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**PREVIEWS**

Previews highlight research articles published in the current issue of STEM CELLS TRANSLATIONAL MEDICINE, putting the results in context for readers.

STEM CELLS TRANSLATIONAL MEDICINE

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Extracellular vesicles (EVs) comprise a heterogeneous group of lipid bilayer-delimited particles released from most, if not all, cells that play crucial roles in cell-to-cell communication through the transport of cargos that include various RNA species (including microRNAs), proteins, lipids, and DNA. Diverse populations of EVs display significant differences in size, morphology, composition, and/or biological mechanisms depending on their cell type of origin and associated physiological status. The interaction of EVs with target cells induces various effects, such as the stimulation of specific signaling pathways or the provision of trophic support,[1] and a range of studies have also established the crucial role of secreted EVs in the success of cell-based therapies; therefore, they may represent a safe and effective cell-free approach to the treatment of various diseases and disorders.[2,3] A vast range of stem and somatic cell types secrete elevated amounts of EVs, and current research aims in this field include the development of clinically-relevant isolation methods and the delineation of strategies to further enhance any inherent therapeutic potential. In our first Featured Article published this month in *STEM CELLS Translational Medicine*, Cardoso et al describe an optimized approach to the scalable and clinically-compatible manufacture of EVs from umbilical cord blood mononuclear cells that significantly accelerate wound healing.[4] In a Related Article published recently in *STEM CELLS*, Harting et al reported on the therapeutic capacities of mesenchymal stem cell (MSC)-derived EVs following inflammatory stimulation in a study that aimed to create a foundation for the enhanced treatment of inflammatory injuries and diseases.[5]

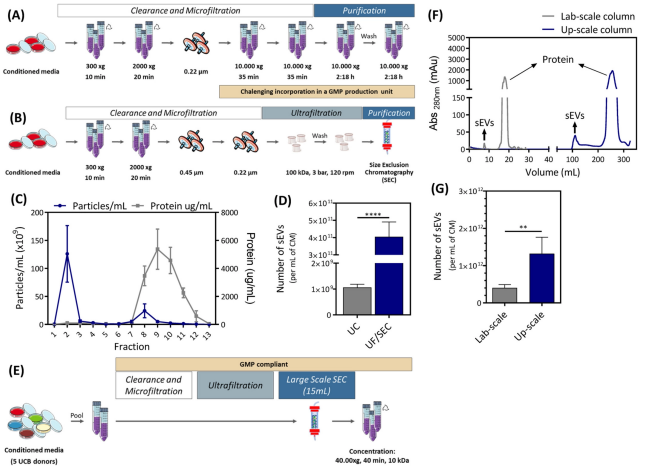
Cells of the innate immune system known as innate lymphoid cells (or ILCs) play crucial regulatory functions in immune responses to commensal microorganisms and pathogens at mucosal barriers, tissue inflammation, and adaptive immunity through the production of effector cytokines in response to a range of stimuli.[6] ILCs are currently categorized into three main groups—ILC1s, ILC2s, and ILC3s[7]—that each possess unique developmental, phenotypic, and functional characteristics while displaying broad similarities to T cell subsets (Th1, Th2, and Th17, respectively). In general, studies have underscored the importance of the proper function of tissue-resident ILCs to tissue homeostasis, morphogenesis, metabolism, repair, and regeneration[7]; however, ILC dysfunction has been established in inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, asthma, atopic dermatitis, and multiple sclerosis. Current research aims in the relatively young ILC field include defining the differences between circulating and tissue-resident ILCs and neonatal and adult ILCs and exploring the role of ILCs (specifically ILC2s[8]) in stem cell-based immunomodulatory therapies for inflammatory diseases. In our second Featured Article published this month in *STEM CELLS Translational Medicine*, Bennstein et al report the unique identity of cord blood-derived circulating ILCs compared to adult circulating and tissue-resident ILCs in a study that could significantly improve our understanding of neonatal innate immunity.[9] In a Related Article published recently in *STEM CELLS*, Fan et al demonstrated that induced pluripotent stem cell-derived MSCs (iPSC-MSCs) inhibited ILC2 function with the assistance of regulatory T cells through the inducible costimulator (ICOS)-ICOS ligand (ICOSL) signaling pathway.[10]

