Negative Effects of Age-Related Chronic Inflammation on Skeletal Stem Cells

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Purpose: Osteoprogenitor cells (OPCs) are not resistant to the aging process. We hypothesize that age-related chronic inflammation contributes to a decline in the osteogenic capacity of OPCs.

Methods: Young, 12-week-old and aged, 52-week-old C57BL/6J mice were used. Aged animals were randomly distributed into control and treatment groups (n = 5 each). Animals in the control group received regular drinking water, while animals in the treatment group received sodium salicylate water for 8 weeks. The inflammatory status of the 3 groups was assessed using a multiplex platform screening for pro- and anti-inflammatory cytokines. FACS (fluorescence-activated cell sorting) was employed to identify the effect of chronic low-level inflammation on OPC number. Proliferation and osteogenic differentiation were assessed in vitro. Two-tailed Student t tests and Mann-Whitney U test were used to determine significant differences between data sets. Significance was attained at $P < 0.05$.

Results: First, we confirmed the presence of inflamm-aging in aged mice using multiplex analysis, and showed that the process is reversible by NSAID (nonsteroidal anti-inflammatory drug) treatment. Next, we aimed at understanding how aging effects OPC number using flow cytometry, which revealed that OPCs made up 0.38% and 0.017% of cells from young and aged mice, respectively. Next, we assessed whether OPC frequency changes as a result of suppressed inflamm-aging. Mesenchymal stem cells (MSCs) from 3, 12, and 12-month-old NSAID-treated animals were subjected to flow cytometry. OPC frequency declined with aging; however, after an 8-week course of NSAID treatment, we noticed a 2-fold increase in OPCs. We then aimed at testing whether NSAID treatment of aged mice resulted in a restoration of the osteogenic potential of the OPCs. Quantitative real-time polymerase chain reaction of the MSCs from NSAID-treated mice showed an increase in osteogenic gene expression compared to untreated aged mice. To further characterize this increase in osteogenic potential, we analyzed MSCs in vitro after treating them with osteogenic differentiation media. Mineralization assays and expression analysis showed decreased osteogenesis of aged cells, while treatment with sodium salicylate recovered this decline and resulted in restoration of the osteogenic potential.

Conclusion: These experiments demonstrate for the first time that age-related chronic inflammation is responsible for the decreased proliferative and osteogenic potential of aged OPCs and that this process is reversible by anti-inflammatory treatment.