Local Environment Changes Driving Progression to Non-union

Michalis Panteli, MD*; James Vun, MBCHB, MRCSED;*
Robert Michael West, DPHIL (OXON), MSc; Elena Jones, PhD;
Ippokratis Pountos, MD; Peter Giannoudis MD, BS
University of Leeds, Leeds, UNITED KINGDOM

Purpose: The aim of this study was to investigate the proliferation, osteogenic potential, and biological functions of mesenchymal stem cells (MSCs) isolated from patients with established atrophic, aseptic nonunions.

Methods: Following informed written consent, samples (peripheral blood, nonunion tissue, bone away from the nonunion site) were collected from 10 patients. Inclusion criteria were adult patients (18 to 65 years old) having revision surgery for an atrophic nonunion of the femur or tibia, with no evidence of infection or having any risk factors for developing a nonunion. Proliferation, osteogenesis, gene expression (a panel of 84 genes involved in osteogenesis was examined), and protein secretion of MSCs isolated from the nonunion site were investigated and compared to that of MSCs isolated from bone away from the nonunion site. To investigate the effect of circulating cytokines on the functions of MSCs, we compared the proliferation and osteogenic potential of MSCs cultured with standard medium, compared to autologous serum medium. Furthermore, we assessed the serum concentrations of the cytokines found to be under- or overregulated in nonunion tissue.

Results: No differences were found in terms of MSC content and proliferation between nonunion and uninvolved bone regardless of medium used, but culture with medium containing patient's own serum led to superior proliferation both in nonunion and uninvolved bone MSCs ($P<0.001$). The osteogenic differentiation of P3 cells was comparable in the cells isolated from bone and those isolated from nonunion tissue (calcium assay: $P=0.446$; alkaline phosphatase [ALP] activity: $P = 0.963$). Comparing the messenger RNA expression of samples at baseline, ICAM [intercellular adhesion molecule] 1, MMP [matrix metalloproteinase] 10, and GLI1 were found to be overexpressed in nonunion MSCs, whereas EGF [epidermal growth factor], IGF [insulin-like growth factor] 2, MMP8, and COL [collagen] 14A1 were underexpressed. Following osteogenic stimulation, only IGF2 and EGF genes were underexpressed in nonunion MSCs. Investigating the above molecules on a protein level, MMP-8 and IGF-2 were reduced in the nonunion tissue ($P<0.001$). On the contrary, the concentration of Dkk-1 was found to be increased in the nonunion-derived MSCs ($P = 0.011$).

Conclusion: Nonunion tissue and unaffected bone contain MSCs with comparable proliferation and osteogenic potential. Comparing the expression of nonunion and bone MSCs at baseline suggest that an inflammatory environment may be the cause of their downregulation, a finding that may support that the cause of the nonunion may be local and not systemic. In other words, the patients have otherwise 'normal' MSCs that can proliferate and differentiate, but at the site of the nonunion their functions are impaired.