Correspondence

All Roses Are Flowers, But Not All Flowers Are Roses

To the Editor:

We read the editorial by Drs. Cornfield and Haddad (1) on our article (2) with considerable interest. The editorial raises important issues, including the importance of accurate definitions for disease states, but it has several misconceptions and errors that we wish to clarify and correct. Drs. Cornfield and Haddad (1) refer to our study as a trial. Although the information was collected as part of a weaning trial, this article is not a report of a trial. It is a descriptive study across nine centers for 6 consecutive months of patients who were mechanically ventilated for more than 24 hours (2).

Drs. Cornfield and Haddad (1) have used the term respiratory failure to refer specifically to acute respiratory distress syndrome (ARDS), which is incorrect. The editorial also incorrectly states that we were not specific in defining ARDS and that the incidence of ARDS was probably higher than reported. We defined ARDS as a Pa_{O_2}/FI_{O_2} ratio of 200 or less with bilateral infiltrates and no evidence of heart failure (2). Although 52% of our patients had a Pa_{O_2}/FI_{O_2} ratio of 200 or less during their stay, only 7.6% met the criteria for ARDS at 24 hours after admission. Timmons and colleagues (3) found that ARDS was found in only 39% of patients with acute hypoxemic respiratory failure, but they did not include any patient with acute ventilatory failure without hypoxia.

Drs. Cornfield and Haddad stated: "Implicit in the objective of the trial is a goal of determining the normative incidence and natural history of respiratory failure in children" (1). We clearly stated that our objective was to describe those children receiving mechanical ventilation for greater than 24 hours across large pediatric referral centers in North America to determine the feasibility of conducting clinical trials to improve the health outcomes of this population (2). The editorial (1) also criticizes our exclusion criteria. We excluded these patients for the express purpose of defining a population in which clinical trials may be undertaken. Patients with pulmonary hypoplasia, bone marrow transplantation, and abnormal vascular tone may not be suitable candidates for a clinical trial of a therapy to improve outcomes from acute respiratory failure. There have been at least three major clinical trials in children with respiratory failure (4–6). Our exclusion criteria are consistent with published studies.

Drs. Cornfield and Haddad (1) have criticized us for not including trauma patients. That is not correct. Trauma was reported in 30 patients (9.6%), and 25 had traumatic brain injury (2). In addition, they point out the difference in mortality rates between our study and that published in the literature (4–6). The studies on high-frequency oscillation and inhaled nitric oxide only included patients whose oxygenation index was greater than 13 and 15, respectively (4, 6). Clearly, these patients had severe disease. The surfactant study had an overall mortality of 11.9% for patients with an oxygenation index greater than 7 (5). Our lower mortality rate might have been secondary to inclusion criteria with a broad range of severity and to improved management strategies in an era of lung-protective ventilation.

We disagree with the conclusion that the significance of the study is diminished. In fact, we would state that it is exactly the opposite for the following reasons. First, it defines a group of children who can be potentially enrolled in clinical trials. Second, it provides an estimate of the population size that can be studied. For example, it would not be feasible to conduct a randomized controlled trial in all children with respiratory failure using mortality as the primary outcome because the mortality is low, especially in infants with bronchiolitis, the single most common diagnosis in this population of children. If we focus on a subset of patients with a higher mortality rate, such as those requiring mechanical ventilation with a clinical diagnosis of ARDS after 24 hours of mechanical ventilation, such a study would have to enroll patients from multiple centers to achieve sufficient power. Third, the editorial (1) points out the heterogeneity in the population requiring acute mechanical ventilator support for more than 24 hours and shows that half of these children are younger than 1 year of age. We believe that our study (2) did meet its intended goal: to provide future clinical investigators an estimate for conducting clinical trials in children with acute respiratory failure. In addition, our study provides estimates of individual subsets of patients with acute respiratory failure who may be eligible for different clinical trials.

Conflict of Interest Statement: S.V., A.R., J.H., P.F., I.C., R.G., and P.L. have no declared conflict of interest.

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Fungal Exposure and Lower Respiratory Illness in Children

To the Editor:

The limitation of data concerning clinically relevant exposures to agents, including fungi, in the indoor environment has been identified as an important problem by scientists studying build-

ing-related illness (1, 2). Despite the attempts to objectively characterize fungal exposure, the recent study of lower respiratory illness (LRI) among children by Stark and colleagues (3) appears to have important limitations in exposure assessment.

