Virchows Archiv A Pathol Anat (1990) 417:291–297

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistological study on collagenous proteins of benign and malignant human cartilaginous tumours of bone

Yoshimichi Ueda [1], Yoshio Oda [1], Hiroyuki Tsuchiya [2], Katsuro Tomita [2], and Isao Nakanishi [1]

Departments of [1] Pathology and [2] Orthopaedics, School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa City, Ishikawa 920, Japan

Received February 12, 1990 / Accepted May 21, 1990

Summary. The immunohistological distribution of collagen types I, II, III, V and VI in human benign and malignant cartilaginous tumours of bone was examined with regard to their aggressiveness. The matrix of enchondromas consisted of type II collagen distributed diffusely, and type VI predominantly localized in the immediate surroundings of the cells. Types I, III and V collagen were not found. These findings were similar to the distribution of collagenous proteins in normal hyaline cartilage where each lobule was consistently rimmed by types I and V collagen. In grade 1 chondrosarcomas, the main collagenous components of matrix were also types II and VI collagen. Type II was sometimes found in the cytoplasm of tumour cells and type VI tended to lose territorial localization. In addition, type I collagen was demonstrated consistently and type V in some cases. In grade 2 chondrosarcomas, type II collagen was demonstrated not only in the matrix but occasionally in the cytoplasm of tumour cells. Type VI was dispersed in the intercellular areas. The other collagenous proteins such as types I, III and V were also present in the matrix. In grade 3 chondrosarcomas, type II collagen was localized predominantly in the cytoplasm of tumour cells and in the adjacent matrix. Type VI was markedly decreased with complete loss of pericellular distribution, whereas types I, III and V were constantly present in the matrix. Those alterations in the distribution of collagen types correlated well with the aggressive behaviour of the tumours. The findings suggest that distribution of different collagen types in cartilaginous tumours reflects the immaturity of the tumour cells and is a useful indicator of their aggressiveness.

Key words: Chondrosarcoma – Chondroma – Collagen – Immunohistochemistry

Introduction

It is generally considered to be difficult to predict the biological behaviour of cartilaginous tumours of bone by morphological findings. Some biochemical analyses of extracellular matrix components (Sweet et al. 1976; Matsuno 1979; Mankin et al. 1980a, b; Herwig et al. 1986) and cellular DNA content (Kreicbergs et al. 1982) have been tried in order to find a more objective means for determining a degree of malignancy. However, there are no valid criteria at present.

Collagen, in addition to proteoglycans, is the main structural component of cartilaginous tumours (Mankin et al. 1980a). It is now recognized as a family of proteins of at least 13 genetically distinct subtypes, each of which is localized tissue-specifically (Miller and Gay 1987; Gordon et al. 1987; Sandberg et al. 1989). Our previous study on the different collagen types in normal epiphyseal plates and osteosarcomas with various histologies demonstrated that it was possible to localize different collagen types using type-specific anti-collagen antibodies even in formalin-fixed, paraffin-embedded cartilaginous tissue sections, after predigestion both by protease and by hyaluronidase (Ueda and Nakanishi 1989). In the present study, we have examined the immunohistological localization of different collagen types in benign and malignant carilaginous tumours with different degrees of malignancy using type-specific antibodies against types I, II, III, V and VI collagen, in order to elucidate any possible correlation between the distribution of different collagen types and their biological behaviour.

Materials and methods

We studied six cases of enchondroma, four cases of well-differentiated chondrosarcoma (grade 1), three cases of moderately differentiated chondrosarcoma (grade 2) and two cases of poorly differentiated chondrosarcoma (grade 3), all of which were treated in our orthopaedic department between 1979 and 1989 (see Table 1). Three cases were within Ollier's disease and one case was of Maffucci's syndrome. Clinical information, including follow-up data, was obtained by review of the patient's charts. Representative tissue samples obtained at the operations (curettage, local excision, or amputation) were available in all cases. The histological grading was according to Evans et al. (1977) and Ericksson et al. (1980), and clinical staging was according to Enneking (1986).

