13. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, *et al.* Genome-wide cell-free DNA fragmentation in patients with cancer. *Nature* 2019;570:385–389.
14. Ding SC, Lo YMD. Cell-free DNA fragmentomics in liquid biopsy. *Diagnostics (Basel)* 2022;12:978.
15. Gao Q, Zeng Q, Wang Z, Li C, Xu Y, Cui P, *et al.* Circulating cell-free DNA for cancer early detection. *Innovation (Camb)* 2022;3:100259.
16. Wang S, Meng F, Li M, Bao H, Chen X, Zhu M, *et al.* Multi-dimensional cell-free DNA fragmentomic assay for detection of early-stage lung cancer. *Am J Respir Crit Care Med* 2023;207:1203–1213.

Nuclear Factor-κB Keeps Basal Cells Undifferentiated in Congenital Diaphragmatic Hernia

Among the congenital anomalies impacting lung development, congenital diaphragmatic hernia (CDH) is among the most common, affecting 1 in 2,500 pregnancies, with the most severe defects having mortality rates exceeding 50% (1) and survivors having substantially impaired lung function (2). There are at least three notable derangements in the CDH lung that contribute to morbidity and mortality. First, the mass effect of abdominal organs entering the chest causes hypoplasia of ipsilateral and contralateral lungs. In a multivariate analysis of magnetic resonance imaging–derived body measurements of 63 third-trimester fetuses with CDH, only percent predicted lung volume was predictive of the need for extracorporeal membrane oxygenation and survival to discharge (3). In addition, not only the lung parenchyma but also the conducting airway cross-sectional area are reduced and are prone to dynamic collapse (4). Second, the pulmonary microvasculature is severely pruned in CDH (5), and pulmonary hypertension is common (6). In a cohort of 220 infants with CDH, B-natriuretic peptide (a marker of atrial distention) and echocardiographic measures of pulmonary hypertension were significantly associated with mortality (7). Third, and perhaps less well known, the lung in general, and the epithelium, in particular, are less mature (8, 9) and have altered protein homeostasis (10). In this issue of the *Journal*, Wagner and colleagues (pp. 1214–1226) use basal cells from the tracheal aspirates of infants with CDH and a rat CDH model to show that nuclear factor (NF)-κB signaling plays a key role in this respiratory epithelial cell dysfunction (11).

Although the embryonic lung epithelium is derived from epithelial progenitor cells (12), the canonical stem cell of the fetal conducting airway epithelium is the same as in the adult conducting airway epithelium: the P63 + basal cell. The key finding of Wagner and colleagues is that basal cells of infants with CDH have an epigenetic predisposition to inflammasome activation that is absent in the basal cells of term and premature infants. Through a series of *in vitro* and *in vivo* experiments with correlation in CDH and non-CDH human lungs, NF-κB activity prevented the differentiation of basal cells into club and ciliated cells and preserved the immature epithelial phenotype in cell culture and in fetal rat airways. Thus, NF-κB is responsible for maintaining the CDH lung epithelium in a persistently immature state. In this study, general and specific inhibition of NF-κB with dexamethasone and JSH-23, respectively, restored basal cell differentiation.

Although the authors are to be commended for their creative approach in culturing basal cells from the tracheal aspirates of recently born infants, they did not definitively show that the behavior of basal cells that become dislodged from the basement membrane is representative of basal cells at large, except insofar as to show that fetuses with CDH have an expansion of basal cells compared with non-CDH fetuses. However, there are two reasons to think this is likely the case. First, like the basal cell culture model, expansion and impaired differentiation of basal cells were observed in the rat CDH model. Second, in human subjects with CDH, whatever process caused the observed epigenetic changes in the detached basal cells was likely present before birth and throughout the epithelium. It would be interesting to evaluate the tracheal epithelium of CDH and control fetal specimens for these same epigenetic marks and to test whether the loss of such marks occurs during maturation.

