Immunoidentification of Type XII Collagen in Embryonic Tissues

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Abstract. We have generated a monoclonal antibody against a synthetic peptide whose sequence was derived from the nucleotide sequence of a cDNA encoding α1(XII) collagen. The antibody, 75d7, has been used to identify the α1(XII) chain on immunoblots of SDS-PAGE tendon extracts as a 220-kD polypeptide, under reducing conditions. Amino-terminal amino acid sequence analysis of an immunopurified cyanogen bromide fragment of type XII collagen from embryonic chick tendons gave a single sequence identical to that predicted from the cDNA, thus confirming that the antibody recognizes the type XII protein. Immunofluorescence studies with the antibody demonstrate that type XII collagen is localized in type I–containing dense connective tissue structures such as tendons, ligaments, perichondrium, and periosteum. With these data, taken together with previous results showing that a portion of the sequence domains of type XII collagen is similar to domains of type IX, a nonfibrillar collagen associated with cross-striated fibrils in cartilage, we suggest that types IX and XII collagens are members of a distinct class of extracellular matrix proteins found in association with quarter-staggered collagen fibrils.

THE extracellular matrix molecules known as collagens represent a highly diverse group of proteins, containing molecules that range from the long rod-like fibrillar collagens (types I, II, III, V, and XI) to molecules that are either short or contain flexible domains (types IV and IX) (see reference 13). A recent addition to the collagens is type XII collagen which has been discovered by cloning cDNA from chick embryonic tendon mRNA (6). Exhibiting partial similarity to type IX collagen, the sequence of α1(XII) has prompted the speculation (18) that type XII collagen may be associated with type I collagen containing fibrils as type IX collagen is associated with type II–containing fibrils in cartilage (5, 21, 22). The presence of type XII collagen as protein in embryonic tendons was confirmed by Dublet and van der Rest (3). These investigators isolated pepsin-resistant fragments from embryonic tendons and demonstrated that the amino acid sequence of a tryptic peptide derived from one of these fragments was in agreement with the conceptual translation product predicted from the nucleotide sequence of the type XII cDNA, pMG377 (3, 6).

In this article we describe the generation of a monoclonal antibody against a synthetic oligopeptide, whose sequence was derived from the nucleotide sequence of pMG377. This peptide sequence is located within the carboxy-terminal nontriple-helical domain of α1(XII) chains, and it was selected because of its low level of similarity with the corresponding domains in α1(IX) and α2(IX) chains. The monoclonal antibody, 75d7, has been used to identify the α1(XII) chain in tendon extracts as a polypeptide of 220 kD, roughly twice the size of the α1(IX) collagen chain. Amino acid sequence analysis of immunopurified cyanogen bromide fragments demonstrates that 75d7 indeed reacts with type XII collagen. Immunofluorescence studies with this antibody demonstrate that type XII collagen is localized in type I–containing dense connective tissues such as tendons, ligaments, perichondrium, and periosteum.

Materials and Methods

Peptide Selection

A sequence of 12-amino acid residues from within the 76-amino acid residue-long carboxy-terminal, nontriple-helical (NC1) domain of α1(XII)[1] was selected for antibody production. The peptide sequence derived from the nucleotide sequence of the cDNA pMG377 (6), FPEPYVPESGPYC, was selected based on its hydrophilicity characteristics calculated as described by Hopp and Woods (7), and a low level of similarity with other known collagen sequences. The carboxy-terminal cysteinyl residue was added for coupling purposes (10).

Synthesis of the Oligopeptide

The peptide was synthesized by the solid-phase procedure of Merrifield (16) with the aid of an automated peptide synthesizer (model 990; Beckman In-

1. In the original publication, describing the discovery of α1(XII) collagen (6), the length of the carboxy-terminal nontriple helical (NC1) domain of α1(XII) is given as 28-amino acid residues. However, we have recently resequenced both strands of the cDNA pMG377 and have discovered that an extra nucleotide was incorrectly inserted in the original sequence close to the stop codon. By removing this nucleotide the open reading frame that defines NC1 does not end after 28-amino acid residues, but continues to include 76-amino acid residues before a stop codon is reached. In this paper, the correct amino acid sequence of NC1 is indicated as part of the sequence in Fig. 3.