The method of defining fungal exposure used in that study places emphasis on specific fungal types, but does not allow for any characterization of the total fungal count in the areas being evaluated. As described in the study by Stark and colleagues (3), a home could have a "high fungal level" when persons in other homes could easily be exposed to greater concentrations of total fungi. For example, a home with an airborne *Aspergillus* level of 100 cfu/m^3 but no other fungi would be considered to have a "high fungal level," whereas a home with an *Aspergillus* level of 37 cfu/m^3, *Cladosporium* of 177 cfu/m^3, and *Penicillium* of 130 cfu/m^3 (mean airborne concentrations from the study, yet totaling 240 cfu/m^3) would not be "high" because none of those concentrations exceed the 90th percentile. Given the uncertainties concerning mechanisms of illness related to fungi, misclassification bias would be an important consideration for this type of exposure assessment.

We agree with the authors that another important limitation of their exposure assessment involves fungal samples being taken only once at the beginning of the survey, whereas information on the outcome measures were collected every 2 months for a year. It remains unclear whether there is any clinical relevance of measures of fungal exposure taken up to 12 months before LRI (or any other health effect).

In the article by Stark and colleagues (3), there appears not to have been any assessment of exposure to environmental tobacco smoke (ETS), whether at home or in daycare settings. Because ETS is known to be associated with increased morbidity in children (4), some assessment of ETS among the study participants would be important.

Finally, the authors draw conclusions concerning the relationship of measured fungal concentrations in houses with the presence or absence of water damage or visible mold in those houses. The methods of assessment of water damage/visible mold are not described, but the assessment appears to be based on parental self-report. We question the usefulness of such occupant self-report and suggest that an objective assessment of the indoor environment for moisture (perhaps using a moisture meter) would be more appropriate.

In summary, problems with assessment of exposure to fungi in the indoor environment, such as those pointed out above, are certainly not unique to the study in question. However, it is important to point out these limitations because they call into question the ability of the authors to draw conclusions concerning (*1*) appropriate types of evaluation techniques for indoor environments (the need for fungal sampling) and (*2*) the relationship of illness to exposure to fungi in the indoor environment.

Conflict of Interest Statement: D.B.T. and E.H.P. have no declared conflict of interest.

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From the Authors:

There is no consensus on a correct manner of characterizing potential fungal exposure in the home. Each method of estimating exposure through fungal measurement has significant limitations that would generally result in an underestimate of health effects by attenuating associations (1) (bias to the null). Because our starting point was examining the prospective associations between lower respiratory illness (LRI) and the presence of home levels of individual taxa that were relatively high for the cohort (>90th percentile for the cohort), it was sensible to follow these analyses by testing the *a priori* hypothesis that having a relatively high level of any one of the taxa might increase the risk for LRI. Drs. Trout and Page present an equally valid but different hypothesis: that even if one does not have a relatively "high" level of any one taxon, having an absolutely high total fungal count in the home may be associated with health effects. By examining "total" fungal counts exclusively, one would place emphasis only on the most abundant taxa—taxa that grow well on the plate or are abundant in ambient air or in the carpet/floor coverings. For example, homes with relatively high dust *Alternaria* levels (90th percentile, 8,333 cfu/g) but low yeasts (90th percentile, 58,000 cfu/g) would not be considered to have high fungal levels. We tested the alternative hypothesis that being in the 90th percentile of total fungi (for the taxa presented in the article [2]) predicted LRI, and found that being in homes with greater than 90th percentile for total airborne fungi predicted marginally higher risk for LRI (relative risk = 1.46; 95% confidence interval: 1.00–2.15), but being in homes with greater than 90th percentile for total dust-borne fungi did not. This suggests that in our cohort, having relatively high levels of known taxa, many of which have documented irritant or allergenic properties, is more predictive of infant LRI than having absolutely higher total levels of fungi, regardless of taxon composition. An additional limitation of using total culturable fungi is that many fungi that could be involved in exposure may be intrinsically unculturable, and thus are not included in the total count, constituting a significant confounder for total counts, but not for counts of specific culturable taxa.