The interplay of lung development and inflammation is complex, and this paper adds to our understanding of how best to use antiinflammatory agents to optimize lung function in CDH. Chorioamnionitis accelerates lung maturation, and prenatal corticosteroids improve alveolar type 2 cell function (13), but late prenatal corticosteroids for fetuses with CDH do not improve survival or lung function (14). There is a growing understanding of how inflammatory signaling interacts with progrowth programs such as transforming growth factor-β (15) and how macrophages influence lung development (16). Mesenchymal stem cells (MSCs) and their extracellular vesicles attenuate many NF-κB signaling cascades and have been variably effective at reducing injury and improving outcomes in a host of conditions (17). MSCs and MSC extracellular vesicles improved lung development in the rat nitrofen CDH model (18, 19). However, the effects may not be purely antiinflammatory, because Zani and colleagues recently demonstrated that extracellular vesicles from amniotic fluid MSCs acted via transfer of microRNAs to improve lung development (20). It is notable that in the present study, Wagner and colleagues administered dexamethasone on three consecutive days during the pseudoglandular stage of lung development. Negative human studies administered four doses during the late saccular stage of lung development (14). Therapies that aim to improve lung structure and

Supported by National Institutes of Health, National Heart, Lung, and Blood Institute, grant R01HL141229.

Originally Published in Press as DOI: 10.1164/rccm.202302-0290ED on March 8, 2023

cell composition would need to begin sooner and to be administered more frequently than has been done in the past.

Understanding how antiinflammatory therapies impact other CDH treatments is important. There is a curious similarity in the cellular phenotype of CDH lung and the lung cell populations of experimental animal fetuses subjected to fetal tracheal occlusion. Fetal tracheal occlusion is a prenatal surgical procedure for CDH in which a fetoscopically placed balloon temporarily prevents egress of epithelial secretions with resultant growth of the lung. The procedure improves survival in fetuses with severe CDH (12). Inflammatory cytokines were increased in an ovine model of tracheal occlusion with and without surgical CDH (2), and basal cells were increased in a mouse model of tracheal occlusion without CDH (13). This finding was attributable to Yap and not NF-κB. Humans with congenital high airway obstruction also had basal cell expansion (13). Although the basal cell epigenetic landscape is not known in these cases, it could be that prenatal airway inflammation in general leads to basal cell persistence and that the phenotype is not restricted to CDH. With the emergence of newer technologies, we can better test such hypotheses and gain a better understanding of how the respiratory epithelium matures *in utero* and how best to target NF-κB to improve neonatal lung health. ■

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

Brian M. Varisco, M.D.
Division of Critical Care Medicine
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio
and
University of Cincinnati College of Medicine
Cincinnati, Ohio

ORCID ID: 0000-0003-0180-2577 (B.M.V.).