Formalin-fixed, paraffin-embedded sections, some of which had been demineralized with 0.5 M EDTA (pH 7.5), were used

Offprint requests to: Y. Ueda

Table 1. Fifteen cases of cartilaginous tumours

Case	Age (years)	Sex	Location	Histological grade	Stage	Treatment	Results
1[a]	11	M	Rt-hand phalanges, multiple	G0	2	En bloc excision	NED 6 years after surgery
2	17	F	Lt-hand metacarpus IV	G0	1	Curettage	NED 5 years 6 months after surgery
3	5	F	Rt-hand middle phalanx IV	G0	1	Curettage	NED 4 years 6 months after surgery
4	31	F	Lt femur	G0	2	Curettage	NED 4 years 3 months after surgery
5	57	M	Lt humerus	G0	2	Curettage	NED 1 year 9 months after surgery
6	51	M	Lt humerus	G0	1	Curettage	NED 10 months after surgery
7	56	M	Rt costal region	G1	IB	En bloc excision	Multiple recurrences
8[a]	46	F	Lt ischium	G1	IB	Hemipelvectomy	Local recurrence 3 years 6 months after surgery
9	55	M	Rt tibia	G1	IA	Wide excision	NED 4 years after surgery
10	30	F	Lt femur	G1	IA	Wide excision	NED 4 years 4 months after surgery
11[a]	60	F	Rt femur	G2	IIB	Wide excision	Local recurrences 6 months, and 2 years 6 months after first surgery
12	65	M	Rt femur	G2	IIB	Wide excision	NED 10 years 1 months after surgery
13	53	F	Rt femur	G2	IIB	Wide excision	NED 4 years after surgery
14	17	F	Lt femur	G3	IIB	Disarticulation	Died 8 months after surgery due to metastases to lung and brain
15[b]	46	F	Rt scapula	G3	IIIB	En bloc excesion	Died 1 month after surgery due to metastases to lung

M, Male; F, female; Lt, left; Rt, right; NED, no evident disease; [a] Ollier's diesease, [b] Maffucci's syndrome

for immunohistochemistry. This was performed by the avidin-biotin methods (Hsu et al. 1981) (Vector Laboratories, Burlingame, Calif., USA), and sections were counterstained with haematoxylin. They were preincubated in 0.01% protease (type XXIV, Sigma, Mo., USA) in TRIS-HCl buffer (pH 7.6) for 10 min at 37° C, and in 0.05% bovine testicular hyaluronidase (Sigma) in phosphate buffer (pH 5.5) for 2 h at 37° C. Primary antibodies were applied overnight at 4° C. Affinity-purified monospecific antibodies to collagen types I, II, III, V and VI were those described previously (Oda et al. 1988; Ueda and Nakanishi 1989). The working dilutions of antibodies to collagen types I, II, III, V and VI were 10, 20, 10, 5, 100 respectively. For control, appropriately diluted normal rat or rabbit serum was applied instead of the primary antibodies.

Results

The clinical details are summarized in Table 1. In cases of enchondroma (G0) which were characterized by uniform-sized cells with small dark round nuclei distributed regularly in abundant hyaline cartilage-like matrix (Fig. 1a), no recurrence developed after curettages or en bloc excision. Four cases of chondrosarcoma (grade 1 histologically) showed fairly cellular chondroid tissues, with scanty cells with one or two plump nuclei (Fig. 2a). In two of them, local recurrences occurred after en bloc excision or hemipelvectomy. Three cases of chondrosarcoma grade 2 showed more cellular proliferation with myxoid matrix. Tumour cells with plump bizarre hy-

Table 2. Composition of collagen types in cartilaginous tumours

Case	Grade	Immunoreactivity with type-specific anti-collagen antibodies				
		I	II	III	V	VI
1	G0	−	++	−	−	++[t]
2	G0	+	++	−	−	++[t]
3	G0	−	++	−	−	++[t]
4	G0	−	++	−	−	++[t]
5	G0	−	++	−	−	++[t]
6	G0	+[a]	++	−	−	++[t]
7	G1	+	++[c]	−	+	++[t]
8	G1	++	++[c]	−	+[c]	+
9	G1	+	++[c]	−	−	++
10	G1	+	+[c]	−	±	++
11	G2	++	++[c]	++	+	+
12	G2	++	+[c]	−	−	±
13	G2	++	+[c]	+	+	++
14	G3	++	+[c]	+	++	±
15	G3	++	+[c]	+	++	+