2. *Abbreviation used in this paper*: PVDF, polyvinylidene difluoride.

struments Inc., Palo Alto, CA). Protected BOC amino acids were purchased from Peninsula Laboratories, Inc. (Belmont, CA) and coupled sequentially to a benzhydrylamine resin. The desired peptide was initially purified by filtration through a Sephadex G-25 column (4 × 60 cm), previously equilibrated with 0.1 M acetic acid. The remaining peptide fraction was pooled, lyophilized, and further purified by reverse phase HPLC. Separation was obtained using an HPLC system (Beckman Instruments, Inc.) an ODS-3 (1 × 25 cm) semi-preparative column (Whatman Inc., Clifton, NJ). Buffer A was 0.05% trifluoroacetic acid and buffer B was 0.05% trifluoroacetic acid in acetonitrile. The gradient was 20–30% B over 30 min at 2.0 ml/min. The amino acid composition of the final peptide was determined with an analyzer (model 121MB Automatic; Beckman Instruments, Inc.), and the amino acid sequence was confirmed by automatic Edman degradation (model 890M; Beckman Instruments, Inc.). The amino acid content found was ±10% theoretical, and amino acid sequence analysis confirmed the peptide structure.

Immunization and Antibody Production

The synthetic peptide was coupled through the carboxy-terminal cysteinyl residue to keyhole limpet hemocyanin as described (10). Lyophilized, activated peptide (5 mg) was coupled to 4 mg of hemocyanin (Polysciences, Inc., Warrington, PA) in 2 ml of 20 mM sodium phosphate buffer, pH 7.5. After incubation at room temperature for 3 h, peptide–hemocyanin complexes were separated from uncoupled peptide by gel filtration on Sephadex G-50 (fine) (1 × 25 cm) equilibrated with 20 mM sodium, pH 7.5. For generation of antibodies, 50–100 μg of peptide–hemocyanin complex was mixed in 0.5 ml of complete Freunds adjuvant (Gibco Laboratories, Grand Island, NY) and injected intraperitoneally into Balb/c mice. Booster injections were given at 2-wk intervals in incomplete Freunds adjuvant. A total of three to four booster injections were given. Mice were periodically bled from the tail vein for screening of sera. When sera were positive in ELISA against peptide–BSA conjugates, subcutaneous injections of 10–20 μg without adjuvant were given twice, one 5 d before fusion and the second 3 d before fusion. Mouse splenocytes were fused with P3 myeloma cells using standard methodologies.

Immunoscreens

Hybridoma supernatants were screened by a dot assay. BSA–peptide or embryonic chick tendon extracts were spotted onto nitrocellulose. The nitrocellulose filter was treated with 5% nonfat dried milk in PBS. Dots were then incubated for 1 h in 50 μl of supernatant. After rinsing in PBS (3 × 5 min), the filters were incubated for 1 h in peroxidase-conjugated goat anti-mouse IgG (Boehringer Mannheim Biochemicals, Indianapolis, IN) in PBS containing 5% normal goat serum. After additional washes, the membranes were treated with 3-3′ diaminobenzidine (0.5 mg/ml) in PBS containing H_2O_2 (0.03%).

Immunofluorescence was performed on 7-μm cryostat sections, which had previously been digested with testicular hyaluronidase (3.3 mg/ml in phosphate buffer, pH 6.0) for 0.5 h at 37°C. Primary antibody, hybridoma culture supernatants or ascites fluid diluted 1:1,000 (~5.0 μg/ml), incubations were for 1 h at room temperature. After washes (3 × 5 min), the secondary antibody was applied (fluorescein-goat anti-mouse IgG, Boehringer Mannheim Biochemicals). Culture supernatants from nonantibody producing hybridomas were collected and used as negative control for primary antisera. Monoclonal antibodies against chicken types I, II, and type IX collagen were generously provided by Dr. T. Linsenmayer (Tufts University, Boston, MA) and Dr. R. Mayne (University of Alabama, Birmingham, AL), respectively.

