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Processes of Osteophyte Formation in Guinea Pigs with Spontaneous Osteoarthritis

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In this study, we investigated osteophyte formation processes in guinea pigs with spontaneous osteoarthritis, histochemically and immunohistochemically. Serial thin frontal sections of right knee joints were prepared from Hartley guinea pigs aged 1, 3, 5, 8, 12 and 18 months. The severity of osteoarthritis was evaluated by safranin-O staining, and the animals were classified into 3 groups: mild, moderate and severe. In addition, immunostaining was performed by using primary antibodies against the proliferating cell nuclear antigen (PCNA), type-I, -II and -III collagens, insulin-like growth factor 1 (IGF-1) and IGF-1 receptor. In the mild group, there was fibrous connective tissue continuous with the synovial membrane and covering the margins of the articular cartilage of the medial tibial condyle. This tissue contained spindle-shaped fibroblastic-like cells. These cells were positive for PCNA, type-I and -III collagens, IGF-1 and IGF-1 receptor. In the moderate group, the chondrocytes beneath the fibroblastic-like cell layer had proliferated and were clustered together. These chondrocytes were also positive for PCNA, type-I and -III collagens, IGF-1 and IGF-1 receptor. In the severe group, this marginal area had been replaced by type-II collagen-positive chondrophytes, which further changed to osteophytes due to the process of endochondral ossification. In guinea pigs, fibroblastic-like cells at the margins of the articular cartilage of the knee joints seemed to be totipotent immature mesenchymal cells. These cells may be the precursors of osteophytes, and IGF-1 appears to be involved in their formation.

Key words: guinea pig; insulin-like growth factor 1; osteoarthritis; osteophyte formation

The pathological changes in osteoarthritis include articular cartilage degeneration and proliferative changes, such as osteophyte formation and sclerosis of the subchondral bone. Sclerosis of the subchondral bone is regarded as a reactive phenomenon to excessive loading, but little is known about the mechanism of osteophyte formation (Jewell et al., 1998). It has been reported that acromegalic osteoarthropathy, which is categorized as secondary osteoarthritis, is characterized by hyperplasia of the articular cartilage and marked osteophyte formation (Bluestone et al., 1971; Jaffe, 1972; Johanson et al., 1983; Resnick, 1988). Insulin-like growth factor 1 (IGF-1) is thought to induce the characteristic pathology of this disease (Lieberman et al., 1992). IGF-1 has been demonstrated to promote mitosis and differentiation of articular chondrocytes, and to induce matrix synthesis (Ash and Francis, 1975; McQuillan et al., 1986; Luyten et al., 1988; Trippel et al., 1989). These facts suggest that IGF-1 may be generally involved in osteophyte formation in cases of osteoarthritis other than acromegalic osteoarthropathy, but this issue remains to be clarified.

Because osteophytes generally appear later than cartilage degeneration, the early processes of osteophyte development cannot be examined in human specimens, although observation of mature osteophytes is possible. We examined the processes of osteophyte formation in guinea

Abbreviations: IGF-1, insulin-like growth factor 1; PCNA, proliferating cell nuclear antigen

pigs, which show histological changes very similar to those in human osteoarthritis (Bendele et al., 1989; Okada et al., 1992; de Bri et al., 1996; Tokuda, 1997), using histological, histochemical and immunohistochemical techniques.

Materials and Methods

Preparation of experimental materials

One-, 3-, 5-, 8-, 12- and 18-month-old Hartley guinea pigs (Charles River Japan Inc., Tokyo, Japan) were sacrificed by intraperitoneal injection of pentobarbital sodium (150 mg/kg). We examined two 1-month-old (1 male, 1 female), three 3-month-old (1 male, 2 females), four 5-month-old (1 male, 3 females), four 8-month-old (2 males, 2 females), two 12-month-old (1 male, 1 female) and two 18-month-old (1 male, 1 female) guinea pigs. The right knee joints from these animals were fixed in 10% neutral formalin in a 90˚ flexed position for 3 days. Next, the knee joints were decalcified with 10% EDTA (pH 7.4) for 4 weeks at room temperature. The specimens were confirmed by soft X-ray that the specimens had been completely decalcified. After being dehydrated with ascending grades of ethanol and xylene, the specimens were embedded in paraffin. These specimens were cut into serial sections 7-μm thick each in the frontal plane passing through the midline between the medial and lateral menisci. These sections were subjected to hematoxylin and eosin, safranin-O and immunohistological staining.

