GENE EXPRESSION DURING POSTEROLATERAL LUMBAR SPINE FUSION: EFFECT OF BMP-2. Scott D. Boden, MD, Michael A. Morone, MD, PhD, George Martin, MD, Greg Hair, PhD, and Michelle Racine. From the Emory Spine Center, Emory University School of Medicine, and the Veterans Affairs Medical Center, Atlanta, Ga.

INTRODUCTION: Posterolateral spine arthrodesis may result in failure to achieve a solid bony union in up to 35% of patients. Recent studies using a validated rabbit model have characterized the healing process using quantitative histomorphometry. However, little is known from a molecular biology perspective about expression of bone and cartilage-related genes during this process. In addition, the role of bone morphogenetic proteins is unclear. Using current molecular biology techniques (RT/PCR) small quantities of tissue can be used to study the expression of specific genes. The goals of this investigation were: 1) To describe the temporal and spatial pattern of bone and cartilage-related gene expression; 2) To correlate the gene expression patterns with the histologic healing patterns; and, 3) To describe the effect of bone morphogenetic protein-2 (BMP-2) on gene expression during spine fusion healing.

MATERIALS AND METHODS: Part I: After approval by the IACUC, 20 adult New Zealand white rabbits underwent L4-5 posterolateral intertransverse process spine arthrodesis using autogenous iliac crest bone graft. Rabbits were euthanized at 2 days, 4 days, 1,2,3,4,5,6,or 10 weeks following surgery. Part II: 14 adult NZW rabbits underwent arthrodesis using autogenous iliac crest bone that had been soaked in 1.5 mg/ml rhBMP-2 solution (Genetics Institute, Cambridge, Ma). Rabbits were euthanized at the same time points as above.

RNA Extraction. Fusion masses were harvested and divided into thirds (two transverse process outer zones and one central zone). Each third was then frozen in liquid nitrogen. Total RNA was extracted using 4 M guanidine isothiocyanate. RNA was extracted from iliac crests harvested from 4 animals to serve as a control for baseline gene expression at time 0 in the fusion mass. The RNA then underwent reverse transcription/polymerase chain reaction (RT/PCR) to study the expression of various genes. Unique PCR primers were designed using sequence information available in Genebank. The number of PCR cycles was 22 for bone morphogenetic protein-2 (BMP-2), BMP-4, BMP-6, Type I collagen, Type II collagen, osteopontin, osteonection, osteocalcin, alkaline phosphatase, and GAPDH (a housekeeping gene). All PCR products were separated on a 12% acrylamide gel and exposed on a phosphorimager. The intensities were normalized to that of GAPDH and then normalized to the relative intensity of each mRNA PCR product at time zero. Quantitation was performed on 2-3 PCR replicates of three independent samples from separate RT reactions and expressed as mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA), with Bonferroni’s post hoc multiple comparison test, was used to detect differences at different time points. All findings described below reached statistical significance defined as p < .05.

RESULTS: Part I: A unique temporal and spatial pattern of osteoblast-related gene expression was observed based on RT/PCR analysis of RNA from the different zones of the fusion mass. During the second and third weeks a significant increase was seen in Type I collagen gene expression. Osteopontin (OP) and osteonectin (ON) were both increased by 1 week, with osteopontin peaking in week 3 (150-fold increase) and osteonectin in week 2 (175-fold increase). A 28-fold increase in osteocalcin PCR product expression was seen in weeks 3-4 in the outer zones. Gene expression in the central zone of the fusion mass lagged 1-2 weeks behind that of the two outer zones correlating with the central lag effect previously observed in the histologic healing sequence.

Expression of several BMPs was also studied. In the outer zones, increased BMP-2 expression was seen in weeks 2-6 with peak expression in weeks 3-4 (40-fold increase). BMP-4 demonstrated a different pattern with a 40-fold increase in week 1 that decreased significantly by week 3. BMP-6 had an early increase on day 2 (54-fold) and a second peak (100-fold) in weeks 4-5. These findings suggest unique time patterns of expression and possibly unique roles for various BMPs during spine fusion.

Part II: Soaking the autogenous bone graft with rhBMP-2 had a dramatic impact on the gene expression during spine fusion healing. Most notably, there was a significantly greater increase in BMP-6 expression on day 2 (95-fold) in the outer zone and an earlier second BMP-6 peak in the central zone at week 2 (instead of week 4). In addition, expression of other bone-related genes described in Part I was seen earlier and at higher levels in the presence of rhBMP-2. The previously observed lag effect in the central zone was minimized with the addition of rhBMP-2 which may explain the decreased...
nonunion rate described in previous animal studies of spine fusion using rhBMP-2.

DISCUSSION: We have shown that it is possible to reproducibly measure gene expression in spine fusion masses using RT/PCR technology. BMP-6 was the earliest of the BMPs to show increased expression and may be a critical factor in the initiation of spine fusion healing. Soaking of autograft with rhBMP-2 increased the early BMP-6 peak and decreased the central lag effect which may explain why fusions with rhBMP-2 heal faster and with less nonunions. In addition to helping understand how rhBMP-2 may function in vivo, these baseline gene expression data will facilitate the design of experiments with fusions enhanced (ultrasound, electrical stimulation) or retarded (nicotine, NSAIDs) to elucidate the potential mechanisms of action of these agents and to design gene-specific biologic strategies to more effectively manipulate the spine fusion healing process.