Preparation of Collagen

Leg tendons, dissected from 17-d chick embryos, were extracted for 24 h at 4°C, after homogenization, in 50 mM Tris-HCl, pH 8.0, containing 1 M NaCl and protease inhibitors (1 mM PMSF, 1 mM *p*-aminobenzamidine, 10 mM *N*-ethylmaleimide, and 10 mM EDTA). By immunoblotting analysis of SDS polyacrylamide slab gels, immunoreactive protein was found to be abundant in the 1 M NaCl soluble fraction. The 1 M NaCl extract was precipitated with ammonium sulfate, 40% saturation, and the precipitate was centrifuged (12,000 *g*, 20 min). The pellet was washed three times with 75% ethanol containing 1.0% potassium acetate, freeze dried, and kept at −20°C.

Cyanogen Bromide Cleavage and Clostridiopeptidase A (EC 3.4.24.3) (Bacterial Collagenase) Digestion

The washed ammonium sulfate pellet was suspended in 70% formic acid and treated with CNBr (12 mg/ml), under nitrogen for 4 h at 30°C. The resulting solution was diluted 10× with water and freeze-dried. The CNBr peptides (6 mg/ml) were suspended in a solution of 50 mM Tris-HCl, pH 7.6, 0.2 M NaCl and 5 mM $CaCl_2$. The sample was then denatured by heating at 100°C for 1 min. Aliquots were cooled and mixed with an equal volume of bacterial collagenase (250 U/ml) (Advance Biofactures, Lynbrook, NY) in the same buffer made 10 mM in *N*-ethylmaleimide. Control samples were mixed with buffer alone. After 5 h at 37°C with occasional shaking, SDS was added to a final concentration of 2%. The sample was boiled for 3 min and dialyzed against electrophoresis sample buffer.

Gel Electrophoresis and Immunoblotting of Polypeptides

SDS polyacrylamide slab gel electrophoresis was performed according to Laemmli (11) and samples were reduced with 1% β-mercaptoethanol. After electrophoresis, the gel was soaked in transfer buffer (10 mM 3-[cyclohexylamino]-1-propanesulfonic acid, 10% methanol, pH 11.0) for 5 min. The gel, sandwiched between a sheet of immobilon™ polyvinylidene difluoride (PVDF)[2] membrane (Millipore Continental Water Systems, Bedford, MA) and several sheets of blotting paper (also equilibrated in transfer buffer), was assembled into a blotting apparatus (Trans-Blot™ Cell; Bio-Rad Laboratories, Cambridge, MA) and electroblotted for 20 h at 60 V in transfer buffer (19). The PVDF membrane was reacted with antibody as described above. The collagen molecular mass markers (cyanogen bromide peptides of type I collagen) were stained with 0.1% Amido black in methanol/acetic acid/water (45:7:48) for 10–15 min. The blots were then destained in methanol/acetic acid/water (45:7:48) for 5–10 min.

Results

Monoclonal Antibodies against Type XII Collagen

As a step in mapping the expression of type XII collagen we have chosen to develop a monoclonal antibody against an oligopeptide derived from the conceptual translation product of the cDNA pMG377. The selected target peptide has a sequence from within the carboxyl nontriple-helical domain of α1(XII) (Fig. 1). The stretch of 12-amino acid residues in the peptide exhibits little or no similarity to sequences of known matrix proteins (including all other collagens). The synthetic oligopeptide was conjugated to keyhole limpet hemocyanin and used as an antigen to generate monoclonal antibodies. One antibody, 75d7, reacts with an epitope within the synthetic peptide and recognizes the native as well as SDS denatured antigen from tendons of chick embryos. In addition, it

