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RESEARCH LETTER

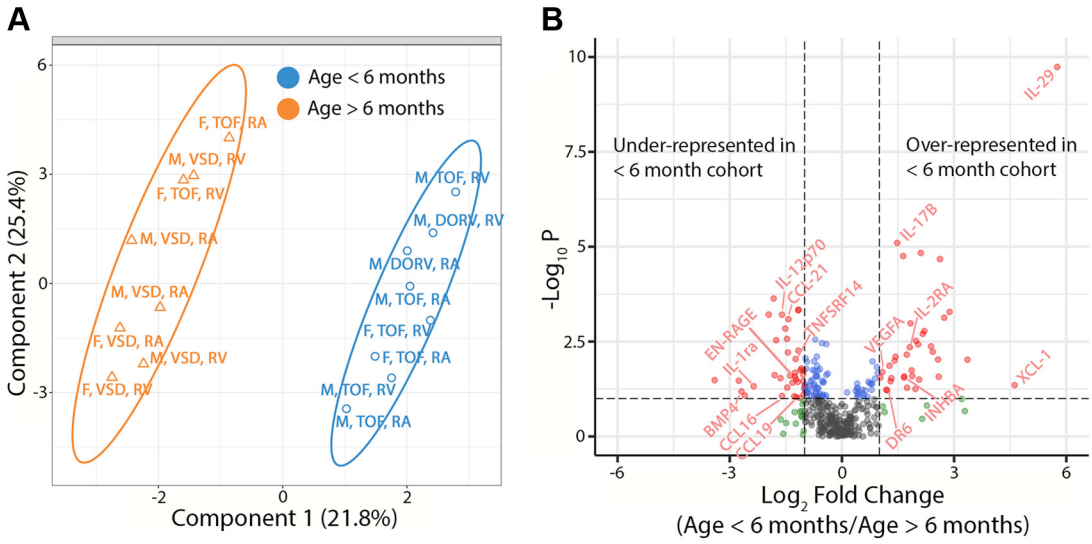
Mesenchymal Stromal Cells Isolated From Patients With Congenital Heart Disease Reveal an Age-Dependent Proinflammatory Phenotype

The morbidity associated with the repair of congenital heart disease (CHD) is highest in very young children, reflecting the greater complexity of morphological lesions requiring early intervention, and a potential inherent risk inversely associated with patient age. We hypothesize that normally reparative mesenchymal stromal cells (MSCs) in the neonatal myocardium secrete a specific repertoire of paracrine factors that condition a hyperinflammatory response to surgical intervention. To test this idea, MSCs were isolated from CHD myocardia during surgical repair under an institution approved protocol. MSCs were isolated from the right atria and right ventricles in 8 patients (total 16 samples) and grouped according to age: <6 months (<6 months; mean age: 3.6 months; median: 4.5 months; interquartile range: 2.0 months) and >6 months (>6 months; mean age: 13.5 months; median age: 10.8 months; interquartile range: 4.8 months). The diagnoses in the <6-month group included tetralogy of Fallot (n = 3) and double-outlet right ventricle (n = 1); the >6-month group included tetralogy of Fallot (n = 1) and ventricular septal defect (n = 3). MSCs were phenotyped using flow cytometry and shown to express the MSC markers CD90 and CD105 at similar levels (~75%; $P = 0.80$), confirming that the cells represented comparable populations. To determine the in vivo reparative effect, secreted factors were collected from MSCs and were intramyocardially injected into a rat left anterior descending occlusion infarct model (300 ng total protein), in accordance with animal protocol approval (#518128). The reparative efficacy of each patient-specific secretome, based on improvement in fractional shortening at 4 weeks after occlusion, revealed a strong inverse relationship to the age of the patient from which the secretomes were obtained ($R = -0.75$, $P < 0.001$), confirming the adverse effect of younger age on MSC-mediated repair capacity.