Evaluation of osteoarthritis

By using safranin O-stained specimens from each animal, the degree of degeneration of the medial condyle of the tibia, in which cartilage degeneration develops earliest, was evaluated based on the histological-histochemical grading system

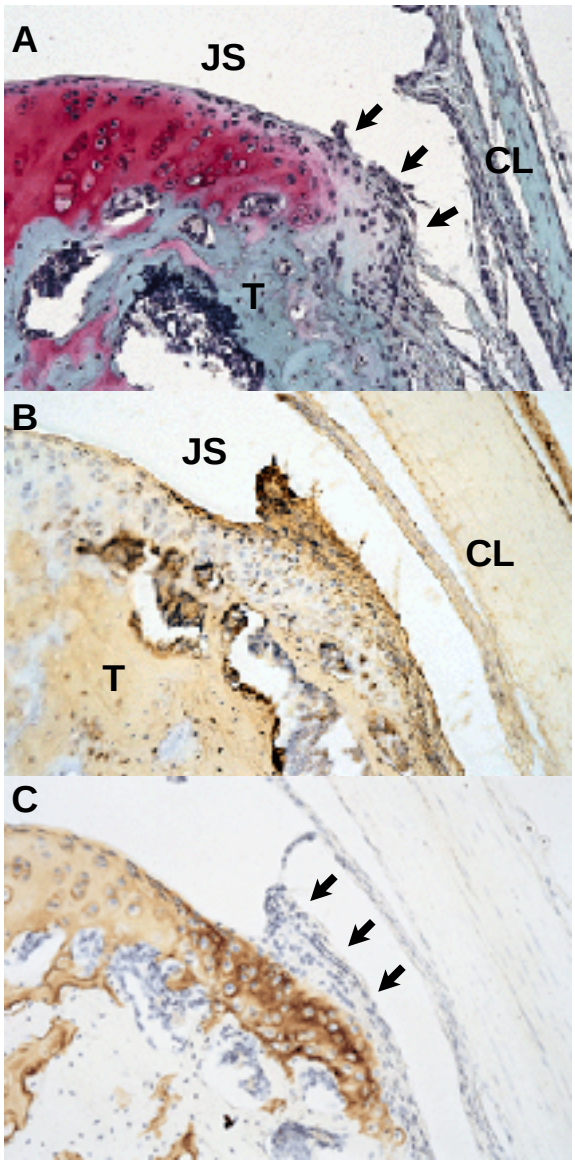
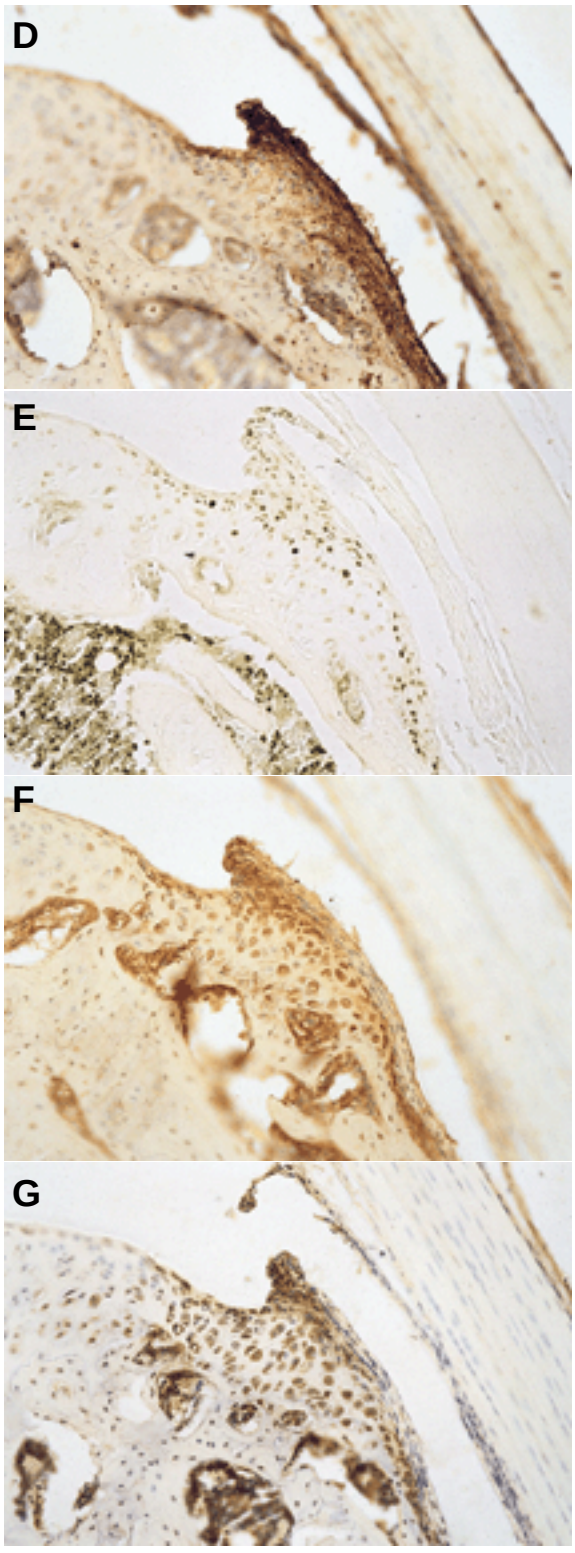


