Cell Therapy: A Novel Treatment Approach for Bronchopulmonary Dysplasia

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**Key Words**
bronchopulmonary dysplasia, lung, preterm, stem cell, cell transplantation

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AEC</td>
<td>Alveolar epithelial cell</td>
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<tr>
<td>AF-SC</td>
<td>Amniotic fluid stem cell</td>
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<tr>
<td>AM-MSC</td>
<td>Amniotic membrane–derived mesenchymal stem cell</td>
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<tr>
<td>BPD</td>
<td>Bronchopulmonary dysplasia</td>
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<tr>
<td>EPCs</td>
<td>Endogenous progenitor cells</td>
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<td>ESCs</td>
<td>Embryonic stem cells</td>
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<tr>
<td>hAECs</td>
<td>Human amnion epithelial cells</td>
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<tr>
<td>hAMMSCs</td>
<td>Human amnion membrane–derived stem cells</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<tr>
<td>IPS</td>
<td>Induced pluripotent stem cell</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>MSCs</td>
<td>Mesenchymal stem cells</td>
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<tr>
<td>RDS</td>
<td>Respiratory distress syndrome</td>
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<tr>
<td>SP-C</td>
<td>Surfactant protein C</td>
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<tr>
<td>TTF</td>
<td>Thyroid transcription factor</td>
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<tr>
<td>UCB-SC</td>
<td>Umbilical cord blood–derived stem cell</td>
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<tr>
<td>UCS-SC</td>
<td>Umbilical cord stroma–derived stem cell</td>
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**Abstract**

Bronchopulmonary dysplasia (BPD) is a major cause of substantial lifelong morbidity in preterm infants. Despite a better understanding of the pathophysiology of BPD and significant research effort into its management, there remains today no effective treatment. Cell-based therapy is a novel approach that offers much promise in the prevention and treatment of BPD. Recent research supports a therapeutic role for cell transplantation in the management of a variety of acute and chronic adult and childhood lung diseases, with potential of such therapy to reduce inflammation and prevent acute lung injury. However, considerable uncertainties remain regarding cell therapies before they can be established as safe and effective clinical treatments for BPD. This review summarizes the current literature investigating cell therapies in lung disease, with particular focus on the various types of cells available and their specific properties in the context of a future therapy for BPD. Pediatrics 2012;130:1–11
Over recent decades, improvements in the pre- and postnatal care of the preterm neonate have seen significant reductions in mortality and acute morbidities such as neonatal respiratory distress syndrome (RDS). However, longer-term sequelae of preterm birth remain a concern in these infants. In particular, lung immaturity and consequent bronchopulmonary dysplasia (BPD) remains a major cause of morbidity and death. With a chronic respiratory health burden secondary only to asthma and far exceeding that of cystic fibrosis, BPD is also associated with serious comorbidities, including cerebral palsy and neurodevelopmental delay.

When Northway first reported BPD more than 40 years ago, he observed a disease in near-term infants with RDS treated with mechanical ventilation using high ventilator pressures and high inspired oxygen concentrations. This resulted in pulmonary fibrosis and airway smooth muscle hypertrophy, causing respiratory failure, ventilator dependence, and significant mortality. However, once this was recognized and the pathogenesis was understood, improvements in neonatal respiratory support all but banished this “old” form of BPD to the annals of neonatal medicine. More recently, the improved survival of very preterm infants has seen it replaced by a “new” BPD, a chronic lung disease reflecting disruption and arrest at a much earlier stage of lung development.

The new BPD is a clinical diagnosis, defined as >21% oxygen dependency for ≥28 days’ duration assessed at 36 weeks’ postmenstrual age. It occurs almost exclusively in very preterm infants born before 30 weeks’ gestation. Approximately 1 in 3 of those infants develop BPD. Even higher rates of BPD are seen in extremely preterm infants, with almost two-thirds of infants born at 22 to 25 weeks’ gestation affected. BPD is characterized by diffuse pulmonary inflammation, with alveolar and vascular simplification and an arrest of lung development at the late canalicular to early saccular stages. This results in a reduced surface area for gas exchange and chronic pulmonary embarrassment. Paradoxically, the incidence of RDS in these infants is often low, despite the immaturity of the canalicular lung. This is likely due to a higher incidence of chorioamnionitis at these extremely low gestational ages and the widespread use of antenatal corticosteroids, particularly repeated doses in very preterm pregnancies. Both infection and corticosteroids increase surfactant production and improve lung compliance. They also both arrest alveolar development, and over time the immature lung becomes incapable of supporting increasing respiratory requirements. This results in the need for supplemental oxygen and ventilation, which in turn stimulate lung inflammation, exacerbating the structural deficits and alveolar arrest, leading to BPD.