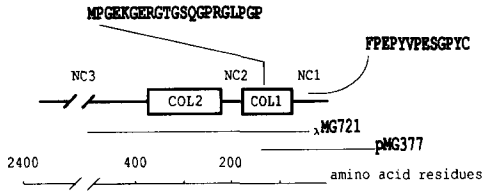

Figure 1. Diagram showing the putative amino acid sequence domain structure of type XII collagen partially deduced from two overlapping cDNA clones (*outlined in solid line*). The peptide sequence from within the NC1 domain that was selected as target for monoclonal antibody production is shown. The cysteinyl residue (*C*) at the carboxyl end of the peptide was added to facilitate coupling to hemocyanin. Also shown is the methionyl residue located at position 139 counted from the carboxyl end of α1(XII) and the 20-residue sequence after it. This 20-residue sequence is in perfect agreement with the amino acid sequence determined by Edman degradation of the immunoisolated cyanogen bromide fragment of tendon type XII collagen.

provides a striking immunofluorescence staining of several dense connective tissues (see below).

Immunoblotting analysis of SDS polyacrylamide gels of 1 M NaCl extracts of 17-d chick tendons revealed an immunoreactive polypeptide with an apparent molecular mass of 220 kD under reducing conditions (Fig. 2, lanes *1* and *5*). This immunoreactive polypeptide, which exhibited sensitivity to clostridiopeptidase A (bacterial collagenase) (Fig. 2, lanes *2* and *6*), we propose represents the α1(XII) chain. While α1(I) and α2(I) collagen chains, as well as a number of other proteins, are clearly visible in Coomassie-stained SDS-PAGE gels, the antibody 75d7 exhibited no reactivity with these proteins. In addition to the 220-kD polypeptide there is an immunoreactive band toward the top of the gel. Whether this very high molecular mass immunoreactive band represents α1(XII) which has been cross-linked to another matrix molecule is currently under examination.

To find out whether the antigen recognized by 75d7 in tendon extracts is indeed α1(XII), we have examined the products of cyanogen bromide cleavage of tendon extracts for immunoreactive peptides. There are four methionyl residues within the pMG377-derived sequence. These residues are located at amino acid residue positions 15, 24, 40, and 139, counted from the carboxyl end of α1(XII) (see Fig. 3). The 75d7 epitope is located between residues 51 and 63 (Fig. 3). Thus, treatment of α1(XII)-containing tissues with cyanogen bromide should release a 99-residue-long immunoreactive fragment. This fragment contains the two cysteinyl residues

Translated sequence of carboxyl end of α1(XII)

GFQGPPGERGMPGEKGERGTGSQGPRGLPGPPGPQGESRTGPPGSTGSRG

PPGPPGRPGNAGIRGPPGPPGYCDSSQCASIPYNGQGFPEPYYPESGPY

QPEGEPPIVPMESERREDEYEDYGVEMHSPEYPEHMRWKRGLSRKAKRKP

Figure 3. Translated sequence of the carboxyl end of α1(XII) contains a portion of the COL1 domain and all of the NC1 domain. The open triangle denotes the junction of these domains. The location of cysteinyl residues presumed to be involved in interchain cross-linking are located near the COL1-NC1 junction (*asterisks*). The peptide sequence from within the NC1 domain that was selected as target for monoclonal antibody production is shown (*solid underline*). The four methionyl residues (•) are shown, one within the COL1 domain and three within the NC1 domain. As described in the text, amino acid sequence analysis of cyanogen bromide peptides purified by affinity chromatography with the antibody 75d7, gave a sequence of 20 residues in perfect agreement (except for hydroxylation of susceptible prolyl [*P*] and lysyl [*K*] residues) with the DNA-derived sequence (*dashed underline*).

at the carboxyl end of α1(XII) chains, believed to be involved in interchain disulfide bonding. Immunoblots of SDS gels with cyanogen bromide extracts of tendons should, therefore, show a positive band at ~11 kD (reduced). A nonreduced sample should show a positive band in a higher molecular mass region. As shown in Fig. 4, the immunoblotting results with 75d7 are consistent with these predictions. In a reduced sample the antibody recognizes a major band at 11 kD, and this band is (as predicted) sensitive to treatment with bacterial collagenase. In an unreduced sample 75d7 recognizes

