lagens. Moreover, the nuclei of these fibroblastic-like cells were positive for PCNA (Fig. 1E). Type-II collagen was not noted in either fibroblastic-like cells or fibrous connective tissue (Fig. 1C). IGF-1 (Fig. 1F) and IGF-1 receptor (Fig. 1G) showed almost the same distribution, being identified in the cytoplasm and areas around fibroblastic-like cells in the fibrous connective tissue. The results described above were obtained in all 5 animals. Chondrocytes noted in a layer deeper than fibroblastic-like cells were negative for PCNA in all but 1 animal, and the cartilage matrix surrounding the chondrocytes was positive for type-II collagen. These chondrocytes were positive for type-I collagen in 3 of 5 animals and for type-III collagen in 1 animal, but no animals were positive for PCNA and type-I and -III collagens at the same time.

In the weight-bearing regions of the cartilage, the cartilage matrix was almost homogeneously positive for type-II collagen. Chondrocytes in the tangential zone were positive for type-I collagen (2/5 animals), IGF-1 (5/5 animals) and IGF-1 receptor (5/5 animals), almost continuously from the articular margins. Chondrocytes in the tangential zone were positive for PCNA in only 1 animal.

Moderate group

Fibroblastic-like cells in the fibrous connective tissue were positive for type-I (Fig. 2B) and -III (Fig. 2D) collagens and PCNA (Fig. 2E) in all animals, as well as in the mild group. In chondrocytes clustering in the layer deeper than fibroblastic-like cells in the fibrous connective tissue, type-I and -III collagens and PCNA were identified in 4 animals. The extracellular matrix surrounding these chondrocytes was positive for type-II collagen (Fig. 2C). IGF-1 (Fig. 2F) and IGF-1 receptor (Fig. 2G) were identified in fibroblastic-like cells in the fibrous connective tissue and chondrocytes in the layer deeper than fibroblastic-like cells in all animals.

In the superficial layer of the weight-bearing regions of the cartilage, type-I (6/7 animals) and -III (5/7 animals) collagens were noted in the degenerated cartilage matrix. Staining for type-II collagen was not heterogeneous, and the degenerated cartilage matrix in the superficial layer of the weight-bearing regions showed stronger staining. Neither IGF-1 nor IGF-1 receptor was observed in the tangential zone or the deeper cartilage layer.

Severe group

At the articular margins, the bone component in the osteophytes was positive for type-I collagen (Fig. 4B) (5/5 animals). Fibroblastic-like cells in the fibrous connective tissue around

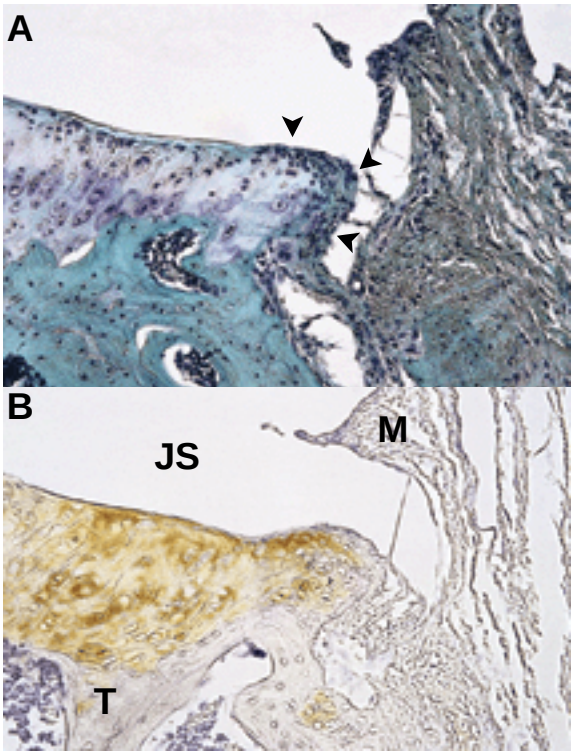

Fig. 3. Safranin-O staining (**A**) reveals a hump-like process (arrowheads) with a thick calcified zone from the medial articular margins of the tibial plateau in the severe group of 12-month-old animals with end-stage osteoarthritis. Chondrocytes are noted in the process, but not stained by safranin O. A distinctive tide mark is observed in a layer deeper than the cartilage layer (**A**). The calcified zone below the tide mark is positive for type-II collagen (**B**), suggesting that the hump-like process is chondrophyte. JS, joint space; M, medial meniscus; T, tibia. Original magnification, × 200.