Whether such changes are irreversible or the lung is capable of resolution and catch-up growth remain to be determined. However, studies to date suggest that for many children, the lung is irreversibly damaged, meaning that the respiratory compromise that begins neonatally continues into adolescence and beyond. For example, follow-up studies of children who had BPD as a neonatal show that they have higher than average rates of chronic cough and wheeze, airway hyper-responsiveness, and lung function abnormalities throughout childhood. These children are also more likely to present to hospital with severe respiratory infections and oxygen dependency than similar preterm born children who did not develop BPD. Improvement in the lives of future children born very preterm requires an effective prevention and/or treatment of BPD. Unfortunately, such a therapy has not yet been forthcoming. However, the increasing interest in cell therapies, particularly as modulators of inflammation and with their substantial regenerative capacity, offers the potential of a cell-based prevention and cure for BPD, that might be able to mitigate lung injury, support epithelial regeneration, and “reboot” lung development.

### CELL THERAPIES IN LUNG DISEASE

Interest in cell therapies began in the early 1900s, when Alexander Maximow coined the term “stem cell” to describe his theory of hematopoiesis in which all blood cells developed from a common precursor cell. A stem cell is a cell that displays a multi-lineage differentiation potential and has the ability to self-renew. Stem cells and cells with stem cell–like properties have been identified in embryonic tissue, bone marrow, most adult tissues, and, more recently, in gestational tissues including the placenta, umbilical cord, and fetal membranes. The enthusiasm with which cell therapies have been embraced over recent years is clear: A search on the US National Institutes of Health clinical trial Web site for “stem cell” elicits >3000 completed or ongoing clinical trials of cell therapy.

With regard to the lung, considerable preclinical and clinical evidence supports the potential utility of cell therapy to treat various chronic lung diseases, including chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, acute adult lung injury, and chronic severe asthma. More recently, investigators have considered the application of cell therapies in pediatric lung diseases such as BPD and cystic fibrosis. In this review, we discuss the evidence for cell therapy in child lung injury, with a particular focus on BPD.
such cell therapy options is detailed in Table 1.

**What Are the Cell Therapy Options, and What Is the Evidence?**

**Embryonic Stem Cells**

Embryonic stem cells (ESCs) are pluripotent cells capable of generating cells from all 3 germ layers (endoderm, mesoderm, and ectoderm). Given this capacity, by the in vitro generation of tissues and organs, ESCs may provide the answer to growing waiting lists for organ transplantation. However, ESCs are a controversial starting point for any cell therapy because they require the destruction of human embryos, typically from stock that is excess to requirements in assisted conception services.25,28

Could ESCs be useful as a lung therapy? In vitro, mouse-derived ESCs have been shown to differentiate into type II alveolar epithelial cells (AEC-IIs) and, when cocultured with fetal lung buds, will form pseudo-glandular epithelial structures expressing surfactant protein C (SP-C), a marker of functional AEC-IIs.30