++, Constantly and strongly positive; +, constantly positive; ±, variably and faintly positive; −, negative; [t] predominantly in the territorial region; [c] positive in the cytoplasm; [a] positive in osseous metaplastic areas

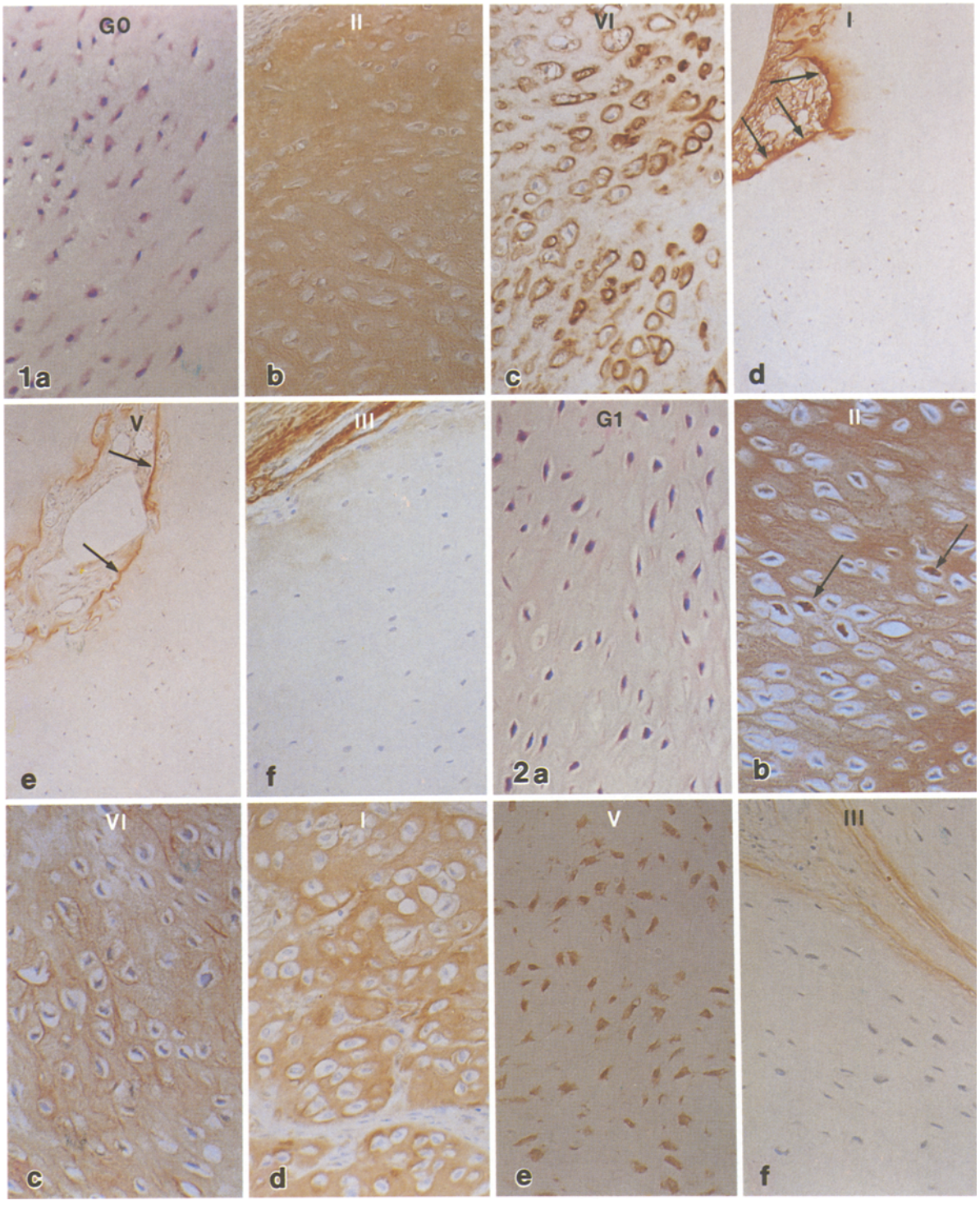

Fig. 1a–f. Benign enchondroma (case 1). **a** Haematoxylin and eosin (H&E) stain; **b** immunostain with antibody to collagen type II; **c** type VI; **d** type I; **e** type V; **f** type III. Matrix is stained diffusely with antibody to type II collagen, and predominantly in territorial regions with antibody to type VI collagen (**b, c**). *Arrows* show rimming of enchondromatous lobules with types I and V collagen (**d, e**). ×150

Fig. 2a–f. Grade 1 chondrosarcoma (case 8). **a** H&E stain; **b** immunostained with antibody to collagen type II; **c** type VI; **d** type I; **e** type V; **f** type III. Matrix is reacted with antibodies to type I collagen as well as types II and VI (**b–d**). Some tumour cells show positive reaction with antibodies to types II (*arrows*) and V collagen (**b, e**). Tumour lobules are devoid of rimming by type I collagen (**d**). Type III is demonstrated in fibrous bands between tumour lobules (**f**). ×150