## FEATURED ARTICLES

### Clinically-Relevant Extracellular Vesicles Accelerate Diabetic Wound Healing

The clinical development of EV-based therapies requires the development of standardized methods for isolation and purification. While differential ultracentrifugation currently represents the gold-standard method, this strategy suffers from problems related to contamination, time constraints, and sample loss. Researchers led by Joana Simões-Correia (Exogenus Therapeutics, S.A., Cantanhede, Portugal) sought to avoid these problems with an approach that combined ultrafiltration and size exclusion chromatography (UF/SEC)[11,12] for the scalable and clinically-compatible isolation of small EVs (or sEVs, smaller than 200 nm) from umbilical cord blood mononuclear cells. As reported in their recent *STEM CELLS Translational Medicine* article,[4] Cardoso et al demonstrated significant improvements to production time, standardization, scalability, EV yield, and contamination levels when

implementing an optimized UF/SEC approach for the isolation of EVs from cells cultured under ischemic conditions compared to EVs isolated by differential ultracentrifugation. A detailed analysis of isolated EVs demonstrated the expected morphology and surface marker expression profile; furthermore, EVs carried proteins, lipids, and RNA species with known anti-inflammatory and regenerative capacities, suggesting that the UF/SEC methodology supports the isolation of EVs with sustained therapeutic potential. The authors confirmed this hypothesis by demonstrating how EV treatment promoted angiogenesis and extracellular matrix remodeling in vitro and significantly accelerated wound healing by modulating inflammation, angiogenesis, and extracellular matrix remodeling in a diabetic mouse model in vivo. Overall, these findings highlight UF/SEC as an exciting alternative to differential ultracentrifugation that supports the standardization and scalability required for the large-scale production of clinically-relevant EVs.

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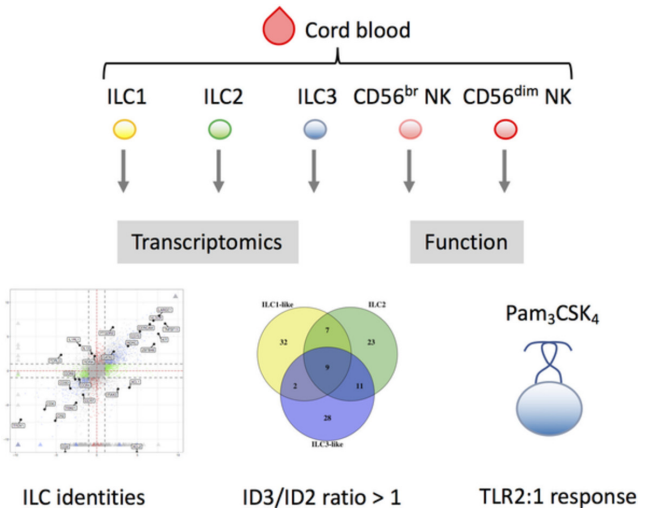
distinguished at the transcriptional level. Furthermore, cord blood ILCs transcriptionally resembled neonatal T cells rather than natural killer cells and displayed a unique signature of DNA binding inhibitor (ID) transcription factors when compared to adult circulating and tissue-resident ILCs. At the functional level, the team revealed that the cord blood ILC1 and ILC2 subsets failed to respond to specific cytokine stimulation in the same manner as tissue-resident ILCs, indicating functional immaturity; however, the cord blood ILC3 subset expressed toll-like receptors and responded to their stimulation through significantly increased proliferation and cytokine secretion. Overall, these data provide new insight into the development and function of neonatal circulating ILCs and suggest a crucial role of the ILC3 subset in neonatal innate host defense.

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## Characterization of Circulating Innate Lymphoid Cells from Neonates

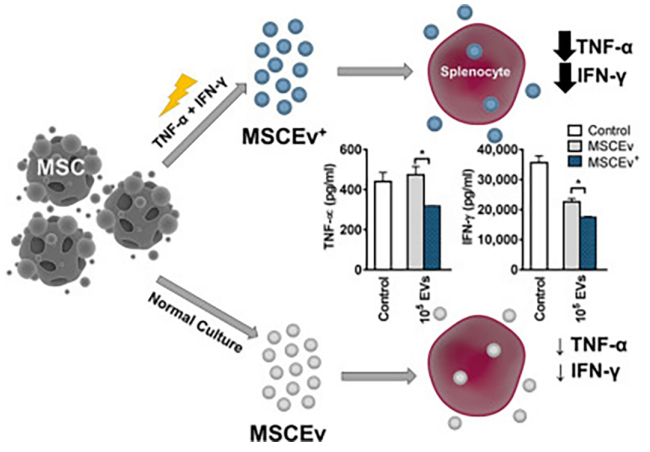
Research in mice has suggested a vital role for circulating neonatal ILCs in ensuring the location-specific innate immune defense through interactions with the environment and other immune cells.[13,14] To further our understanding of human neonatal circulating ILCs, researchers from the laboratory of Markus Uhrberg (Heinrich-Heine University Düsseldorf, Germany) took a systems biology approach and performed in-depth bulk RNA sequencing and functional analysis on ILCs from cord blood samples with the help of the team's recently developed staining protocol for the identification of cord blood ILCs and natural killer cells.[15] In their new *STEM CELLS Translational Medicine* article,[9] Bennstein et al report that while all three cord blood ILC types share the expression of certain factors, including CD28, CCR4, and SLAMF1, they could be easily