Mouse-derived ESCs can also generate tracheobronchial epithelium in vitro, evidenced by their differentiation into Clara, basal, intermediate, and ciliated cells, suggesting that they can generate cells from the entire respiratory tract, not only lung itself. Furthermore, these cells formed tight barrier junctions that function in a manner similar to native mouse airway epithelium.31 Similarly, differentiation down a respiratory lineage has been demonstrated by human ESCs with the expression of thyroid transcription factor (TTF-1), a marker of early lung development, and aquaporin-5 and SP-C, markers of alveolar epithelial type I cells (AEC-Is) and AEC-IIs, respectively. Promisingly, these surface markers were expressed in an ontogeny that mimicked the process of normal lung progenitor cell development with TTF-1 expression preceding that of SP-C and then aquaporin-5. In short, at least in vitro, ESCs look capable of providing a source of cells for lung and airway regeneration. However, the very property that makes ESCs attractive, their pluripotency, may also present a problem: the potential to form tumors within the recipient.26,33 To circumvent this, it has been suggested that rather than administrating undifferentiated ESCs, the delivery of ESC-derived differentiated and lineage committed cells, alveolar epithelial cells (AECs) in the case of lung, might be preferable, if effective.34 In that regard, the administration of human ESC–derived AEC-IIs to mice with bleomycin-induced pulmonary fibrosis reduced structural injury, repaired the pulmonary epithelium, and improved lung function.34 Furthermore, human ESC-derived AEC-IIs can differentiate into type I AECs, suggesting that these cells will be able to regenerate damaged alveolar epithelium.35 In the setting of perinatal, rather than adult, lung injury, ESC-derived AECs can rescue pulmonary hypoplasia and vascular disruption in fetal lung explants in vitro.36 This suggests that ESCs, or ESC-derived AECs, may be capable of enhancing septation, saccule formation and alveologenesis in the preterm lung, that is, to be able to regenerate “arrested” immature lung. However, whether this promise can be

**TABLE 1 Summary and Characteristics of Common Cell Therapies**

<table>
<thead>
<tr>
<th>Stem Cell Type</th>
<th>Source</th>
<th>Evidence in Lung Repair</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic SCs</td>
<td>Excess human embryos from IVF</td>
<td>Generate type I and II AECs in vitro9,10,32; ESC-derived AECs rescue bleomycin-induced lung injury in vivo34, and pulmonary hypoplasia in vitro35</td>
<td>Totipotent</td>
<td>Tumorigenic; Ethical restrictions; Limited source; Require expansion</td>
</tr>
<tr>
<td>Bone marrow SCs</td>
<td>Bone Marrow</td>
<td>Generate type II AECs in vivo31; Rescue hyperoxia-induced injury in neonatal mouse lung43</td>
<td>Pluripotent; Antiinflammatory; Track record in preclinical and clinical studies</td>
<td>Tumorigenic; Risk of chimerism; Invasive collection; Require expansion</td>
</tr>
<tr>
<td>Umbilical cord SCs</td>
<td>Cord Blood; Wharton's Jelly</td>
<td>Generate type II AECs in vivo37; Rescue hyperoxia-induced injury in neonatal rat lung in vivo37; Rescue bleomycin-induced lung injury in vivo50</td>
<td>Multipotent; Antiinflammatory; Easily accessible; Track record in preclinical and clinical studies</td>
<td>Limited yield; Require expansion</td>
</tr>
<tr>
<td>Amniotic fluid SCs</td>
<td>Amniotic fluid</td>
<td>Generate type II AECs in vivo38, Pro-angiogenic1,50</td>
<td>Multipotent; Antiinflammatory; Low antigenicity</td>
<td>Invasive collection (amniocentesis); Require expansion</td>
</tr>
<tr>
<td>Amniotic membrane SCs</td>
<td>Membrane stroma; Membrane epithelium</td>
<td>Favor adipogenic, neurogenic, vasculogenic, and hepatic potentials50,52,54; Generate type I and II AECs in vitro9,30; Rescue bleomycin-induced adult lung injury and fetal lung injury induced by ventilation38 and LPS35 in vivo</td>
<td>Pluripotent</td>
<td>Variable engraftment rate</td>
</tr>
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delivered awaits in vivo evidence in models of BPD. Those experiments certainly need to be undertaken before clinical application.