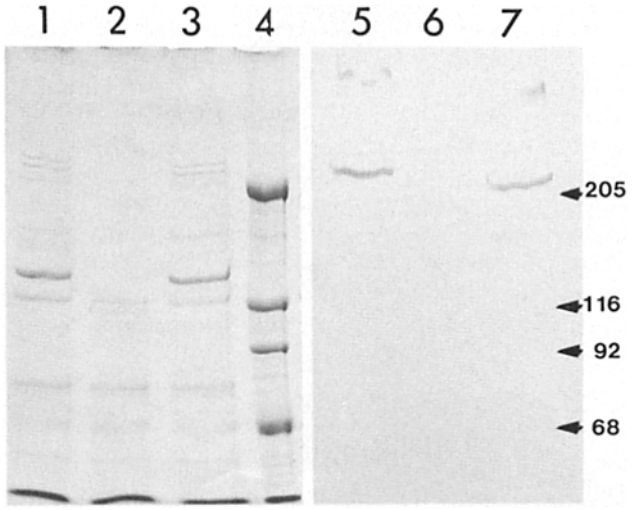

Figure 2. Immunoblotting analysis of SDS polyacrylamide gels of 1 M NaCl extract of day-17 chick tendons reveals an immunoreactive polypeptide migrating with an apparent molecular mass of 220 kD under reducing conditions. Lanes *1–4* were stained with Coomassie, lanes *5–7* were transferred to a PVDF membrane and immunostained with the antibody 75d7. The positions of globular molecular mass markers are indicated on the right. Lanes *1* and *5*, 1 M NaCl extract of tendons. Lanes *2* and *6*, extracts digested with bacterial collagenase for 5 h at 37°C. Lanes *3* and *7*, collagenase buffer and temperature controls. Note the collagenase sensitive, 75d7 immunoreactive, 220-kD polypeptide. In addition there is a very high molecular mass immunoreactive band which appears to migrate with proteins >300 kD, which may represent a nonreducible cross-link of α1(XII) chain to another molecular component.

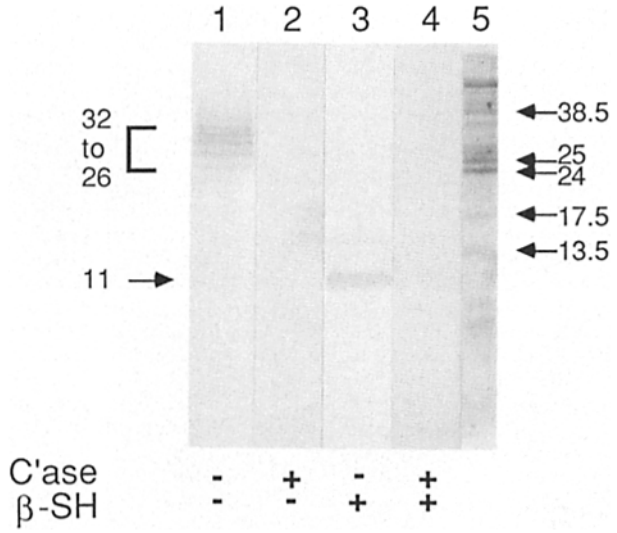

Figure 4. Gel electrophoresis and immunoblotting of the CNBr digest of crude type XII collagen. Wells 1 to 4 were loaded with 40 μg of the digest. The samples were reduced with β-mercaptoethanol (*β-SH*) and digested with collagenase (*C'ase*) as noted under the figure. After electrophoresis, lanes *1* to *4* were blotted and reacted with the antibody 75d7. Lane *5* shows CNBr peptides of type I collagen used for molecular mass determination and stained with Amido black. As noted in the text, both unreduced and reduced immunoreactive CNBr fragments are sensitive to bacterial collagenase (lanes *2* and *4*).

