Stem Cells for Extreme Prematurity

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Abstract

Regenerative medicine is a bourgeoning field promising to repair damaged organs and thus has created high hopes in neonatology to curb some of the complications due to extreme preterm birth. Extensive laboratory investigations over the past 15 years have tried to harness the regenerative potential of a variety of (stem) cell-based therapies. Most preclinical studies have focused on experimental neonatal lung and brain injury. These promising results lead to the initiation of phase I clinical trials for chronic lung disease of prematurity and severe intraventricular hemorrhage, two of the most devastating complications of extreme preterm birth. Despite this relative rapid clinical translation, major gaps persist in our understanding of the biology of these putative repair cells and our ability to predict the quality and thus the efficacy of the cell product. This review will provide a brief overview of the various cell-based therapies that have been investigated in experimental neonatal lung injury and the remaining challenges in utilizing these new, disruptive therapies to their full extend to realize the promise of regenerative medicine in neonatology.

Keywords
- lung
- injury
- stem cells
- regenerative medicine
- preterm birth

Bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, remains the main complication of extreme prematurity.[1] Dramatic improvements in perinatal care have substantially increased neonatal survival from 34 weeks' gestation since the original description of BPD in 1967[2] to 22 to 24 weeks' gestation nowadays. The ransom of success is the increasing challenge of protecting an ever more immature and fragile lung from the multiple deleterious effects of inflammation, oxidative stress, mechanical ventilation, and suboptimal growth.[3] Even so the lung has robust repair potential, the life-long consequences of interfering with early lung development remain unknown. Increasing evidence in the literature suggests reduced lung function, airway hyperreactivity,[4] and impaired lung vascular growth,[5] as well as other organ impairment[6] justifying the need for exploring effective early interventions that are currently lacking.

Recent insights into the regenerative potential of stem cells[7] have created excitement in neonatology for the treatment of complications of extreme prematurity including BPD. Stem cells are defined as cells capable of self-renewal and differentiation into at least one other cell type. However, it is not these fundamental properties that seem to underlie their mechanism of action. Preclinical and some clinical evidence do not support the local proliferation and differentiation of these cells into lung cells for repair. The therapeutic benefits seem to emanate from a by-stander effect: the secretion of repair modulating factors in the microenvironment,[8] cell-to-cell interactions via the exchange of micro-organelles, and release of extracellular vesicles (EVs).[9] Given the excitement in the field, a panoply of cell-based therapies have emerged.

Brief Appraisal of Cell-Based Therapies Investigated in Preclinical and Clinical Studies of Neonatal Lung Injury

Mesenchymal Stromal Cells—The Front Runner

Definition

Mesenchymal stromal cells (MSCs) are plastic adherent, multipotent cells (they can be induced to become cartilage, bone, or

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fat) that express certain cell surface markers (CD105, CD73 and CD90, and lack of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules) as defined by the International Society for Cellular Therapy.[10] Originally described in the bone marrow,[11] MSCs have now been shown to exist in all organs including the umbilical cord tissue and cord blood.[12]

Rationale and Biological Plausibility

MSCs are potent immune-modulatory cells, but also exert pleiotropic effects that target numerous other pathophysiological mechanisms that contribute to BPD: MSCs are antifibrotic, antioxidant, and proangiogenic.[8] Other logistical aspects make them appealing as an off-the-shelf ready-to-use cell therapy product as MSCs express HLA class I, but not HLA class II, and thus do not elicit alloreactive lymphocyte proliferation, enabling allogeneic transplantation of MSCs. Furthermore, evidence suggests that MSCs are more abundant in the tracheal aspirate of preterm infants that go on to develop BPD versus those that do not.[13] These MSCs seem to acquire by default a profibrotic phenotype suggesting they may lose their repair potential and/or even contribute to the disease pathogenesis. These findings are corroborated by observations of human fetal lung mesenchymal cells exposed to hyperoxia in vitro[14] and in resident lung MSCs isolated from neonatal rats exposed to 14 days of hyperoxia.[15]

Preclinical Evidence

Numerous investigators have demonstrated the lung protective effect of MSCs derived from rat bone marrow[16,17] and human umbilical cord tissue[18] and cord blood[19] in the neonatal rodent model exposed to hyperoxia. Even so various endpoints were assessed including the route of administration, dose–response, timing, long-term effect, and toxicity, as well as some mechanisms of action, most of these studies were exploratory in nature. While a systematic review and meta-analysis of all preclinical studies with MSCs in experimental neonatal lung injury confirmed the therapeutic benefit of MSCs on alveolarization, inflammation, and lung vascular growth, it also revealed some limitations in experimental design, reporting, and risk of bias, as well as the lack of studies in large animals.[20]