**Mesenchymal Stem Cells**

After ESCs, mesenchymal stem cells (MSCs), most commonly derived from bone marrow, are probably the next most extensively investigated stem cell. Friedenstein et al were the first to show that bone marrow–derived stromal cells could be differentiated down nonhematopoietic mesenchymal lineages, including fat, cartilage, and bone. However, it took another 25 years before the full differentiation potential of bone marrow–derived MSCs was recognized, differentiating down endodermal and ectodermal lineages, including lung. Furthermore, it became apparent that MSCs could be isolated from almost any tissue, such that they are now commonly collected from fat as well as bone marrow. With regard to being a possible therapy for lung diseases, including BPD, MSCs possess 3 key properties: an ability to home to sites of injury, multilineage differentiability, and immunomodulatory functions. Several studies have now confirmed that MSCs preferentially traffic to sites of injury, although the precise mechanism(s) by which MSCs do this remain unclear. Nonetheless, it is likely MSCs are responding to chemokine signaling from inflammatory cells as evidenced by their expression of diverse chemokine receptors and their ability to migrate upon stimulation by various chemokine ligands. In the context of a targeted therapy for the lung, MSCs migrate to injured lung both in vitro and in vivo. They also form lamellar bodies and express SP-C, consistent with differentiation into functional AEC-IIs, and are able to engraft and differentiate into alveolar epithelium in vivo. Indeed, unlike ESCs, there is in vivo evidence of the potential therapeutic efficacy of MSCs in experimental models of BPD. Van Haagen and colleagues showed that MSCs improved alveolar and vascular architecture, lung function, and survival in mouse pups exposed to high inspired oxygen concentrations. However, MSCs were only effective when administered early on in the injury process, on postnatal day 4, and not after established injury at day 14. This confirms the observation that, in the bleomycin mouse model of lung fibrosis, MSCs were able to engraft into injured lung, differentiate into type II alveolar cells, and reduce bleomycin-induced lung fibrosis but only if given at the time of bleomycin administration and not a week after. This suggests that, at least in these 2 models of lung injury, MSCs may be able to prevent injury but are unable to repair damaged lung once established.

The ability to prevent but not repair established lung injury, and the inability to reestablish, or “reboot,” alveolar development might hint at how MSCs are likely to be working. Specifically, although MSCs can certainly engraft into damaged lung epithelium and differentiate into lung alveolar cells, they seem to be able to prevent acute lung injury without substantial levels of cell engraftment. This would suggest that their third attribute, the ability to modulate the host immune response to injury, may be their principal modus operandi. In adult mice with Lipopolysaccharide-induced acute lung injury, the administration of MSCs reduced levels of proinflammatory cytokines and prevented both neutrophilia and edema despite a low engraftment rate. Similarly, the administration of bone marrow–derived angiogenic stem cells prevents hyperoxia-induced lung injury despite low engraftment rates. In addition, MSC-conditioned media reduces apoptosis in type II alveolar cells exposed to hyperoxia and overexpression of the vasculoprotective gene angiopoietin-1 in MSCs further rescues lung integrity after LPS-induced injury, observations that imply the role of secreted factors. Consistent with these results, treatment with MSC-conditioned media alone, in the absence of cells, also results in lung repair in hyperoxia-treated mice. That is to say, the presence of the cells themselves is not required for repair, just what the cells secrete.

Unfortunately, not all studies of MSCs in BPD models have been so promising. In one study of hyperoxia-exposed mouse pups, the administration of whole bone marrow isolate resulted in substantial lung engraftment of MSCs, but the cells did not differentiate into epithelial cells. Instead, they unexpectedly undertook a chimeric change generating alveolar macrophages. Furthermore, cell administration did not improve lung injury. Rather, the bone marrow MSC-derived macrophages remained proliferative 8 weeks after transplantation responding to subsequent hyperoxia by reinitiating pulmonary inflammation. In short, they worsened injury. However, the whole marrow cell preparation differs significantly from the population of bone marrow–derived MSCs first described and characterized by Friedenstein and advocated as a cell therapy in regenerative medicine today. Whole bone marrow contains hematopoietic cells whereas bone marrow derived MSCs are purified of hematopoietic cells. It is these cells that are likely to have contributed to the increased inflammation after transplantation and not MSCs. Nonetheless, in preterm babies, the presence of MSCs in tracheal aspirates has been associated with an increased risk of BPD development and increased myofibroblast differentiation. Thus, although some studies suggest promise for selected MSCs as a therapy for BPD, uncertainty remains about the safety of these cells.
and about which subpopulations might confer benefit and which may cause harm. In particular, the unexpected generation of alveolar macrophages from whole bone marrow transplant is a concerning finding and one that requires additional examination before such therapies could be considered clinically.