Fig. 1. Safranin-O staining (**A**) and immunohistological staining (**B**–**G**) show no chondrophyte formation in the medial margin of the tibial plateau in the mild group of 3-month-old animals without degenerative changes due to osteoarthritis. Areas in the fibrous connective tissue (arrows) negative for safranin-O staining (**A**) and for type-II collagen (**C**) are positive for type-I (**B**) and -III (**D**) collagens. The nuclei of fibroblastic-like cells in the fibrous connective tissue are positive for PCNA (**E**), IGF-1 (**F**) and IGF-1 receptor (**G**). CL, medial colateral ligament; JS, joint space; T, tibia. Original magnification, × 200.

[Figs. 1A–C on p. 132 and Figs. 1D–G on p. 133]



Figs. 1D–G. *Continued from the previous page.*

(Mankin et al., 1971). Because pannus formation was not observed in the degenerated cartilage of any of the guinea pigs at any age, this item was excluded. Thus, the degree was graded on a 6-tiered scale instead of a 7-tiered scale, with 13 as the total number of points. According to the cartilage degeneration score, animals were classified into the following 3 groups: the mild group (grades 0–2), the moderate group (grades 3–7) and the severe group (grades 8–13).

Immunohistological staining

Deparaffinized sections were reacted in solution containing bovine testicular hyaluronidase (Wako Kogyo Co. Ltd., Osaka, Japan; 2 mg/mL phosphate-buffered saline, pH 5.3, for 30 min at room temperature). For detection of type-II collagen, pronase treatment (Dako, Kyoto, Japan; 1 mg/mL phosphate-buffered saline, pH 7.3, for 30 min at room temperature) was added (Aigner et al., 1993). Protein treatment was not performed for detection of proliferating cell nuclear antigen (PCNA). For the secondary antibody, enzyme reagent and mixture of substrate and dye, Histofine streptavidin-biotin-PO (M) and (P) kits (Nichirei, Tokyo) were used, and staining was performed with the streptavidin-biotin method. Among primary antibodies, polyclonal anti-type-I collagen antibody (LSL, Tokyo; diluted at 1:500), monoclonal anti-PCNA antibody (Nichirei; non-diluted), monoclonal anti-IGF-1 antibody (Upstate Biotechnology, Lake Placid, NY; 100 µg/mL) and polyclonal anti-IGF-1 receptor antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA; 1 µg/mL) were reacted in a moist chamber maintained at 4°C for about 24 h. Monoclonal anti-type-II and -type-III collagen antibodies (Fuji Chemical Industries, Takaoka, Japan; diluted at 1:1000 and 1:500, respectively) were reacted for 1 h at room temperature. Counterstaining was performed with 3% methylene green for anti-PCNA antibody and hematoxylin for