Clinical Trials

Nonetheless, the promising preclinical data and the apparent safety and lack of engraftment of MSCs have encouraged early phase clinical trials in preterm infants at risk of developing BPD. The first phase I clinical trial tested allogeneic cord blood-derived MSCs in nine preterm neonates born between 23 and 29 weeks' gestational age at 5 to 14 days of life if still on mechanical ventilation.[21] A single intratracheal injection of 10^7 or 2×10^7 MSCs was shown to be feasible and well tolerated without serious adverse events or dose-limiting toxicity. At 2 years follow-up, there were no adverse effects on growth, respiratory, and neurodevelopmental outcomes.[22] Results of a U.S. trial with the same cell product and similar design are pending (NCT02381366). A phase II randomized, double-blinded, multicenter, controlled trial using a low dose is currently underway (NCT01828957). A recent case report of two preterm infants with severe BPD treated at late stages of the disease showed that the repeated intravenous (IV) injection of bone marrow-derived MSCs was feasible and safe, but it did not influence disease outcome.[23] Postmortem lung analysis confirmed absence of lung engraftment of transplanted cells. Well-designed early phase trials over the coming years will provide more information regarding feasibility and safety of routes of administration, timing, and repeated administration.[24]

Human Amniotic Epithelial Cells—The Mimic: Different Cell Type, Same Mechanism of Action

Definition

A large number of human amniotic epithelial cells (hAECs) can be isolated from the amniotic membrane of the placenta after birth. hAECs are stem cell-like cells with self-renewal and multilineage potential.[25] Similar to MSCs, they have limited class IA and II HLA expression and thus present a low risk of allogeneic rejection.

Rationale and Biological Plausibility

hAECs also share similar pleiotropic, mostly anti-inflammatory, but also proangiogenic, properties with MSCs.[25] hAECs do not engraft and exert their therapeutic benefit through a paracrine activity.

Preclinical Evidence

Extensive experimental evidence has demonstrated the lung protective effects of hAECs not only in neonatal rodents exposed to hyperoxia, but also in fetal sheep exposed in utero to lipopolysaccharide or mechanical ventilation.[26–28] Similar to MSCs, the relative ease of cell isolation, expansion and the lack of risk of rejection, and apparent safety profile are appealing characteristics for an off-the-shelf cell product.

Clinical Trials

Given the promising preclinical data, a phase I trial was recently conducted in six preterm infants born at less than 29 weeks' gestation with established severe BPD receiving a single IV infusion of 1 million hAECs/kg.[29] An important lesson from this trial was the adjustment of the infusion protocol after the first infant experienced transient bradycardia and hypoxia during the infusion. This was likely related to a pulmonary microembolic event and did not reoccur in the remaining five infants after changes were made to the cell infusion protocol. This trial showed feasibility and lack of toxicity of a single IV dose of hAECs. The same group is now launching a phase I trial to test the feasibility and safety of a dose escalation (2, 10, and 30 million cells/kg) in 24 preterm infants at high risk of severe BPD still requiring an $FiO_2 \geq 25\%$ while mechanically ventilated or an $FiO_2 \geq 35\%$ while on noninvasive respiratory support at day 14 of life.[30]

Endothelial Progenitor Cells—The Vascular Hypothesis of BPD

Definition

The term endothelial progenitor cell (EPC) is used to describe a cell that can regenerate the endothelial lining of blood

vessels. As for other stem cells, the nomenclature remains ambiguous, leading to controversy in interpreting study results. A recent consensus statement on nomenclature of EPCs suggests the use of precise terminology based on defining cellular phenotype and function.[31] Two distinct and well-defined cell types have been considered as EPCs: endothelial colony forming cells (ECFCs) and myeloid angiogenic cells (MACs). Both promote vascular repair and may thus be considered for therapeutic purposes, although their mechanisms of action appear to be different. ECFCs are characterized by (1) robust proliferative potential, (2) secondary and tertiary colony formation upon replating, and (3) de novo blood vessel formation in vivo when transplanted into immunodeficient mice. Of clinical relevance for neonatology, these cells exist in cord blood and can be expanded for therapeutic purposes.[32] MACs also participate in angiogenesis but do not directly form the endothelial monolayer of new vessels and display various monocyte/macrophage phenotypes and function.

Rationale and Biological Plausibility

Increasing evidence suggests that lung vascular growth and angiogenic growth factors are crucial for lung growth and repair.[33] Interestingly, resident ECFCs are present in the developing human and rat lung[34] and their function is impaired in experimental neonatal lung injury.[35] Combining the data from a recent systematic review indicating a link between impaired EPC function in circulating/cord blood of preterm infants and preterm birth-associated complications[36] suggests that exogenous supplementation of EPCs may be therapeutic. Unlike MSCs, however, ECFCs may be immunogenic, thus requiring autologous transplantation.