Placental Stem Cells

One of the more recent areas of stem cell research has been the recognition that the placental and associated tissues, including the umbilical cord and fetal membranes harbor a variety of stem cells or stem cell–like cells. The characteristics of these various cells vary depending on their origin, but in general they all appear to possess both regenerative capacity and low immunogenicity, likely resulting from their origins in the complex maternal-fetal environment.

Umbilical Cord Stem Cells

Stem cells can be isolated from either cord blood or the stromal perivascular matrix (Wharton jelly) surrounding the umbilical vessels. Directed banking of umbilical cord blood is gaining increasing popularity in developed countries, and many banking programs now also offer banking of the cord itself. Although these programs were originally established as a source of cells for the potential treatment of future hematologic disorders in the child, it is now apparent that these cells have capabilities well beyond that. Both cord blood–derived stem cells (UCB-SCs) and stroma–derived stem cells (UCS-SCs) can be isolated in significant numbers, and both cell populations display multilineage differentiation potential. With regard to lung injury, in the bleomycin-induced adult lung injury model, UCS-SCs have been shown to localize to areas of lung fibrosis and to reduce lung inflammation and injury, including fibrosis, and increase matrix metalloproteinases supporting fibrotic repair; in the neonatal hyperoxia lung injury model UCB-SCs have been shown to be reparative. Although UCB-SCs have been shown to differentiate in vitro into type II AECs, the in vivo reparative effects of both UCS-SCs and UCB-SCs are achieved without any evidence of engraftment or differentiation to a lung cell phenotype in vivo just like MSCs. If proven to be effective in other models of BPD, then cord-derived cells would be particularly attractive because an individual’s own cells could be stored prospectively and used if required.

Amniotic Fluid and Amnion-derived Cells

Within the amniotic fluid there is a mixed population of mesenchymal and epithelial stem cells that have similar potency to adult MSCs but that are much more proliferative. Unlike adult MSCs, amniotic fluid stem cells (AF-SCs) express the stem cell markers Oct4 and SSEA-4, typical of undifferentiated ESCs, and are capable of true multilineage differentiation. Furthermore, likely reflecting their role in maternal acceptance of pregnancy, these cells display enhanced immune privilege, with reduced human leukocyte antigen (HLA) I and II antigen expression. This latter property makes these cells attractive from an allogenic cell therapy perspective, offering the potential that cells from any donor could be used in any recipient. With regard to a lung therapy, AF-SCs express TTF-1 and, upon injection into both embryonic and adult mouse lungs, engraft and differentiate into lung lineages. These cells also secrete proangiogenic factors that might be beneficial in BPD where there is disordered vascularity. Despite the ability to engraft into injured lung and support epithelial regeneration and vasculogenesis, whether AF-SCs are able to repair lung injury in vivo has not been established. Certainly, this is a cell type that merits additional evaluation in the setting of BPD and other lung diseases. However, sourcing cells from amniotic fluid in midpregnancy might be a difficult task, requiring large-scale amniocentesis.

In contrast, amniotic membrane-derived mesenchymal stem cells (AMMSCs) and amniotic epithelial cells (hAECs) can be readily derived from the term placenta. Amniotic MSCs appear to be similar to adult MSCs, expressing pluripotent markers and differentiating down multiple tissue lineages, most favorably into adipogenic, neurogenic, vasculogenic, and hepatic cells. Amnion epithelial cells (hAECs) also possess stem cell–like properties, displaying pluripotency and clonal properties and expressing stem cell surface markers akin to ESCs. Consistent with this, hAECs differentiate in vitro down all tissue lineages including pancreatic, hepatic, neurogenic, cardiomyocytic, and pulmonary lineages. However, unlike ESCs, hAECs do not appear to form teratomas in vivo making them more attractive for clinical use.