The heart is a lucrative source of endogenous factors secreted by MSCs that can mediate beneficial or maladaptive cardiac remodeling.[1] To determine the specific chemokine/cytokines that could mediate the age-dependent effect, secretome components were determined using a protein microarray (RayBio L-507 Human Array, RayBiotech). The protein constituents of the individual secretomes were compared using principal component analysis (**Figure 1A**) illustrating a clear segregation of the 2 age cohorts. It is notable that the cytokine profile constituents are distinctly different between the age groups, dominating minor differences associated with diagnosis, tissue of origin, or sex. Pathway analysis (ClueGO) of proteins differentially identified between the age groupings indicates enrichment of a proinflammatory phenotype in the younger cohort (ie, GO:0004896 [cytokine receptor activity], GO:0048018 [receptor ligand activity], GO:0030546 [signaling receptor activator activity], all $P < 10^{-7}$ [Bonferroni correction]; cytokines identified from GO pathways are labeled in **Figure 1B**). Interleukin (IL)-17B, a member of a family of proinflammatory cytokines that are hyperactivated in autoimmune disorders,[2] was differentially enriched in the <6-month cohort (fold change [FC] = 2.8; $P = 10^{-5}$) (**Figure 1B**). However, IL-1 receptor antagonist, the endogenous inspiration for the engineered IL-1R inhibitor anakinra, which inhibits IL-1-mediated inflammatory responses and dampens NOD-like receptor family pyrin domain-containing 3 inflammasome activation secondary to mitochondrial oxidative stress,[3] had reduced expression in the younger group (FC = 0.19, $P = 0.04$).

IL-29 (or interferon-λ1), which showed the most significant overabundance in the younger age group (FC = 54; $P = 10^{-10}$), signals via IL-28R1 and IL-10R2 to activate the Janus kinase-Signal Transducer and Activator of Transcription pathway (**Figure 1B**).[4] IL-29 exhibits protective properties against epithelial injury caused by various inflammatory disorders,[4]

FIGURE 1 Analysis of Cardiac Progenitor Cell Secreted Cytokines



(A) Segregation of cytokine profiles by age. Unsupervised analysis (principal component analysis) illustrates clear segregation of MSC cytokine profiles by patient age. Individual patient profiles are labeled by sex (M, F), lesion (VSD, TOF, or DORV), and sample site (RA, RV). Components illustrate the data variance along the specified axis. **(B)** Volcano plot of differentially expressed cytokines according to age. Dotted axes show significance limits at 2-fold change (Log2FC = 1) and $P < 0.05$. Cytokines from discussed GO pathways are labeled. BMP = bone morphogenetic protein; CCL = chemokine ligand; DORV = double outlet right ventricle; EN-RAGE = extracellular newly identified receptor for advanced glycation end products binding protein; F = female; FC = fold change; GO = gene ontology; INHBA = inhibin subunit beta A; M = male; MSC = mesenchymal stromal cells; RA = right atrium; RV = right ventricle; TNFSRF14 = tumor necrosis factor receptor superfamily member-14; TOF = tetralogy of Fallot; VEGFA = vascular endothelial growth factor A; VSD = ventricular septal defect; XCL-1 = X-C motif chemokine ligand-1.

consistent with the idea that neonatal MSCs can upregulate IL-29 secretion as a protective mechanism in response to the inflammatory milieu in the context of complex CHD.

The benefits of stem cell/stem cell secretome therapy have been linked to the reparative capacity associated with a subset of CD68-positive macrophages.[5] Secretome-injected rat infarct tissue was examined for changes in immune cell infiltration. Measured at 4 weeks after MI (resolution phase), hearts injected with MSC-secreted factors from >6-month patients showed significantly greater reparative CD68/CD206 double-positive macrophage (M2) infiltration ($P = 0.0001$), correlating with greater functional cardiac improvement (2.2% vs 6.6% improvement in FS, $P = 0.04$). This difference corresponded with pathway analysis highlighting terms for macrophages and monocytes (ie, GO:0030225 [macrophage differentiation], GO:0071677 [mononuclear cell migration], GO:0002548 [monocyte chemotaxis], $P < 0.001$ for all).

Overall, our analysis suggests that MSC-derived secretomes from very young CHD patients feature a more inflammatory chemokine/cytokine profile adverse to cardiac repair, and a reduced capacity to generate a resolving inflammatory cell response, than those from infants older than 6 months. Our findings infer that strategies to reduce the inflammatory response will be especially beneficial in surgical interventions for the youngest CHD patients. This analysis identifies specific MSC factors that represent potential therapeutic anti-inflammatory targets. The IL-1R antagonist anakinra, IL-17 inhibitors, and pegylated interferon-λ1 are testable Food and Drug Administration-approved drugs predicted from this study to therapeutically mitigate the elevated systemic inflammatory response and associated cardiac dysfunction consequent to surgical repair of complex neonatal CHD.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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