Preclinical Evidence

The first observation with bone marrow-derived MACs showed restoration of alveolar and lung vascular growth in hyperoxic neonatal mice.[37] In another study, short-term cultured bone marrow-derived cells with "EPC characteristics" lead to partial recovery in alveolar septal number in this same animal model.[38] Short-term cultured EPCs impaired alveolar growth in normoxic pups and long-term cultured cells caused some aberrant tissue growth raising the importance of safety and long-term studies in the preclinical setting.

IV infusion of human cord blood-derived ECFCs into immune-deficient rats and mice exposed to hyperoxia promotes alveolar and lung vascular growth, and attenuates pulmonary hypertension.[35] These effects persist after 10 months and no tumor formation was noted. Surprisingly, ECFCs do not engraft and seem to act through a paracrine effect since the cell-free ECFC-derived conditioned media exert similar benefit to whole cell therapy.[35] In a bleomycin-induced neonatal lung injury model, ECFC-derived conditioned media had no effect on lung growth but attenuated pH.[39]

Focused preclinical studies are required before clinical translation of this cell type can be considered in neonates.

$CD34^+$ Mononuclear Cells—The Old Fashioned, Straight from the Bone Marrow Transplant Literature

Definition

Hematopoietic stem cell (HSC) transplantation has been practiced for over 50 years in cancer patients to reconstitute bone marrow function. The consideration of cell therapy for regenerative indications is new.

Rationale and Biological Plausibility

Within HSCs, $CD34^+$ represents a marker for engraftment. Earlier studies suggested some lung engraftment and differentiation of bone marrow-derived cells.[40] These observations have now been dispelled[41] and the rationale for using these mononuclear cells (MNCs) should be re-examined.

Preclinical Evidence

Four studies have explored the lung protective effect of MNCs.[42–45] Intranasal inoculation of fresh human cord blood $CD34^+$ MNCs into neonatal mice with apoptosis-induced lung injury resulted in sparse engraftment and very limited alveolar epithelial differentiation yet improved lung growth 1 year after transplantation. IV injection of MNCs from cryopreserved cord blood into hyperoxia-exposed newborn mice significantly attenuated methacholine-induced airway hyperreactivity, and mildly improved alveolarization, lung compliance, and elastance. Interestingly, total nucleated cells had no effect and granular cells caused high mortality and an emphysematous phenotype.[46] Intraperitoneal injection of human umbilical cord blood MNCs in a double-hit mouse model combining antenatal hypoxia and postnatal hyperoxia improved septal thickness and decreased Tgfβ3 mRNA expression and proinflammatory IL-1β. Finally, intratracheal injection of cord blood MNCs in neonatal hyperoxic rats had no effect on alveolarization and lower anti-inflammatory and no angiogenic effects compared with cord blood-derived MSCs or human adipose tissue-derived MSCs.[47] This study highlights an interesting debate about therapeutic potency of various cell therapies.

Human Amniotic Fluid Stem Cells—Don't Throw Away the Bath Water

Definition

The discovery of cells in the amniotic fluid displaying stem cell characteristics provided yet another potential perinatal source for therapeutic cells. These human amniotic fluid stem cells (hAFSCs) express embryonic and adult stem cell markers, expand extensively without feeders, and are multipotent as they can differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages.

Rationale and Biological Plausibility

Previous literature suggests anti-inflammatory effects of hAFSCs[48] as well as the ability to integrate and differentiate into epithelial lung lineages.[49]

Preclinical Evidence

One study so far explored the lung protective effect of hAFSCs.[50] Intratracheal injection after established hyperoxic lung injury in neonatal rats showed low hAFSC retention, improved alveolar and lung vascular structure as well as increased vascular endothelial growth factor expression, suggesting yet again—despite their multipotency—a paracrine mechanism of action. Given the ease of access to other perinatal sources such as cord, cord blood, and placenta, it remains to be tested if hAFSCs provide superior repair properties than MSCs and hAECs.

Induced Pluripotent Stem Cells—Personalized Cell Therapy

Definition

Induced pluripotent stem cells (iPSCs) are differentiated cells that have been genetically reprogrammed to an embryonic stem cell (ESC)-like state by forced expression of a combination of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) important for maintaining the defining properties of ESCs.[51,52]

iPSCs possess desirable characteristics for use as a cell therapy. Like ESCs, iPSCs have the potential to become any cell type in the body. Increasingly robust methods of differentiation into alveolar epithelial type 2 cells (AT2) are described.[53] Human iPSCs (hiPSCs) can be generated from each patient, thus representing an autologous source of cells that overcome the likelihood of immune-mediated rejection. An unlimited number of cells can be generated from hiPSCs as they can be maintained and expanded in vitro.