a cluster of bands with an average molecular mass of 30 kD. These bands are also sensitive to treatment with bacterial collagenase. Additional support for this conclusion that the cyanogen bromide fragments recognized by 75d7 indeed contain the predicted portion of α1(XII) chains, comes from amino acid sequence analysis as described elsewhere (4). Unreduced cyanogen bromide fragments, purified by affinity chromatography on a column containing the antibody 75d7, separated by SDS gel electrophoresis and electroblotted onto a PVDF membrane gave a single amino-terminal amino acid sequence, in perfect agreement with the sequence predicted from the cDNA with cyanogen bromide cleavage at residue 139 (Fig. 3) (4). Collagenase treatment of the unreduced fragment should result in an epitope-containing peptide migrating below 10 kD. Why no reactivity with the antibody is seen in Fig. 4, lane 2 is yet unclear. The faint immunoreactive band migrating above the major 11-kD band in Fig. 4, lane *3* may represent the product of incomplete cyanogen bromide cleavage.

Immunolocalization of Type XII Collagen in Embryonic Tissues

Immunofluorescence with 75d7 demonstrates the type XII antigen to be prevalent within dense connective tissues, which contain type I collagen (Figs. 5–7). Tissues most strikingly positive for type XII collagen include tendons, ligaments and dense connective tissue associated with perichondria, and periostea. These are tissues which also are immunoreactive with antibodies against type I collagen (compare Fig. 5, *A* with *C*). Type I collagen staining, however, is not always associated with staining for the type XII antigen. Smaller connective tissue septa in striated muscle (endomysium) (Fig. 5 *A*) or loose connective tissues which stain with moderate intensity with type I antibodies do not stain for type XII (Fig. 5 *C*). Also, although periosteum and endosteum are positive with 75d7 (Fig. 6), bone matrix is negative. Around tissues that contain types II (Fig. 5 *B*) and IX (Fig. 5 *D*) collagens there is an interesting complementarity to the staining patterns seen with type IX and XII antibodies (Fig. 5, *C* and *D*). For example, in the fibrocartilagenous intervertebral disk, type IX antibodies stain the central region with the highest intensity (Fig. 7 *B*), while 75d7 stains mostly the periphery (Fig. 7 *A*).

Discussion

Monoclonal Antibody against Type XII Collagen

Type XII collagen was recently identified on the basis of the isolation of the cDNA pMG377 from an embryonic chick tendon cDNA library (6). Sequencing of this clone indicated that it encodes the carboxyl region of a polypeptide which is in part similar in sequence to type IX collagen chains. The structure of the peptide, which was given the designation

