INTRODUCTION: Autologous bone is widely accepted as the gold standard for graft material because it generally offers the most predictable and favorable clinical outcomes. However, the procurement of autograft is associated with a high incidence of complications. In addition, the amount of autograft available can sometimes be limited (e.g., in long fusions and in patients undergoing multiple revision surgeries). In an attempt to avoid some of the limitations and complications of autograft, several alternatives have been developed. These include cadaveric allograft, xenograft, and synthetic bone matrix. One major difference between autograft and its alternatives is the addition of a viable, non-antigenic cellular component consisting of osteoblasts, osteocytes, peristeal cells, and marrow elements (including mesenchymal stem cells). The contribution of graft-derived cells to the fusion site has been examined in a number of studies, but there is no consensus regarding their exact contribution, especially during the later phases of graft incorporation. In order to investigate this question, we developed a mouse model of spinal fusion in which male-derived bone graft was transplanted into syngeneic female mice. This procedure allowed us to track the fate of the grafted cells through the use of Y-chromosome painting probes. In addition, this novel mouse model of spinal fusion was characterized radiographically, histologically, and histochemically.

METHODS: Spinal fusion. Eleven Balb/c mice (Jackson Laboratories, Bar Harbor, ME) formed the basis of this study. Animal protocols were approved by the institutional committee on animal research. A posterolateral paraspinal approach was taken. The transverse processes of the first and second coccygeal vertebrae of female mice using a 1:1 mixture of morselized iliac crest and syngeneic male derived bone graft. Histologic, histochemical, and Y-chromosome analysis were performed by hybridizing the slides with a Y-chromosome specific probe. Specimens were fixed in PFA, decalcified, embedded, and cut into 7μm thick slide sections. Histologic analysis was performed by the H&E method. Osteoblastic and osteoclastic activities were determined histochmically using stains for alkaline phosphatase and tartrate-resistant acid phosphatase, respectively. Y-chromosome painting was performed by hybridizing the slides with a dioxigenin-labeled Y-chromosome probe. Hybridized probe was then detected with a fluorochrome conjugated anti-dioxigenin antibody.

RESULTS: One week postoperative. Intraembranous and endochondral bone flowed off of the decorticated transverse processes. The central area consisted of unremodeled and unincorporated graft chunks. Alkaline phosphatase activity was noted on the decorticated transverse processes as well as within graft material located at the periphery of the fusion bed adjacent to the soft tissue envelope. The central portion of the fusion bed was devoid of osteoblastic activity, although TRAP activity was noted in this region, indicating osteoclastic resorption. Y-chromosome painting revealed abundant graft-derived cells at this point. These cells which survived transplantation consisted of osteocytes within cortical pieces of bone graft, marrow cells from the trabecular portions of the graft, peristomal cells lining the bone graft, and osteoblasts. Two weeks postoperative. Early consolidation was noted radiographically and histologically between the transverse processes and the adjacent bone graft, with the formation of marrow spaces in these regions. The central portion of the graft now demonstrated more alkaline phosphatase activity but remained separated from the consolidated areas. Cartilage islands were noted bridging adjacent pieces of bone graft, and also bridging bone graft to decorticated transverse process. Cartilage was also noted in the periphery, adjacent to the dorsal musculature. The cellular contribution of graft remained abundant. Graft derived cells lined the marrow surfaces of the areas which had consolidated. They were also present within the fusion mass as osteocytes, as well as within the cartilage islands. Interestingly, when cartilage islands lay between host and donor tissues, the chondrocytes near the graft were of graft origin, whereas those adjacent to the host tissues were of host origin. Six weeks postoperative. By this point, a solid arthrodesis had been achieved in all of the animals. A well formed, thin cortical rim with a well developed periosteum surrounded the fusion mass. The transverse processes were completely engulfed in the fusion mass. A very mature marrow cavity was present. Graft derived cells consisted of occasional osteocytes but were present in much fewer numbers than during the earlier timepoints.

DISCUSSION: In the present study, we developed and characterized a novel mouse model of posterolateral intertransverse process spinal fusion. The timecourse of fusion was accelerated in mice compared to humans and other animal models. All of our mice had healed radiographically and histologically by 6 weeks. However, not all mice undergoing this procedure fused. Preliminary work (not shown) in which less bone graft was used resulted in a much higher rate of nonunion. In addition, animals that underwent decortication alone without bone grafting also failed to fuse. Because the focus of the present study was to determine the cellular contribution of graft to the fusion, however, a dose response study was not performed and a sufficient amount of bone graft was used to ensure fusion. It is possible that with smaller amounts of graft material, the eventual cellular contribution may be lower.

Fusion occurred through the formation of both endochondral and intramembranous bone. Consolidation and incorporation of bone graft proceeded from the decorticated transverse processes towards the central region. In fact, the central portion of the fusion bed remained unincorporated at two weeks, whereas graft near the transverse processes had already incorporated and, in some areas, formed a marrow space. One potential explanation for this observation is that decortication of the transverse processes liberates cells with osteogenic potential. In our model, bone graft appeared to make two different types of cellular contributions. At one week, the primary contribution consisted of the surviving transplanted cells in and around the pieces of bone graft. By two weeks, however, graft derived chondrocytes arose de novo in areas undergoing endochondral ossification. Other graft derived cells (such as osteoblasts and marrow cells) were also present, but because these cell types had been directly transplanted at surgery, it is unknown whether they arose de novo or were transplanted. By six weeks, after extensive remodeling had occurred, scattered graft derived osteocytes remained present within the cortical rim, suggesting that bone graft does make a lasting cellular contribution to the fusion mass.

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