Rationale and Biological Plausibility

AT2s, a subset of which represent distal lung progenitor cells, promote normal lung growth and repair after injury.[42] AT2 depletion is postulated to contribute to persistent lung injury in BPD.[42–45]

Preclinical Evidence

One study so far explored the lung-protective effect of iPSC-derived AT2s. Proof-of-concept airway delivery of primary murine AT2s prevent hyperoxia-induced impairment in lung function and alveolar growth in neonatal mice.[54] Undifferentiated murine and human (h)iPSCs also preserve lung function and alveolar growth but cause local teratoma formation and systemic cellular infiltration in various organs. Conversely, airway delivery of hiPSC-derived AT2 improves lung function and structure without evidence of tumor formation at 8 months and some degree of engraftment. Further studies need to confirm the mechanism of action and importantly, the safety of differentiated iPSCs. The pace of advances in the iPSC field is impressive—as indicated by the recent description of fail-safe iPSCs[55]—and the ancillary knowledge generated from iPSC biology may lead to a better understanding of organ regeneration.

Overall, these observations indicate stem cell-based therapies as a growing field of investigation with MSCs and hAECs as the front runners since they have entered early-phase clinical trials. A common denominator of all these putative cell products—independent of the original rationale and biological plausibility for using these cells in the first place—is the lack of engraftment and the absence of potency assays to predict therapeutic efficacy in vivo before infusing the product.

Knowledge Gaps

Cell Manufacturing

Clinical-grade cell products have to be manufactured according to good manufacturing practices to ensure a consistent and controlled product according to quality standards. Since the "process is the product," each step along the manufacturing process will affect the quality and thus the therapeutic potency of the product.[56]

For example, preconditioning of bone marrow MSCs by ex vivo exposure to hyperoxia prior to intratracheal injection enhances the lung protective effect in neonatal hyperoxia-induced lung injury in rats.[57] Interestingly, female bone marrow-derived MSCs seem to have greater therapeutic efficacy than male MSCs on lung inflammation and vascular remodeling in this same animal model and this effect is more pronounced in male animals.[58] Conversely, exposure to surfactant reduces MSC viability and combined MSC + surfactant administration does not exert additive lung protective effects on lung structure in hyperoxia-induced lung injury.[59]

These findings indicate that much more needs to be learned about the biology and manufacturing of cell-based therapies, starting with the cell source (adult bone marrow, cord blood, cord tissue, placenta, etc.), isolation–culture–expansion techniques, cryopreservation protocols, the choice of using a frozen versus a fresh cell product, an autologous versus an allogeneic strategy, the cell type, or a cell versus a cell-free product.[60]

Indeed, one exciting new avenue is the recognition that MSCs exert their therapeutic benefit through the release of EVs. Amongst these EVs, exosomes, membrane-derived nano-sized particles that contain proteins and miRNA, have attracted particular attention.[61] Exosomes perform as well as if not better in protecting the lung from hyperoxic injury.[9,62–64] Rigorous preclinical studies need to confirm the initial results of these exploratory studies. However, similar to the actual cell product, manufacturing at the clinical scale and prediction of the bioactivity remain a challenge for the implementation of these "nanotherapies for micropreemies."[65]

Potency Assays

An ideal scenario can be found in the HSC field where CD34 represents a marker for engraftment and thus a potential indicator of successful replenishment of bone marrow function. Unfortunately, cell therapies in regenerative medicine still lack reliable and rapid disease- and mechanism-specific in vitro assays to predict their therapeutic efficacy. In vivo tests are still lacking. Recent advances in 3D lung organoids that replicate an organ-in-a-dish may yield more reliable results than traditional culture techniques.[66] The most promising approach today is the CLinical Indications Prediction (CLIP) scale to predict how donor-to-donor heterogeneity and culture conditions impact the therapeutic efficacy of MSCs[67]:

high Twist1 levels predict proangiogenic properties, while low Twist1 levels predict a more anti-inflammatory activity. Combined with omic technology, a multipanel of factors may achieve accurate prediction of the potency of a given cell product which in turn would substantially benefit our understanding of the mechanism of action and facilitate the manufacturing process.

Conclusion

MSC and hAEC-based therapies have entered early-phase clinical trials in neonatology. Numerous other cell types are being investigated in the laboratory. Nonetheless, these are early days for cell therapy and major gaps in our knowledge persist with regard to the mechanism of action of these various cells. Strong rationale and biological plausibility need to be confirmed in rigorous preclinical studies. Potency assays need to be established to predict in vivo bioactivity and to facilitate and fine-tune the manufacturing process of a given cell product. Well-designed clinical trials—based on robust preclinical evaluation—will permit successful (safe and timely) translation of these potentially game-changing therapies in neonatology.

Conflict of Interest
None declared.

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