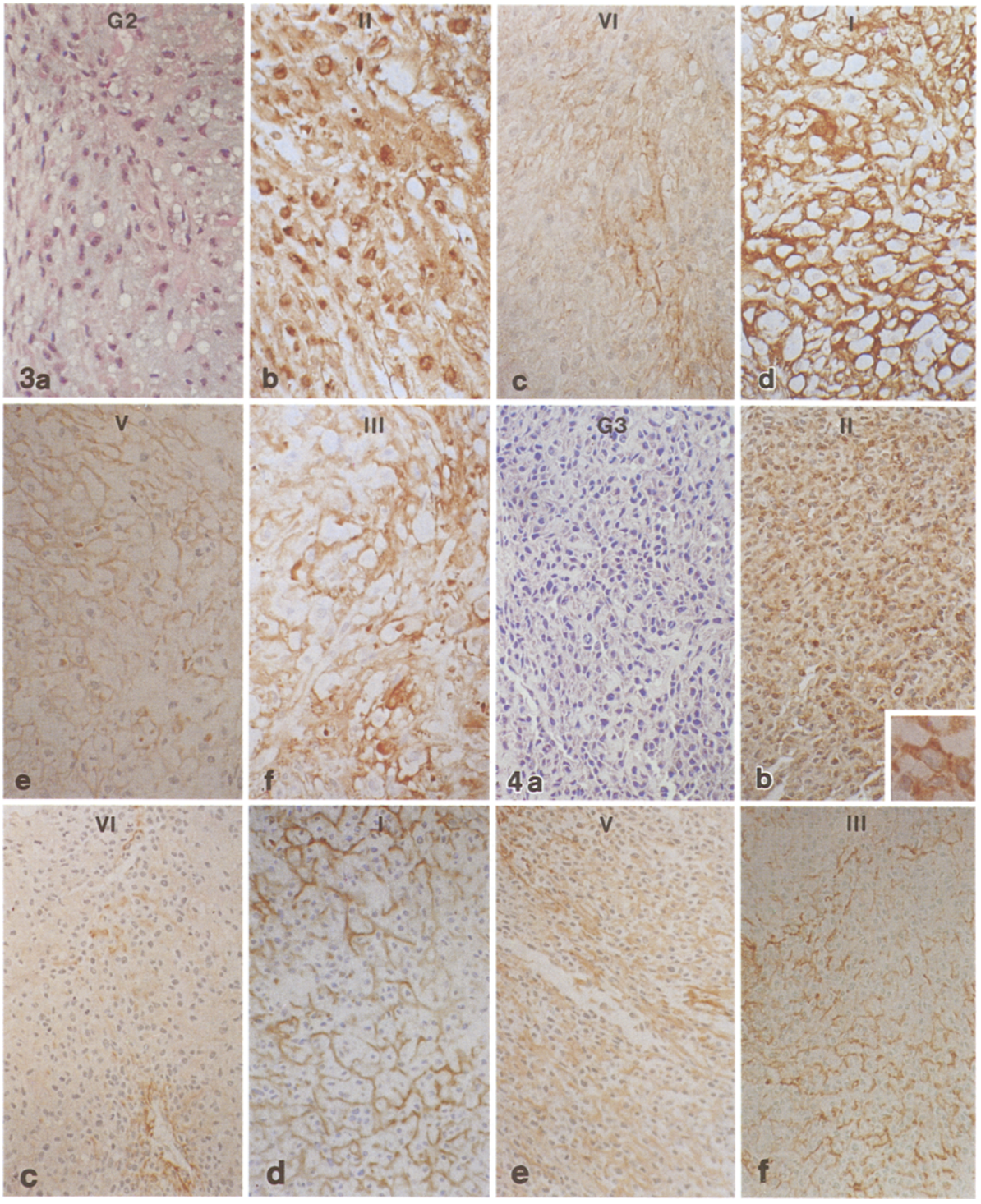

Fig. 3a–f. Grade 2 chondrosarcoma (case 11). **a** H&E stain; **b** immunostain with antibody to collagen type II; **c** type VI; **d** type I; **e** type V; **f** type III. Matrix shows intense reaction with antibodies to collagen types I and III as well as II (**b, d, f**). Types VI and V collagen are also demonstrated in matrix (**c, e**). Tumour cells reacted with antibodies to collagen types II (**b**). ×150

Fig. 4a–f. Grade 3 chondrosarcoma (case 14). **a** H&E stain; **b** immunostain with antibody to collagen type II; **c** type VI; **d** type I; **e** type V; **f** type III. Matrix shows positive reaction with antibodies to collagen types I, V and III (**d–f**). Type II collagen is mainly localized in the cytoplasm of tumour cells and in the adjacent matrix. *Inset* shows the intracellular positive reaction of tumour cells (**b**). Type VI is seen only faintly in the matrix (**c**). ×150; *inset* ×480

perchromatic nuclei were more frequently seen, although mitosis was rare (Fig. 3a). In one, the tumour recurred in spite of wide resection. In grade 3 chondrosarcomas, tumour cells with nuclear abnormalities and considerable mitotic activity were seen with relatively little myxoid stroma. Sometimes fibrosarcoma-like spindle tumour cells were seen at the periphery of the lobules. No foci of osteoid lacework or trabecula produced directly by the tumour cells were found in spite of careful and extensive histopathological examination of the resected tumour tissues.

In case 15, metastatic pulmonary tumours were examined at necropsy, but osteoid production was not detected. Both patients who died with chondrosarcoma died because of pulmonary metastases.

The immunohistochemical staining for different types of collagen in benign and malignant cartilage tumours with various degrees of malignancy is summarized in Table 2.

The hyaline cartilaginous matrix of benign enchondromas reacted intensely and diffusely with antibody to collagen type II; an intracellular positive reaction was rarely observed. The matrix was also stained with the antibody to type VI collagen, and the reaction was much stronger in the regions surrounding enchondroma cells than in the intercellular regions. Collagen types I, III and V were negative in the cartilaginous matrices of most cases of enchondroma except for case 2, in which type I collagen was present in some areas with increased cellularity and myxoid matrix. The immunoreactivity to type I collagen in case 6 was not in the cartilaginous but in the metaplastic osseous regions. Furthermore, the positive reaction to types I and V collagen bounding the lobules of enchondromas was invariably detected (Fig. 1b–f).