As we stated (2), we were limited in that air and dust samples were taken only once in the first 2 to 3 months of each child's life and may not represent integrated exposure over the entire first year of life. However, it is a reasonable hypothesis that exposure to fungi in the first few months of life may influence the immune system or the propensity to respiratory symptoms, or both, over the first year of life. Moreover, it is possible that dust fungi represent, perhaps more than air fungi, the fungal characteristics of the home over longer periods.

We did consider the potential independent or confounding effects of environmental tobacco smoke (ETS) (2). Only 7% of children were exposed to ETS at home and 6% in daycare settings. Thus, whereas other studies with higher smoking rates have found reproducible significant effects of ETS on LRI in infancy, we did not. In univariate analyses, smoking in daycare settings was marginally ($p = 0.12$) associated with LRI; but in our final models, the association was less significant and did not change the magnitude or precision of the estimate of the effect of fungi on risk for LRI.

Moisture meters and point measurements of relative humidity are not gold standards for evaluating home dampness (3, 4). With their acknowledged limitations, questions regarding home dampness are a well established and accepted tool used to evaluate the reproducible associations between dampness and LRI in infancy (4). The combination of exposures that home dampness represents is not fully understood. Dampness likely represents other exposures in addition to fungi, and may represent conditions leading to fungal exposure not detected through culturable fungal methods (5). In cross-sectional studies, parents who have children with symptoms may tend to be biased toward answering positively to home dampness questions, but our study was longitudinal and less prone to this bias.

In summary, although we recognize the limitations of all studies of associations of home fungal levels with LRI, we believe that it is unlikely that the associations that we found between fungi and LRI risk are caused by bias or unmeasured confounders. Confidence in these findings will come if they are reproducible in other studies (4), and more work is needed to develop feasible methods of improving the characterization of early childhood fungal exposure in large-scale birth cohort studies.

Conflict of Interest Statement: D.G. and P.C.S. have no declared conflict of interest. H.A.B. consults for the Sharper Image, which markets an air cleaner designed to address exposure to fungal spores and other airborne contaminants, and receives approximately $6000 per month for these services.

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Toll-like Receptors and Allograft Rejection

To the Editor:

We read with interest the article from Palmer and colleagues (1) demonstrating that recipients heterozygous for a mutation in the toll-like receptor (*TLR*) *4* gene had reduced acute allograft rejection after lung transplantation. The authors also demonstrated that mutations in the allograft had no impact. It would be of interest to investigate whether transplant recipients with the mutated *TLR4* gene (Asp299Gly or Thr399Ile) had defects in adaptive immune function after transplantation in addition to reduced rejection rates. Do lymphocytes from these patients demonstrate reduced interferon-γ production in response to donor antigen? Is there a reduction in the number of donor-specific antibodies in these recipients? These are important questions as work in experimental models has demonstrated that mice that are deficient in an important TLR signal adaptor protein, MyD88, have impaired priming and production of helper T cell type 1 immune responses in infectious and transplant models (2, 3). In our work using *TLR2*-, *TLR4*-, and *MyD88*-deficient animals, we demonstrated that allograft rejection of HY incompatible skin allografts was largely abrogated in the absence of MyD88 (2). *TLR2*-deficient animals had a marginal delay, but *TLR4* recipients did not manifest reduced acute rejection, in contrast to the work by Palmer and colleagues (1). Furthermore, rejection could be reestablished by restoring *MyD88* signaling in either the donor or the recipient. Clearly, there are likely to be large differences between an experimental model and a clinical study. It would be of interest to investigate whether the *TLR4* mutation (or any other TLR mutation) impacts other types of solid organ transplantation.

Conflict of Interest Statement: D.R.G. and B.M.T. have no declared conflict of interest.