In the context of tissue repair, the reparative and antiinflammatory capacity of amnion has been long appreciated. It has been used in wound repair such as corneal ulcers and burns for >50 years. It is only recently, however, that the immune modulatory properties of amnion have been recognized as being key to these reparative and wound-healing properties. In particular, like AF-SCs, hAECs have a low level of MHC I and II antigen expression, affording them immune privilege, and they inhibit leukocyte migration and proliferation. In animal studies of pulmonary fibrosis, human AM-derived stem cells (hAMSCs) reduced neutrophil infiltration and structural injury. Similarly, hAECs reduced inflammation in the lungs of adult mice exposed to...
intranasal bleomycin, leading to less fibrosis and scarring and improved lung function. Although engraftment of hAECs in the injured lung has been shown in immune compromised mice, this has not been observed in immune-competent mice. Indeed, in the latter study, it was suggested that engraftment was not required for lung repair but rather immune modulation was the key mechanism, as is the case with MSCs.

In regard to the developing lung and a possible therapy for BPD, hAECs have been shown to prevent injury in a fetal ovine model of ventilation-induced injury and in an ovine model of in utero infection. Both ventilation-induced injury and chorioamnionitis are important pathways to BPD. That hAECs were able to mitigate injury in both of these large animal models is promising. Interestingly, although engraftment of hAECs was observed in both studies, it was only to a low level at <1%. Instead, it appeared that, like MSCs, hAECs exert their reparative effects principally via modulation of the host inflammatory response. Like umbilical cord–derived cells, if effective, cells isolated from the amnion would be an attractive therapy for BPD because an individual’s own cells could be used.

**Induced Pluripotent Stem Cells**

In 2006, Takahashi and Yamanaka were the first to report that fibroblasts treated with pluripotent transcription factors form clonal cells with a pluripotency akin to ESCs and referred to these as induced pluripotent stem cells (iPS). This technology promises custom-made stem cells, avoiding the donor-matching issues that limit allogeneic transplantation. In regard to the injured lung, iPSs have been suggested as a vector for gene therapy in diseases such as cystic fibrosis and α-1-antitrypsin deficiency. However, current evidence of their efficacy in animal models of these and other lung diseases is lacking, and to date no preclinical data are available regarding either the safety or efficacy of these novel cells in lung injury.

**Lung Progenitor Cells**

Like all tissues, the lung harbors its own stem cells or endogenous progenitor cells (EPCs) that are likely to have important roles in normal lung development and may have roles in repairing injury. Although uncertainty remains, it would appear that there are probably 4 or 5 distinct niches of EPCs within the lung allowing targeted repair of the lung from the major airways down to the alveoli. Indeed, it is the use of different mouse models of lung injury that each damage different zones of the respiratory tract that has allowed the identification of a hierarchy of EPCs. However, although these studies have afforded important insights into lung development and repair, to date there has been no report of using these cells as a therapy for BPD. Most likely this is because of the inherent difficulties in isolating, purifying, and expanding sufficient cells for administration. Nonetheless, a better understanding of how EPCs are mobilized and proliferate could well lead to an effective therapy for BPD. Interestingly, in preterm infants at increased risk of BPD, the number of lung EPCs has been shown to be reduced. This may underlie the inability of these infants to regrow their lung beyond the acute phase of injury.

**CELL THERAPIES FOR BPD**

With such a diverse menu of cells for regenerative medicine in intractable lung diseases, it is clear that cell therapies should be a focus of research in the coming years. However, an understanding of the limitations of each therapy is required to best tailor research strategies toward the most effective, safe, and clinically amenable options. Although experimental data supporting cell therapy in lung diseases are growing, only a few studies have assessed the therapeutic benefits in models of BPD, and to date, no clinical trials have been undertaken. Given their clinical track record in hematologic disease, epithelial disorders, and wound repair, it is not surprising that bone marrow, umbilical cord, and placental-derived cells have been the focus of preclinical studies so far. However, which of these cell types are most appropriate as a therapy for BPD remains uncertain. In this regard, it is useful to consider the pathologic mechanisms in BPD and the mechanisms through which each type of cell may exert their effects.