In grade 1 chondrosarcomas the hyaline cartilaginous or myxoid matrix also showed intense reaction with antibody to type II collagen. Type II collagen was sometimes demonstrated in the cytoplasm of tumour cells. Type VI collagen existed not only in the pericellular areas but also diffusely in the interstitium. In addition, type I collagen was shown to be present constantly in the matrix of grade 1 chondrosarcoma. Type V collagen was also recognized in some cases. In case 8, the immunoreaction to type V collagen was localized predominantly in the cytoplasm of tumour cells. No positive staining for type III collagen was detected. Some tumour lobules infiltrating the bone marrow were devoid of rimming by types I and V collagen (Fig. 2b–f).

In grade 2 chondrosarcomas, type II collagen was demonstrated not only in the myxoid matrix but also occasionally in the cytoplasm of tumour cells. In some cases, immunoreactivity to type II collagen decreased significantly. Type VI collagen was distributed mainly in intercellular areas with a fibrillary pattern and most of the pericellular distribution was abolished. The myxoid matrix also showed intense reaction with antibodies to collagen types I, III, and V. Rimming by types I and V collagen was hardly found (Fig. 3b–f).

In grade 3 chondrosarcomas, immunoreactivity to type II collagen in the tumour matrix was markedly diminished, being localized in the cytoplasm of tumour cells and the adjacent matrices. Type VI collagen was hardly discernible, whereas collagen types I, III and V were constantly demonstrated in the tumour matrix. There was no rimming of tumour lobules by types I and V collagen at all (Fig. 4b–f).

Normal rat or rabbit serum, used as negative control, showed no significant positive reaction.

Discussion

The major structural components of the cartilaginous tissues are collagenous proteins and proteoglycans. Recent progress in biochemical research on collagenous proteins has clarified that those in hyaline cartilage are composed of type II, the main structural component and the other minor collagens such as types IX, X and XI (Miller 1985; Miller and Gay 1987; Burgeson 1988). Immunofluorescence studies have located these various collagen types in specific regions of cartilaginous tissues where their presence presumably reflects their different structural and metabolic roles (Ayad et al. 1984; Schmid and Linsenmayer 1985; Poole et al. 1988; Kwan et al. 1989; Mendler et al. 1989). Previous immunohistological investigation on the localization of different collagen types in formalin-fixed, paraffin-embedded sections of epiphyseal cartilage using type-specific antibodies to collagen types I, II, III, IV, V and VI disclosed the same findings as previous immunofluorescence studies (von der Mark and von der Mark 1977; Ayad et al. 1984): type II collagen was distributed diffusely in the matrix, while type VI was mainly confined to the territories around cartilage cells; neither collagen type I, III, IV nor V was demonstrated in the cartilaginous matrix (Ueda and Nakanishi 1989).

The present study revealed that the composition and distribution of different collagen types in benign enchondroma (G0) were quite similar to those of normal hyaline cartilage. In chondrosarcoma, in contrast, the following alterations in composition and distribution of collagen types were recognized. There was the appearance of additional types of collagen, such as types I, V and III, not present in normal hyaline cartilage; a loss of the peculiar collagenous pattern of cartilage characterized by diffusely distributed type II and pericellular localized type VI collagen; and the appearance of the intracellular immunoreactivity to type II collagen. As shown in Table 2, the extent of these changes seems to have a relationship with histological grading.

The loss of the particular distribution of collagen types II and VI seen in normal hyaline cartilage appeared to correspond with immaturity of the tumour cells, and the appearance of various additional collagen types in chondrosarcomas also appears to represent the immaturity of the tumour cells rather than an aberrant expression associated with malignant transformation, since types I and III have been reported to be expressed in the early phase of chondrogenesis (Reddi et al. 1977; Tacchetti et al. 1987) and types I, III and V collagen are observed in and around the proliferating chondrocytes of human embryonal limb bud (unpublished data).

Of the various additional collagen types, the appearance of type III collagen in less-differentiated chondrosarcomas has been pointed out by Remberger and Gay (1977) and Matsuno (1979). In the present study, most cases positive for type III collagen resulted in local recurrence or distant metastases. Therefore, the presence of type III collagen in chondrosarcoma may imply aggressive behaviour of the tumour.

