basic and clinical researchers in this field should collaborate early and extensively to ensure that the most "translatable" science is moving forward.

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Reply

From the Editorialists:

Drs. Karumanchi and Parikh highlight the conflicting effects of activation of the Toll-like receptor 4 by LPS on peripheral blood leukocyte gene expression in mice and patients. This analysis is limited by many of the same methodological concerns raised in our editorial (1). In addition, because of safety concerns, the dose of LPS that Seok and colleagues (2) used to treat mice was ~100,000-fold less than the lethal dose in mice (~40 mg/kg), and a correspondingly low dose was administered to humans. Thus, the relevance of these studies to patients with sepsis is limited. The issue of the contribution of Toll-like receptor signaling in sepsis will be difficult to unravel as these receptors are likely to be activated by both primary (pathogen-related) and secondary (neutrophil extracellular traps and release of mitochondrial DNA) events that contribute to the pathogenesis of sepsis (3). The suggestion by Karumanchi and Parikh to use cultured human cells is one valid approach; however, culturing immune cells on plastic and other artificial factors imposed by *ex vivo* culture may skew the immune response of these cells. Therefore, we continue to stress the need for development of novel animal model systems, including the humanized mouse and others as preclinical models of human sepsis (4).

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