## RELATED ARTICLES

## Stimulated MSC-Derived Extracellular Vesicles Display Enhanced Anti-Inflammatory Capacities

The reported therapeutic relevance of MSC-derived EVs[16] and their relative safety regarding systemic application when compared to MSCs themselves[17] has fostered significant interest in their clinical translation as a safe and effective approach to the treatment of a range of disorders. In a recent *STEM CELLS* article, researchers led by Matthew T. Harting (University of Texas McGovern Medical School, Houston, Texas, USA) described a clinically-relevant approach to the production and isolation of EVs with enhanced anti-inflammatory capabilities. In their exciting study, the authors stimulated MSCs with pro-inflammatory cytokines (TNFα and IFNγ) in the hope that subsequently secreted EVs would possess enhanced anti-inflammatory properties. To explore this hypothesis, the team compared EVs secreted from stimulated (MSCEv+) and unstimulated MSCs (MSCEv)

after an enhanced isolation strategy that employed a clinically-relevant tangential flow filtration system. While remaining similar regarding size and surface marker expression, pro-inflammatory stimulation significantly altered the protein composition, cytokine profile, and RNA content of MSC-derived EVs. Excitingly, these alterations endowed them with a heightened immunomodulatory capacity that prompted a significant reduction in pro-inflammatory cytokine release from activated splenocytes when compared with EVs secreted by unstimulated MSCs thanks, in part, to a differential uptake mechanism and the elevated expression/activity of Cyclooxygenase 2 and Prostaglandin E2. Overall, the authors hoped that their relatively simple, reliable, and scalable approach might foster the development of safe and effective EV-based treatments for inflammatory injuries and diseases.

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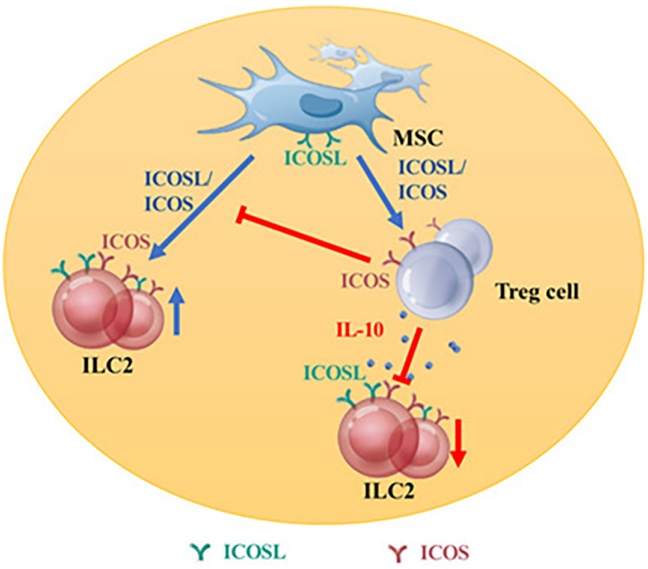
## Immunomodulatory MSCs Induce Regulatory T Cells to Suppress Innate Lymphoid Cells

Researchers from the laboratory of Qing-Ling Fu (Sun Yat-sen University, Guangzhou, China) recently described how iPSC-MSCs alleviated type 2 inflammation by modulating T lymphocyte subsets in allergic rhinitis patients and animal disease models,[18-20] a phenomenon controlled by ICOS-ICOSL-mediated signaling.[21] To explore the impact of MSC therapy on the ILC2 subset, given their role as critical controllers and effectors of type 2 inflammation,[8] the Fu team evaluated the immunomodulatory effects of iPSC-MSCs cocultured with peripheral blood mononuclear cells exposed to pro-inflammatory cytokines that potently activate ILC2 cells to model the effector phase of allergic airway inflammation. Their hypothesis stated that iPSC-MSCs would significantly inhibit ILC2 proliferation and activation and thereby suppress the development of allergy-specific inflammation. In a recent *STEM CELLS* article,[10] Fan et al discovered that iPSC-MSCs effectively downregulated the expression of pro-inflammatory, allergy-associated cytokines and reduced the number of activated ILC2s. Further experiments with purified peripheral blood mononuclear cell populations provided evidence that iPSC-MSCs did not directly suppress ILC2 activation and instead inhibited ILC2 function through a regulatory T cell-mediated mechanism. Specifically, iPSC-MSCs activated regulatory T cells through an ICOS-ICOSL interaction, with the activated regulatory T cells then suppressing ILC2 function through the secretion of the anti-inflammatory cytokine Interleukin-10. Overall, these fascinating findings provided new insight into how the regulation of ILC2s may represent an effective means to treat inflammatory disorders such as allergic rhinitis.

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