It is very interesting to note that type II collagen was demonstrated not only in the matrix but also in the cytoplasm of tumour cells in chondrosarcomas, while cytoplasmic reaction was rarely found in enchondromas. In non-neoplastic tissues, proliferating chondrocytes in the centre of human embryonal limb buds also presented a positive reaction to type II collagen (unpublished data), although it was not detected in chondrocytes in normal neonatal epiphyseal cartilage (Ueda and Nakanishi 1989). Thus, neoplastic chondrocytic cells which are active both in proliferation and production of type II collagen may show intracellular immunoreaction to type II collagen. In grade 3 chondrosarcomas, immunoreaction to type II collagen was predominantly localized intracellularly, whereas it was scanty in the extracellular matrix. The intracellular immunoreaction to type II collagen may be associated with either abortive production or abnormality in secretion in highly malignant tumour cells.

In the differential diagnosis between enchondroma and grade 1 chondrosarcoma is the most perplexing problem for pathologists. Structural features such as encasement of tumour lobules by lamellar bone in enchondromas and permeation of the tumour mass in the bone marrow in grade 1 chondrosarcomas are considered to be more reliable than the cyclogical factors such as cellularity and the frequency of binucleated cells (Mirra et al. 1985). In the present study, types I and V collagen were consistently seen to form a boundary at each lobule of enchondromas, whether or not this was encased by lamellar bone histologically. This rimming in areas without obvious bone might represent a remnant of the preexisting bony encasement which becomes atrophic due to compression by enchondromatous lobules. However, some tumour nests in grade 1 chondrosarcomas were not bound by type I or type V collagen and rimming was not observed at all in grade 2 and 3 chondrosarcomas. The immunohistological evaluation of rimming by types I and V collagen seems to facilitate the differential diagnosis between enchondroma and low-grade chondrosarcoma.

The differential diagnosis between chondrosarcoma and chondroblastic osteosarcoma is another important problem. Histopathologically they resemble each other, and a clear-cut differentiation depends on demonstration of osteoid production directly by tumour cells. The peculiar composition of different collagen types is useful in identifying tumour osteoid in osteosarcoma (Ueda and Nakanishi 1989). However, the present study showed that an immunohistochemical approach to collagenous proteins in their chondroid matrices was not helpful in the differential diagnosis between chondrosarcoma and chondroblastic osteosarcoma, because the collagenous composition of chondrosarcoma, especially grade 2, was closely similar to that of chondroblastic portions of conventional osteosarcoma, as previously reported (Ueda and Nakanishi 1989).

The present immunohistochemical study of different collagen types in benign and malignant cartilaginous tumours may provide data helpful in judging the degrees of maturation and biological activity of tumour cells. It could be useful in the differential diagnosis between enchondroma and chondrosarcoma.

Acknowledgements. We wish to thank Prof. F. Matsubara for providing us materials for chondrosarcomas, and Prof. A. Roessner, Gerhard-Domagk-Institut für Pathologie (Münster, FRG), for kindly reviewing the manuscript.

References

Ayad S, Evans H, Weiss JB, Holt L (1984) Type VI collagen but not type V collagen is present in cartilage. Coll Rel at Res 4:165–168

Burgeson RE (1988) New collagens, new concepts. Annu Rev Cell Biol 4:551–577

Enneking WF (1986) A system of staging musculoskeletal neoplasms. Clin Orthop Relat Res 204:9–24

Eriksson AI, Schiller A, Mankin HJ (1980) The management of chondrosarcoma of bone. Clin Orthop Relat Res 153:44–66

Evans HL, Ayala AG, Romsdahl MM (1977) Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. Cancer 40:818–831

Gordon MK, Gerecke DR, Olsen BR (1987) Type XII collagen: Distinct extracellular matrix component discovered by cDNA cloning. Proc Natl Acad Sci USA 84:6040–6044

Herwig J, Roessner A, Buddecke E (1986) Isolation and characterization of proteoglycans and glycosoaminoglycans from human chondrosarcoma. Exp Mol Pathol 45:118–127

Hsu SM, Raine L, Fanger H (1981) A comparative study of the peroxidase-antiperoxidase method and avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibody. Am J Clin Pathol 75:734–738