the osteophyte were positive for type-I and -III collagens (Fig. 4D) and slightly positive for PCNA (Fig.4E) (5/5 animals). Type-II collagen was not observed in the fibrous connective tissue (Fig. 4C). IGF-1 (Fig. 4F) and IGF-1 receptor (Fig.4G) were noted in the fibrous connective tissue of all animals.

In the weight-bearing regions, the staining intensity for type-II collagen in the cartilage matrix tended to decrease with osteoarthritis progression. The staining intensities for type-I and -III collagens were increased mainly in the superficial layer of the degenerated cartilage compared with those in the mild and moderate groups. No PCNA-positive cells were detected in the weight-bearing regions of the cartilage. The superficial matrix of the degenerated cartilage was positive for IGF-1 and IGF-1 receptor (5/5 animals).

Synovial membrane

IGF-1 and IGF-1 receptor showed the same staining pattern, a comparatively homogeneous distribution in the cytoplasm of lining cells of the synovial membrane. There was no intergroup difference in this staining pattern.

Discussion

A study in human knee joints by Allard et al. (1990) has suggested the presence of fibrous connective tissue that is connected to the synovial membrane, is not stained with safranin O, and differs from articular cartilage at the margins. Using a rabbit model for experimental osteoarthritis, Moskowitz and Goldberg (1987) have demonstrated that cells with a very high regenerative capacity are present in the junction between the synovial membrane and perichondrium or between the synovial membrane and periosteum at the margin of the articular cartilage, and these cells are involved in osteophyte formation. Telhag and Lindberg (1972) also reported similar results. Moreover, an immunohistological study has demonstrated that fibroblastic-like cells in the fibrous connective tissue produce a connective tissue matrix mainly composed of type-I and -III collagens (Aigner et al., 1995). The presence of type-I and -III collagens was confirmed in totipotent immature mesenchymal cells of the cartilagenous anlage during the embryonic period (Linsenmayer et al., 1973; von der Mark et al., 1976; von der Mark and von der Mark, 1977). In this study, we confirmed the presence of fibroblastic-like cells that were embedded in the fibrous connective tissue and were positive for type-I and -III collagens, but were negative for type-II collagen at the cartilagenous margins of the knee joint in guinea pigs. It was also demonstrated that these cells were positive for PCNA as well, indicating that they have a high mitotic activity. Consequently, fibroblastic-like cells at the margins of the articular cartilage seemed to be totipotent immature mesenchymal cells based on the features of the staining pattern for collagens and on having differentiating potential.

Fibrous connective tissue was present at the articular margins, and fibroblastic-like cells presumed to be totipotent immature mesenchymal cells were identified in this connective tissue in all animals irrespective of the degree of osteoarthritis. Mitotic activity of chondrocytes observed in the layer deeper than these fibroblastic-like cells varied according to the degree of osteoarthritis. In the mild group, no mitotic activity of the chondrocytes at the margins was enhanced. In the moderate group, however, chondrocytes with an enhanced mitotic activity had proliferated and clustered. Around these chondrocytes, a cartilage matrix positive for type-II collagen was identified. In the severe group, in which osteoarthritis had progressed further, a hump-like process with a thick calcified zone, which was considered to be the chondrophyte, was observed. Furthermore, this area had been substituted by bone tissue positive for type-I collagen. These findings suggest that the mechanism of osteophyte formation is mediated by endochondral ossification.

IGF-1 and IGF-1 receptor showed almost the same distribution in all animals. This result demonstrates that chondrocytes with an IGF-1 secretory activity and those with IGF-1 receptor exist in the joints. In particular, totipotent immature mesenchymal cells at the margins of the