**Selecting a Targeted Cell Therapy**

The key mechanism underpinning BPD is inflammation, leading, in turn, to dysregulated deposition of extracellular matrix proteins, such as collagen and elastin, alveolar simplification, and disrupted vasculogenesis. Thus, any successful cell therapy is likely to need to be effective in 2 ways. First, the therapy should be able to modulate lung inflammation. MSCs, umbilical cord stem cell, amniotic fluid–derived mesenchymal stem cell, and hAMSCs have all been shown to inhibit inflammatory cell migration and proliferation and reduce the production of proinflammatory cytokines. These properties are particularly important in the preterm infant, whose immune response may be overwhelmed by a relative lack of antiinflammatory mediators, predisposing to chronic inflammation. Interestingly, both MSCs and hAECs express interleukin-1ra and interleukin-10 antiinflammatory cytokines that could provide the infant with a mechanism of immune regulation otherwise not available to them.
Furthermore, quenching inflammation in the preterm lung would be expected to alter fibroblast-myofibroblast differentiation, reducing fibrosis and dysplastic changes. In this way, cell therapies could regulate structural remodeling in BPD.

The second key attribute likely necessary for a successful cell therapy in BPD is the capacity to regenerate, or facilitate regeneration of, a functional lung epithelium to replace destroyed tissue. Each of the cell types discussed in this review has in vitro and/or in vivo capacity to differentiate into pulmonary precursor and alveolar epithelial cells. This is most notable for ESCs, with creation of not only distal but also proximal functional pulmonary epithelium to replace destroyed tissue. This is most notable for ESCs, with creation of not only distal but also proximal functional pulmonary epithelium. Induced pluripotent stem cells present the next highest potential for tissue regeneration, with a potency akin to ESCs, and the added capacity for patient-specific cell development, maximizing engraftment and minimizing immune rejection. Interestingly, hAMSCs harbor the next highest pluripotency after ESCs and iPSCs, expressing the largest range of stem cell markers, with the capacity to differentiate in vitro into functional alveolar epithelium. However, as previously discussed, engraftment of these cells in animal studies has been variable, and current evidence suggests that both AM-MSCs and hAECs are not capable of in vivo epithelial integration in sufficient numbers to substantively regenerate lung epithelium. Despite this, hAMSCs are still capable of reducing alveolar injury, further suggesting that paracrine factors are more important in cell-mediated repair than the contribution of cell-derived grafts. Similarly, despite a capacity for MSCs and UCBS-SCs to develop an AEC-II phenotype, engraftment rates in BPD models vary, with the majority of studies reporting little or no in vivo engraftment. In summary, although the capacity for incorporation and differentiation in vivo has been noted for all cell types, structural repair does not appear dependent on engraftment. Studies should now begin to focus on factors released by the cells to affect these responses and to determine whether the administration of conditioned media, media in which the desired cell type has been grown and that contains soluble factors secreted by the cells, would be sufficient to avoid the associated risks with allogeneic cell delivery. Furthermore, whether these cells are able to stimulate proliferation of native lung stem cells has not been addressed. It is possible that exogenously delivered cells may induce activation of endogenous lung stem cells, which are known to be reduced in BPD and other chronic lung injury and thereby accelerate repair.

