Reply to Dr. Weiskirchen

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TO THE EDITOR: We would like to thank Dr. Weiskirchen (5) for his interest in our recent publication (3). As we mentioned in the DISCUSSION and as pointed out by Dr. Weiskirchen, the absence of protein data in our adenovirus model represents a limitation of our study. Regarding the RNA expression, we also expected to find a greater difference in ccn1/cyr61 gene expression in the mice instilled with AdCyr61 compared with the control group. Although inadequate gene transfer is a possibility, we would like to point out other possibilities. The RNA was extracted from whole lung preparations. However, the adenovirus was delivered by intratracheal instillations, which result in a patchy distribution of the instillate, leaving large areas of the lung without exposure to the adenovirus. Therefore, only a portion of the measured lung was expected to show increased expression of CYR61. In addition, only the airway and alveolar epithelial cells were expected to be exposed to the adenovirus, and they represent only a fraction of the total lung cells. These two reasons would result in lower total lung expression of ccn1/cyr61, even if local expression at the target cells was robust. Further loss-of-function studies of CYR61 in the lungs will be required to confirm our results.

Dr. Weiskirchen expressed a desire for additional information regarding the cloning and purification of the adenovirus. Additional details on the method we used for vector generation, cloning, and production of recombinant adenovirus can be found in the supplementary materials and methods of Ren et al.’s paper (4). For the purification process we used cesium chloride gradient ultracentrifugation as described by Chartier et al. (2). Although we agree that the presence of trace of cesium in our adenovirus cannot be completely excluded, we believe that it didn’t impact our results because both adenoviruses, AdCtr and AdCyr61, were purified by the same method and we did not observe any toxic effects in the control group.

Dr. Weiskirchen is interested in the weight loss we observed in the group of mice instilled with AdCyr61. Rapid weight loss is a common finding of acute lung injury models and is likely due to a combination of decreased food and water intake and increased insensible losses. Finally, we thank Dr. Weiskirchen for pointing out two mistakes regarding CYR61 definition that were made during the editing process and were not present in the submitted manuscript.

REFERENCES