Kreicbergs A, Boquist L, Borssen B, Larsson SE (1982) Prognostic factors in chondrosarcoma. A comparative study of cellular DNA content and clinicopathologic features. Cancer 50:577–583

Kwan APL, Dickson IR, Freemont AJ, Grant ME (1989) Comparative studies of type X collagen expression in normal and rachitic chicken epiphyseal cartilage. J Cell Biol 109:1849–1856

Mankin HJ, Cantley KP, Lippiello L, Schiller AL, Campbell CJ (1980a) The biology of human chondrosarcoma. I. Description of the cases, grading, and biochemical analysis. J Bone Joint Surg [Am] 62:160–176

Mankin HJ, Cantley KP, Schiller AL, Lippielo L (1980b) The biology of human chondrosarcoma. II. Variation in chemical composition among types and subtypes of benign and malignant cartilage tumors. J Bone Joint Surg [Am] 62:176–188

Mark K von der, Mark H von der (1977) The role of three genetically distinct collagen types in enchondral ossification and calcification of cartilage. J Bone Joint Surg [Br] 59:458–464

Matsuno T (1979) Morphological and biochemical studies of collagen on benign and malignant cartilage tumors (in Japanese). Hokkaido Assoc Orthop Traum 24:1–15

Mendler M, Eich-Bender SG, Vaughan L, Winterhalter KH, Bruckner P (1989) Cartilage contains mixed fibrils of collagen type II, IX, and XI. J Cell Biol 108:191–197

Miller EJ (1985) Recent Information on the chemistry of the collagens. In: Proceedings of 2nd international Conference on the Biochemistry and Biology of Mineralized Tissues. Ebsco Media, Birmingham, Ala., pp 80–93

Miller EJ, Gay S (1987) The collagens: an overview and update. Methods Enzymol 144:3–41

Mirra JM, Gold R, Downs J, Eckardt JJ (1985) A new histologic approach to the differentiation of enchondroma and chondrosarcoma of the bones. A clinicopathologic analysis of 51 cases. Clin Orthop Relat Res 201:214–237

Oda Y, Kawahara E, Minamoto T, Ueda Y, Ikeda K, Nagai Y, Nakanishi I (1988) Immunohistochemical studies on the tissue localization of collagen types I, III, IV, V, and VI in schwannomas. Correlation with ultrastructural features of the extracellular matrix. Virchows Arch [B] 56:153–163

Poole CA, Wotton SF, Daunce VC (1988) Localization of type IX collagen in chondrons isolated from porcine articular cartilage and rat chondrosarcoma. Histochem J 20:567–574

Reddi AH, Gay R, Gay S, Miller EJ (1977) Transitions in collagen types during matrix-induced cartilage, bone, and bone marrow formation. Proc Natl Acad Sci USA 74:5589–5592

Remberger K, Gay S (1977) Immunohistochemical demonstration of different collagen types in the normal epiphyseal plate and in benign and malignant tumors of bone and cartilage. Z Krebsforsch 90:95–106

Sandberg M, Tamminen M, Hirvonen H, Vuorio E, Philajaniemi T (1989) Expression of mRNAs coding for the α1 chain of type XIII collagen in human fetal tissues: comparison with expression of mRNAs for collagen types I, II, and III. J Cell Biol 109:1371–1379

Schmid TM, Linsenmayer TF (1985) Developmental acquisition of type X collagen in the embryonic chick tibiotarsus. Dev Biol 107:373–381

Sweet MBE, Thonar EJ-MA, Immelman AR (1976) Glycosoaminoglycans and proteoglycans of human chondrosarcoma. Biochem Biophys Acta 437:71–86

Tacchetti C, Quarto R, Nitsch L, Hartmann DJ, Cancedda R (1987) In vitro morphogenesis of chick embryo hypertrophic cartilage. J Cell Biol 105:999–1006

Ueda Y, Nakanishi I (1989) Immunohistochemical and biochemical studies on the collagenous proteins of human osteosarcomas. Virchows Arch [B] 58:79–88