INTRODUCTION

Distraction osteogenesis (DO) is a technique that has been widely utilized by surgeons for limb lengthening and in the correction of deformities and nonunions. The two most notable characteristics of this process are that bone is primarily formed by an intramembranous process and that the new tissues are highly vascularized. In order to test the functional role of vascularization in promoting bone formation and assess the relative importance of the two different vascular endothelial growth factor receptors in mediating vascular tissue and bone formation during DO, we used antibody blockade to both VEGFR1 and VEGFR2 and compared this to single antibody blockade to only VEGFR2.

RESULTS

In collaboration with the ImClone Corporation through a materials transfer agreement. Antibodies to VEGFR1 (MF1) and VEGFR2 (DC101) were provided by the ImClone Corporation through a materials transfer agreement.

*Surgical, Distraction and Injection Protocols. All animal research was performed under an approved IACUC protocol. The surgical method of murine DO was performed as previously described. Antibodies were delivered by intraperitoneal injections every third day after perfusion with a suspension of lead chromate in silicone rubber.

**Assessment of Bone and Vascular Formation By µCT. Bone formation and vasculature were assessed by µCT. After removal of the fixators, bones are imaged via µCT at a resolution of 12 µm using a Scanco µCT 40 system (Scanco Medical, Basserdorf, Switzerland). Specimens are immersed in a 60-40 solution of ethyl alcohol and saline during scanning in order to minimize decomposition of the tissue throughout the duration of the scan (~70 minutes). Measurements of total bone volume fraction and mineral density are made directly from µCT image data of each specimen. Vasculature was imaged by µCT after perfusion with a suspension of lead chromate in silicone rubber.

**Molecular Analysis. Tissue samples were collected and mRNA expression was analyzed for the following time points: day 0 (normal bone) day 7 (end of latency), day 10 (latency and 3 days of active distraction), day 17 (latency and 10 days of active distraction), day 20 (latency, active distraction and 3 days of consolidation) and day 31 (latency, active distraction and 14 days of consolidation). Bone formation was assessed by mRMA assay for a set of extracellular matrix protein mRNAs including osteopontin (OPN), bone sialoprotein (BSP), collagen types 1A1 and 1A2. Expression of various angiogenic receptors including flt1, flk1, Nrp1 and Nrp2 were also assessed across both normal and antibody blockade.

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**ACKNOWLEDGEMENTS

NIH AR049410; University of Alabama at Birmingham

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53rd Annual Meeting of the Orthopaedic Research Society

Paper No: 0180