**Selecting a Practical Cell Therapy**

Aside from the theoretical evidence supporting the use of each of these individual cell types, practical aspects of the preparation and delivery of the various cells are likely to influence which cell type might find favor. The major considerations in clinical application of cell therapies include the capacity for donor rejection and need for immunosuppression, the capacity to form tumors, the ethical and practical restrictions surrounding the acquisition of cells, and the infrastructure available to ensure quality control and good manufacturing practices in clinical use. Compared with ESCs, in which donor immunologic incompatibility is a major concern, MSCs and cells from gestational tissues have a reduced risk of immune rejection, attributed to their low expression of MHC class I and II antigens and, in hAMSCs, the lack of highly polymorphic HLA-A, B, C, and DR antigens and T-cell costimulatory factor B7.1,45,101 Also, AM-MSCs and hAECs express HLA-G, a potent immune regulator, making these cells a more attractive cell therapy option, avoiding the need for immunosuppression. The very property that makes stem cells attractive as therapies in regenerative medicine, namely, pluripotency, may also be their downfall when applied clinically. Embryonic SCs are highly tumorigenic, with the extended culture times required for cell banking further increasing the risk of genomic instability and tumor formation.26,102 A case of tumor formation in a patient receiving ESCs for a neurologic condition has already been reported.103 This is also a potential limitation of both MSCs and iPSCs.26 Mesenchymal stem cells in particular present a conundrum, with both anti- and protumorigenic properties. Numerous studies have demonstrated the propensity of MSCs to promote tumor growth and metastasis in vivo, including various sarcomas and Kaposi sarcoma and also as a metastatic niche in breast cancer.104–107 Cases of tumor formation after bone marrow and fat transplants have been reported in human subjects.111,112 However, MSCs have also proven beneficial in some cancer models, suppressing tumor formation in Ewing113 and Kaposi114 sarcoma and also as a delivery vehicle for genetic and chemotherapies.115,116 Although the evidence to date continues to support both roles of MSCs in malignancy, their ability to secrete factors influencing tumor formation, metastasis, and angiogenesis dependent on the microenvironment into which they are delivered necessitates caution and additional investigation to better characterize the mechanisms through which MSCs may contribute to tumor formation in the setting of cell therapy. In contrast, despite having a pluripotency and clonogenicity similar to ESCs, studies to date in both animals and humans have not identified a tumorigenicity of hAECs akin to ESCs and MSCs.65 One of the greatest limitations in the application of stem cells clinically has
been the ethical debate regarding their acquisition. In particular, the derivation and use of ESCs have been condemned by some authorities and banned by some governments because they require the destruction of human embryos. This has limited their availability for both research and clinical application. Similarly, although MSCs are now applied in various clinical trials, their procurement is invasive and cell numbers retrieved are low; only 10 MSCs are obtained per 1 million total bone marrow cells extracted with even lower numbers in older donors. These cells then require additional in vitro expansion to obtain a sufficient number for transplantation. The recognition that MSCs can be derived from adipose tissue may make future MSC isolation less invasive and more successful in terms of yields.

Nonetheless, cells obtained from gestational tissues appear particularly attractive. The umbilical cord and placenta are essentially medical waste, being discarded at birth. As such, this is a noninvasive and potentially limitless source of cells. In particular, the amniotic membrane yields plentiful cells, with an average of 100 million hAECS and 2 million AM-MSCs isolated per amnion.

Finally, in selecting an optimal therapy for BPD treatment, it is important to consider the current recommendations and quality control practices available. For example, one major drawback of MSC therapy is the lack of accepted surface markers for MSC classification, meaning that the purity of isolated populations is currently unknown. Indeed, the report of chimerism of bone marrow–derived stem cells into alveolar macrophages and worsening fibrosis with enhanced myofibroblast differentiation was likely the result of unidentified cells present in impure isolates. Similarly, current procedures for the procurement of ESC-derived differentiated cells provide insufficient purity for clinical use, increasing the risk of malignancy resulting from incomplete differentiation of transplanted cells. Even the quality controls on the use of cord blood–derived stem cells has been criticized, suggesting that culture conditions are currently even less reliable than for bone marrow–derived stem cells. With this in mind, our group has recently published an isolation protocol ensuring good manufacturing practices for the isolation of hAECS using animal-free products for human application.

CONCLUSIONS

Although much research has focused on the potential therapeutic benefit of cell therapies in lung injury and repair, there remain only limited studies of the efficacy of such therapy in bronchopulmonary dysplasia. Available studies suggest that cells derived from the bone marrow, umbilical cord, and amniotic membrane are likely to be the most effective for BPD. From a safety and quality control perspective, the use of amnion-derived stem cells appear to be a safe and abundant source of cells for autologous transplant in infants with BPD, improving on safety concerns surrounding MSCs in particular. However, confirmation of their utility and safety profile in other models of BPD injury are awaited. Finally, whichever cell type(s) are used in BPD therapy, additional research is needed to guide other issues such as optimal dose, route, and timing of cell administration, thereby guiding clinical trials and the development of possibly the first successful therapy for human preterm infants with BPD.

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