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From the Authors:

We thank Drs. Goldstein and Tesar for their comments regarding our study (1). Goldstein and colleagues recently observed that skin allograft rejection did not occur in mice with targeted disruption of *MyD88*, but did occur in those with disruption of toll-like receptor (*TLR*) *2* or *TLR4* (2). In contrast, we found decreased acute rejection in lung transplant recipients heterozygous for either of two mutations in *TLR4* previously associated with endotoxin hyporesponsiveness (3). There are likely several important explanations for the differences between their animal model and our clinical study. First, environmental exposure directly into the allograft makes clinical lung transplant unique. Inhalational exposure to air pollution (including endotoxin), infectious agents (such as gram-negative bacteria), and other noxious toxins occurs on a regular basis after lung transplantation. As a result, *TLR4* may be of particular importance in the initiation of innate and adaptive immune responses after lung transplantation. Genetic differences in *TLR4* signaling, therefore, might exert a greater influence on posttransplant rejection in lung transplant as compared with other organs. Second, in contrast to the murine model used by Dr. Goldstein, in which mice were identical at the major histocompatibility (MHC) loci, almost all human lung allograft recipients have multiple MHC mismatches with the donor (4). MHC matching is not performed because of short cold ischemic times tolerated by lung allografts. The absence of a significant effect with the *TLR4* disruption in the murine model does not address the impact of impaired *TLR4* signaling in the

setting of multiple MHC differences. Finally, we were interested to see that in the study by Goldstein and colleagues (2) there was a trend toward decreased skin allograft rejection in mice with disruption of *TLR4* ($p = 0.13$), with one *TLR4*$^{-/-}$-recipient mouse demonstrating indefinite skin graft survival, providing some experimental support for our results.

We agree that allograft rejection is a complex biological response, and further study of the cellular and humoral response to the allograft in lung transplant recipients with the 299/399 polymorphisms is critical. We look forward to pursuing additional studies that elucidate the mechanisms by which innate and adaptive immunity interact in the setting of human organ transplantation. Further investigation into the immunogenetics of the alloimmune response may greatly enhance our ability to prevent and treat clinical rejection. Ultimately, both our clinical study and the animal work suggest an important and previously unrecognized role for innate immunity in the development of allograft rejection.

***Conflict of Interest Statement*:** S.M.P. and L.H.B. have no declared conflict of interest. D.A.S. has a patent pending on toll-4 assay and has no other declared conflict of interest.

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Forces in Emphysema: Newtonian v Quantum Mechanics

To the Editor:

We read with great interest the recent review of the pathobiology of emphysema by Suki and colleagues (1). In it the authors addressed classic hypotheses regarding the etiologic factors of emphysema—for example, protease–antiprotease, inflammation, mechanical forces, and collagen deposition. We were particularly interested in the authors' position on the role of mechanical forces as merely having blunt physical effects on the lung parenchyma, which overlooks a growing body of evidence that shows how physical forces can have specific molecular physiologic or pathophysiologic effects on the developing and adult lung (2). Workers in our laboratory (3) and others (2) are studying the influence of mechanical forces on normal lung morphogenesis and its possible role in lung pathology. Suki and colleagues (1) have taken the more conservative position that physical forces merely dissect the tissue as if it were a hank of rope under tension (their analogy). We prefer to think of physical forces "molding" the lung during normal morphogenesis, and perhaps explaining how varying tension can explain both ventilation–perfusion matching and the cause of emphysema. The bases for such speculation are myriad: lung morphogenesis is highly plastic, literally being "molded" by amniotic fluid distension *in utero* (4); postnatally, lung structure/function are influenced by physical forces that allow the alveolar wall of the lung to remodel under the influences of distension and contraction, through a common paracrine mechanism; and perhaps even wedge resection for the treatment of emphysema represents a means by which the remaining healthy, but restricted parenchyma, can be salvaged by allowing it to "stretch."

Parathyroid hormone–related protein (PTHrP) is a stretch-regulated paracrine hormone that is necessary for normal lung development; knocking out this gene causes stage-specific failure of lung morphogenesis (5). Recent studies from our laboratory have demonstrated the importance of PTHrP signaling between epithelium and interstitium for alveolar homeostasis (6); in the absence of PTHrP signaling, both the interstitial and epithelial cells readapt in a manner mimicking fibrosis. We acknowledge that this is a minority position, but one that offers the possibility that chronic lung disease is part of the continuum of development, homeostasis, and repair (7), and that as such it may be a reversible (i.e., treatable) process.

***Conflict of Interest Statement*:** J.S.T. and V.K.R. have no declared conflict of interest.

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Dr. Suki was given the opportunity to respond to this letter but declined to do so.