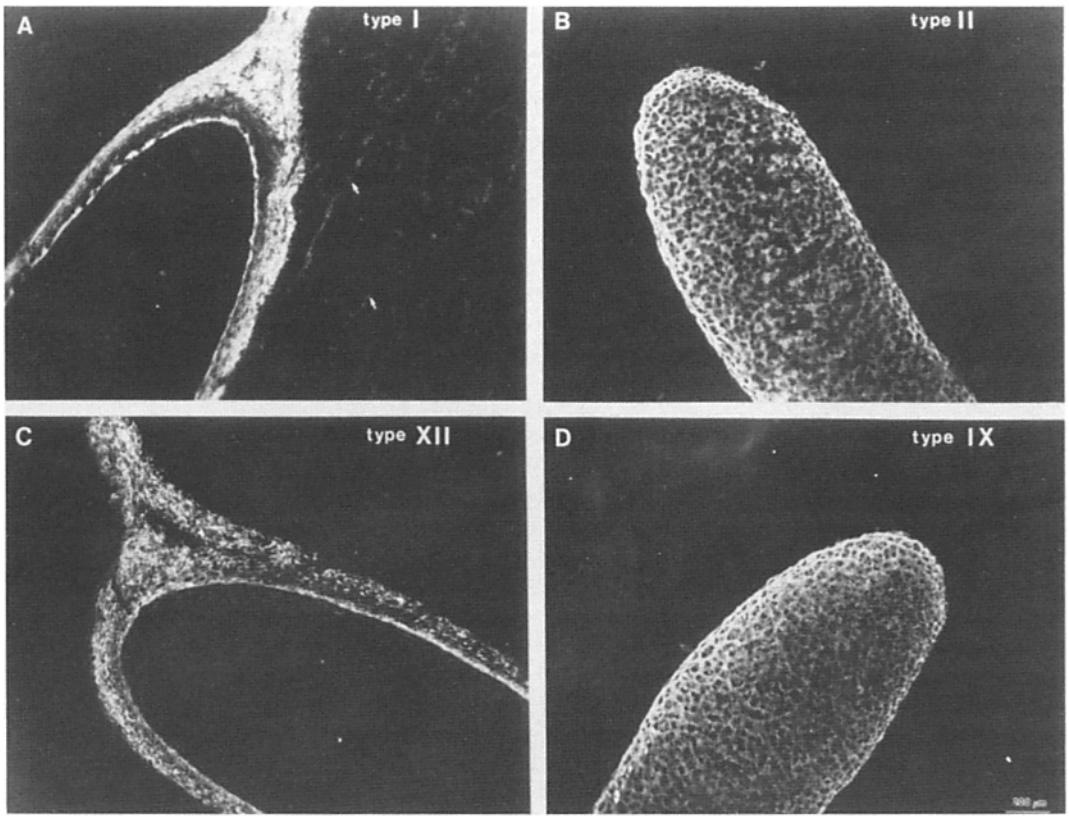

Figure 5. Cryostat sections of 15-d embryonic chick sterna immunostained with monoclonal antibodies against types I (*A*), II (*B*), IX (*D*), and XII (*C*) collagens. Note that cartilage matrix shows positive staining for types II and IX collagens, while perichondrium and attached ligament are positive for types I and XII collagens. Note also that thin septa in striated muscle are positive for type I (*A*, *arrows*) but negative for types II, IX, and XII collagens.

