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Cell Therapy with the Cell or without the Cell for Premature Infants? Time Will Tell

Bronchopulmonary dysplasia (BPD) remains one of the main complications in preterm infants born before 28 weeks' gestational age (GA) (1). Advances in perinatal care since the original description of BPD more than 50 years ago have allowed the survival of preterm infants as young as 22 weeks' GA. The corollary is that these infants are now born at the limit of biological viability because their lungs are still at the late canalicular stage when blood vessels and airways are just becoming juxtaposed. The task of protecting the ever more immature lung is becoming increasingly challenging. In a sense, neonatologists are victims of their own success. Not surprisingly, an increasing number of reports describe the long-term consequences of BPD in young adults. Pulmonary vascular disease, cardiac dysfunction, and emphysematous changes may result from early disruption of normal lung development, impaired repair processes, and early aging (2–4). Although incremental improvements in the use of our current therapies—such as less-invasive surfactant administration, for example (5)—can have an immediate positive impact on the incidence and severity of BPD, additional innovative treatments may be required to prevent and/or repair lung damage to substantially improve the respiratory outcome of micropremies.

Cell therapies for regenerative benefits represent such a promising approach. Mesenchymal stromal cells (MSCs) in particular have attracted attention in part because of their ease of isolation, culture, and expansion and because of their putative pleiotropic effects (6–8). Yet it is the immune-modulatory and reparative effects of MSCs that provided the biological plausibility for these cells to be tested in diseases with a strong inflammatory component such as the acute respiratory distress syndrome (9, 10) and BPD (11). Furthermore, MSCs do not engraft but rather act via a "hit-and-run" mechanism through cell-to-cell contact and the release of bioactive molecules contained in nano-sized particles termed exosomes or small extracellular vesicles (12, 13). These observations opened exciting prospects for cell therapies without the cell.

In this issue of the *Journal* (14), Willis and colleagues (pp. 1418–1432) follow up on their original findings (15) to explore more in detail the molecular mechanisms by which MSC-derived small extracellular vesicles (MEx) exhibit their lung-protective effects in a well-established lung injury model in newborn mice exposed to hyperoxia. Biodistribution studies after intravenous injection revealed that MEx localize mostly to the liver and the lung. MEx interact with lung myeloid cells, restore the apportion of alveolar macrophages, and attenuate proinflammatory cytokine production. In a series of elegant experiments, the group demonstrates that MEx promote an immunosuppressive bone marrow–derived myeloid cell (BMDMy) phenotype: adoptive transfer of MEx-educated BMDMy, but not naive BMDMy, preserved alveolar architecture, blunted fibrosis and pulmonary vascular remodeling, and improved exercise capacity in this model. These findings provide further evidence for the antiinflammatory and reparative mechanisms of action of MSCs and their MEx.

Based on the above results, it is not surprising that MEx were found to accumulate mostly in the liver within 24 hours. Whether the liver could be the exclusive site of further macrophage/myeloid cell education or whether MEx migrate to the BM to directly interact with immune cells in this location deserves further exploration. Likewise, lineage tracing studies may answer the question whether educated cells subsequently migrate from the BM to the lungs or whether MEx only affect circulating immune cells. MEx administration early during the disease process was also able to blunt fibrosis, arguing in favor of early intervention and thus providing some clinical directions for these findings. Finally, it is uncertain whether identification of the MEx biological cargo will be critical for the clinical application of MEx therapy, although more understanding of the RNA and protein components that are most therapeutic might advance more focused therapies for preventing BPD in micropremies.

Although these observations demonstrate that much more needs to be learned about the biology of MSCs and their nanovesicles, the time is ripe for well-designed early-phase clinical trials to test the feasibility and safety of MSC-based therapies in preterm infants at risk of BPD. The results of the very first phase I trials suggest feasibility and short-term safety of a proprietary cord blood–derived MSC product administered as early as 10 days of life via the intratracheal route (16–18). Results of a phase II trial testing this same product in 66 preterm infants at 23–28 weeks' GA did not show a significant improvement in the primary outcome of death or moderate/severe BPD with MSCs compared with placebo (19). In that study, a subgroup analysis suggested an improvement in the secondary outcome of severe BPD (53% [8/15] to 19% [3/16]) with MSCs in the 23 to 24 gestational weeks group, but the study was underpowered, prompting a larger trial focused on these lower GA categories. Other cell products such as human amnion epithelial cells

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have also entered the clinical arena and shown feasibility and short-term safety when administered intravenously in preterm infants with established BPD after 36 weeks' corrected age (20). A current phase I trial is testing the safety and feasibility of higher and multiple doses of human amnion epithelial cells (ACTRN12618000920291). Numerous trials are planned or currently underway to answer the abundant questions about the optimal route, dosing, timing, single or multiple administration, and cell source. How to manufacture an optimal cell product is a research field on its own and will require close cross-disciplinary interactions with bioengineers. Clearly, cell-based therapies for BPD are in their infancy, and parallel work in the laboratory and the clinic will unravel the true potential of this disruptive technology. The current work by Willis and colleagues provides further rationale for testing yet another potential innovative therapy using extracellular vesicles derived from bone marrow MSCs. Well-designed preclinical and clinical studies are warranted to help answer another question: Do micropremies need microtherapies (the cells) or nanotherapies (the extracellular vesicles), or could both approaches be beneficial? ■

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