References

1. Wagner R, Montalva L, Zani A, Keijzer R. Basic and translational science advances in congenital diaphragmatic hernia. *Semin Perinatol* 2020;44: 151170.
2. Miles KG, Powell AW, Critser PJ, Hardie W, O'Neil M, Cash M, *et al.* Long-term exercise and pulmonary function outcomes in a contemporary cohort of children with congenital diaphragmatic hernia. *Pediatr Pulmonol* [online ahead of print] 7 Feb 2023; DOI: 10.1002/ppul.26348.
3. Niemiec SM, Louiselle AE, Phillips R, Gien J, Zaretsky MV, Derderian SC, *et al.* Third-trimester percentage predicted lung volume and percentage liver herniation as prognostic indicators in congenital diaphragmatic hernia. *Pediatr Radiol* 2023;53:479–486.
4. Bates AJ, Higano NS, Hysinger EB, Fleck RJ, Hahn AD, Fain SB, *et al.* Quantitative assessment of regional dynamic airway collapse in neonates via retrospectively respiratory-gated ^{1}H ultrashort echo time MRI. *J Magn Reson Imaging* 2019;49:659–667.
5. Stainsby AV, DeKoninck PLJ, Crossley KJ, Thiel A, Wallace MJ, Pearson JT, *et al.* Effect of prenatal diaphragmatic hernia on pulmonary arterial morphology. *Anat Rec (Hoboken)* [online ahead of print] 23 Jan 2023; DOI: 10.1002/ar.25159.
6. Zani A, Chung WK, Deprest J, Harting MT, Jancelewicz T, Kunisaki SM, *et al.* Congenital diaphragmatic hernia. *Nat Rev Dis Primers* 2022;8:37.
7. Avitabile CM, Wang Y, Zhang X, Griffis H, Saavedra S, Adams S, *et al.* Right ventricular strain, brain natriuretic peptide, and mortality in congenital diaphragmatic hernia. *Ann Am Thorac Soc* 2020;17: 1431–1439.
8. Peiro JL, Oria M, Aydin E, Joshi R, Cabanas N, Schmidt R, *et al.* Proteomic profiling of tracheal fluid in an ovine model of congenital diaphragmatic hernia and fetal tracheal occlusion. *Am J Physiol Lung Cell Mol Physiol* 2018;315:L1028–L1041.
9. Varisco BM, Sbragia L, Chen J, Scorletti F, Joshi R, Wong HR, *et al.* Excessive reversal of epidermal growth factor receptor and ephrin signaling following tracheal occlusion in rabbit model of congenital diaphragmatic hernia. *Mol Med* 2016;22:398–411.
10. Khalaj K, Antounians L, Figueira RL, Post M, Zani A. Autophagy is impaired in fetal hypoplastic lungs and rescued by administration of amniotic fluid stem cell extracellular vesicles. *Am J Respir Crit Care Med* 2022;206:476–487.
11. Wagner R, Amonkar GM, Wang W, Shui JE, Bankoti K, Tse WH, *et al.* A tracheal aspirate-derived airway basal cell model reveals a proinflammatory epithelial defect in congenital diaphragmatic hernia. *Am J Respir Crit Care Med* 2023;207:1214–1226.
12. Hogan BL, Barkauskas CE, Chapman HA, Epstein JA, Jain R, Hsia CC, *et al.* Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell* 2014;15:123–138.
13. Dehaene I, Steen J, Dukes O, Olarte Parra C, De Coen K, Smets K, *et al.* On optimal timing of antenatal corticosteroids: time to reformulate the question. *Arch Gynecol Obstet* [online ahead of print] 4 Feb 2023; DOI: 10.1007/s00404-023-06941-w.
14. Lally KP, Bagolan P, Hosie S, Lally PA, Stewart M, Cotten CM, *et al.*; Congenital Diaphragmatic Hernia Study Group. Corticosteroids for fetuses with congenital diaphragmatic hernia: can we show benefit? *J Pediatr Surg* 2006;41:668–674, discussion 668–674.
15. Holzfurtner L, Shahzad T, Dong Y, Rekers L, Selting A, Staude B, *et al.* When inflammation meets lung development-an update on the pathogenesis of bronchopulmonary dysplasia. *Mol Cell Pediatr* 2022;9:7.
16. Heydarian M, Schulz C, Stoeger T, Hilgendorff A. Association of immune cell recruitment and BPD development. *Mol Cell Pediatr* 2022;9:16.
17. Ting AE, Baker EK, Champagne J, Desai TJ, dos Santos CC, Heijink IH, *et al.* Proceedings of the ISCT scientific signature series symposium, "Advances in cell and gene therapies for lung diseases and critical illnesses": International Society for Cell & Gene Therapy, Burlington VT, US, July 16, 2021. *Cytotherapy* 2022;24:774–788.
18. Yuniartha R, Alatas FS, Nagata K, Kuda M, Yanagi Y, Esumi G, *et al.* Therapeutic potential of mesenchymal stem cell transplantation in a nitrofen-induced congenital diaphragmatic hernia rat model. *Pediatr Surg Int* 2014;30:907–914.
19. Takayama S, Sakai K, Fumino S, Furukawa T, Kishida T, Mazda O, *et al.* An intra-amniotic injection of mesenchymal stem cells promotes lung maturity in a rat congenital diaphragmatic hernia model. *Pediatr Surg Int* 2019;35:1353–1361.
20. Antounians L, Catania VD, Montalva L, Liu BD, Hou H, Chan C, *et al.* Fetal lung underdevelopment is rescued by administration of amniotic fluid stem cell extracellular vesicles in rodents. *Sci Transl Med* 2021; 13:eaax5941.