Introduction: The morphological abnormalities that result from immobilization during joint development have been well documented (1, 2). In addition, previous studies have begun to characterize the molecular biology of normal joint development via in situ hybridization and immunohistochemical techniques (3-6). The objective of this study was to investigate how temporal and spatial patterns of characteristic extracellular matrix (ECM) molecules are altered under conditions of reduced mechanical loading in developing synovial joints. In particular, we focused on ECM molecules whose synthesis is known to be influenced by mechanical stimuli: collagen XII and tenascin-C (7, 8). Because of the demonstrated in vitro mechano-sensitivity of these molecules, we hypothesized that their expression patterns and intensity would be altered under conditions of reduced mechanical stimulation during in vivo joint development.

Methods: Limb immobilization was pharmacologically induced in embryonic chicks starting at day 6 of incubation using a well-established skeletal muscle depolarizing agent, decamethonium bromide (DMB), effectively causing the limbs to develop in the absence of normal mechanical loading (2, 9). DMB (0.02%) was dropped onto the choroidallantoic membrane twice daily, while control embryos received equal volumes of saline. Embryos were sacrificed at daily intervals from 8 to 17 days of incubation, and all handling was in accordance with animal experiment regulations in Switzerland. Using standard techniques of immunohistochemistry with appropriate negative controls and predigestion as necessary, we examined the effects of immobilization on the temporal and spatial patterns of protein expression in the developing interphalangeal joints of the foot and in the knee joint. Specifically, we probed for the presence of the following proteins with purified antibodies against: (1) procollagen I, (2) procollagen II, (3) collagen XII, and (4) tenascin-C. Three different antibodies against tenascin-C were compared, allowing localization of the three tenascin variants, Tn190, Tn200, and Tn230.

Results: In the normal chick joint subsequent to cavitation, the most superficial fibrous layer of the joint surface exhibits positive immunostaining for collagens I, XII, and tenascin. Immediately beneath this fibrous cell layer in the region containing rounded overt chondrocytes, collagen II and tenascin are expressed, with tenascin expression diminishing deeper into the epiphysis and absent in the more mature flat and hypertrophic cell zones. As noted by other investigators (6), the predominant tenascin variant expressed in the epiphyseal cartilage was determined to be the short form, Tn190. In addition, the intra-articular structures of the knee joint stain positively for collagens I, XII, and tenasin, with very high expression of these molecules in tendons, ligaments, menisci, and synovium (Fig. 1L).

In the DMB-treated embryos, changes in molecular expression patterns are observed concomitant with fusion of the joint surfaces and the degeneration of intra-articular structures which accompany joint immobilization. Prior to joint fusion, the population of cells in the fibrous surface layer diminishes in number, with an associated decrease in the extent of expression of collagens I, XII, and tenasin. As the joint fuses, the cells of the fused region differentiate into overt rounded chondrocytes, and no longer express collagens I or XII (Fig. 1R), but are positive for collagen II and the shortest tenasin variant at a level consistent with that of immature epiphyseal chondrocytes in a normal joint.

While collagens I and XII continue to be strongly expressed at other sites within the immobilized joint (Fig. 1R), the level of tenasin expression appears to be further diminished in regions of the chondroepiphysis, synovium, and tendons, as well as within the remnants of the fibrous surface layer of cells. In particular, the largest tenasin variant, Tn230, which is normally strongly expressed at the margins of the joint near the first sites of cavitation (Fig. 2L), is markedly reduced during immobilization (Fig. 2R). Tenasin levels in the DMB-treated embryos remain normal in other sites such as the dermis, however.

Discussion: This study demonstrates that the morphological abnormalities which result from embryonic immobilization during joint formation are associated with altered patterns of molecular expression within the developing joint. Accompanying joint fusion and the degeneration of intra-articular structures is a decrease in the extent of ECM proteins normally expressed by these cell populations (e.g. collagens I, XII). However, while the expression of collagens I and XII persists at normal levels elsewhere within the immobilized joint, tenasin expression is additionally diminished within the chondroepiphysis, synovium, and tendons, as well as within the remnants of the fibrous surface layer of cells. This effect is most notable for the largest tenasin variant, Tn230. The reduction in Tn230 near normal sites of cavitation, in conjunction with the observed absence of complete cavitation under conditions of immobilization, support the hypothesis that the anti-adhesive properties of tenasin may normally contribute to the establishment and maintenance of a fully articular joint (10). These data provide in vivo support of in vitro studies which suggest that tenasin expression is sensitive to external changes in mechanical loading environment (8). However, these data do not support a similar conclusion for collagen XII in vivo. While embryonic immobilization results in altered patterns of collagen XII expression in association with joint fusion and the degeneration of intra-articular structures, no additional decrease in collagen XII is seen within joint structures and cell populations which persist under conditions of reduced mechanical loading during joint formation.

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