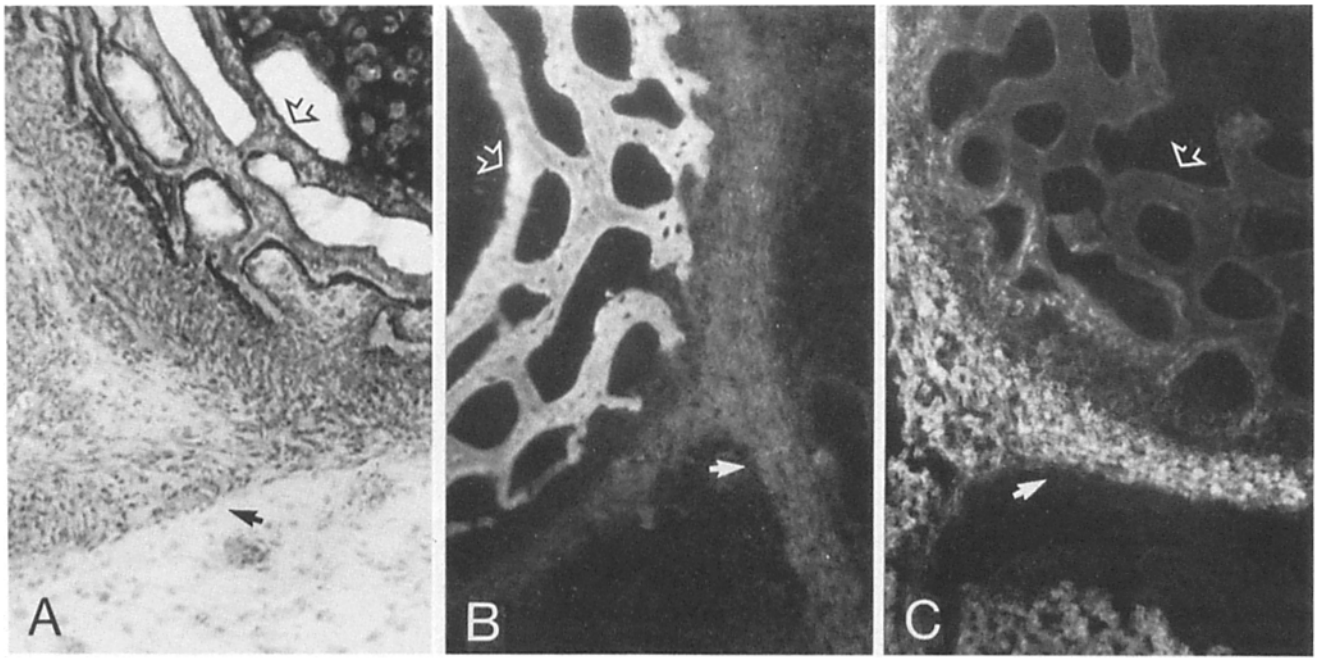

Figure 6. Cryostat sections through a long bone (phalange) of a 19-d-old chick embryo, stained with the monoclonal antibody against type I collagen (*B*) and type XII collagen (*C*). Note the positive staining of tendon (*large arrow*), periosteum (*open arrows*), and the lining of internal cavities (*small arrows*). Note that the bone matrix (the inner surface indicated by triangles) is positive for type I but negative for type XII. Toluidine blue stained section (*A*).

α1(XII), prompted the prediction that it is part of a molecule containing short triple-helical domains and that pepsin extracts of chick embryonic tendons therefore should contain triple-helical pepsin-resistant fragments derived from type XII collagen. Isolation of such fragments has indeed been reported by Dublet and van der Rest (3), and amino acid sequencing of a tryptic peptide from one of the fragments shows complete identity with the pMG377-derived sequence. Therefore, the α1(XII) collagen gene is expressed not only on the mRNA level (as shown by our ability to isolate a cDNA clone) but also on the protein level in embryonic tendons. However, isolation of pepsin-resistant triple-helical fragments of type XII collagen from embryonic tendons (3), although providing evidence for the existence of the protein in this tissue, unfortunately does not provide information about the size of α1(XII) and distribution of the protein in different tissues and organs.

We describe here for the first time the preparation of monoclonal antibodies against type XII collagen and the use of the antibodies for immunoidentification of the protein in tissues and tissue extracts. The antibody 75d7 was elicited by injecting a synthetic peptide into mice and the results of the Western blotting and affinity chromatography/amino acid sequencing clearly demonstrate that it reacts with type XII collagen in embryonic tendons.

Immunofluorescence studies with 75d7 show that type XII collagen is found within type I collagen containing structures such as tendon, ligament, perichondrium, and periosteum (Figs. 5–7). However, type XII collagen staining is clearly not associated with all type I–containing structures. As mentioned above, connective tissue septa in striated muscle or loose connective tissue which stain with moderate intensity with type I antibodies do not stain for type XII (Fig. 5, *A* and *C*). In addition, as shown in Figs. 5 and 7, cartilage and bone matrix appear negative with the 75d7 antibody. We cannot, at present, rule out the possibility that type XII collagen is present in cartilage and bone, but not detected by staining with 75d7 because of masking or degradation/modification of the epitope. Future biosynthetic studies with isolated chondrocytes and osteoblasts should enable us to address this issue. It should be noted that α1(XII) mRNA has been detected in tendon, sternal cartilage, and calvarial bone total RNA preparations by ribonuclease protection assays (6). Therefore, a combination of immunohistochemistry with 75d7 and in situ hybridization with α1(XII) cDNA should also be useful in addressing the question of the level at which type XII collagen expression is regulated.

The Molecular Structure of Type XII Collagen

The amino acid sequence analysis of disulfide-bonded, cyanogen bromide fragments of type XII collagen, purified by affinity chromatography on a column containing the monoclonal antibody 75d7, demonstrates that the carboxyl region of type XII collagen can be isolated with cyanogen bromide (as predicted from the cDNA sequence) as a disulfide-bonded fragment of ~30 kD (4). Since sequence analysis of the 30-kD components indicates a single, unique sequence and 75d7 reacts with a peptide of 11 kD after reduction, it has been concluded that type XII molecules are homotrimers composed of three α1(XII) chains (4).

This conclusion leaves open the question of why the unreduced carboxy-terminal cyanogen bromide peptide of type XII collagen migrates as a cluster of multiple sharp bands on SDS gels (Fig. 4). Since sequence analysis of the peptides gave no indication of a sequence other than the unique

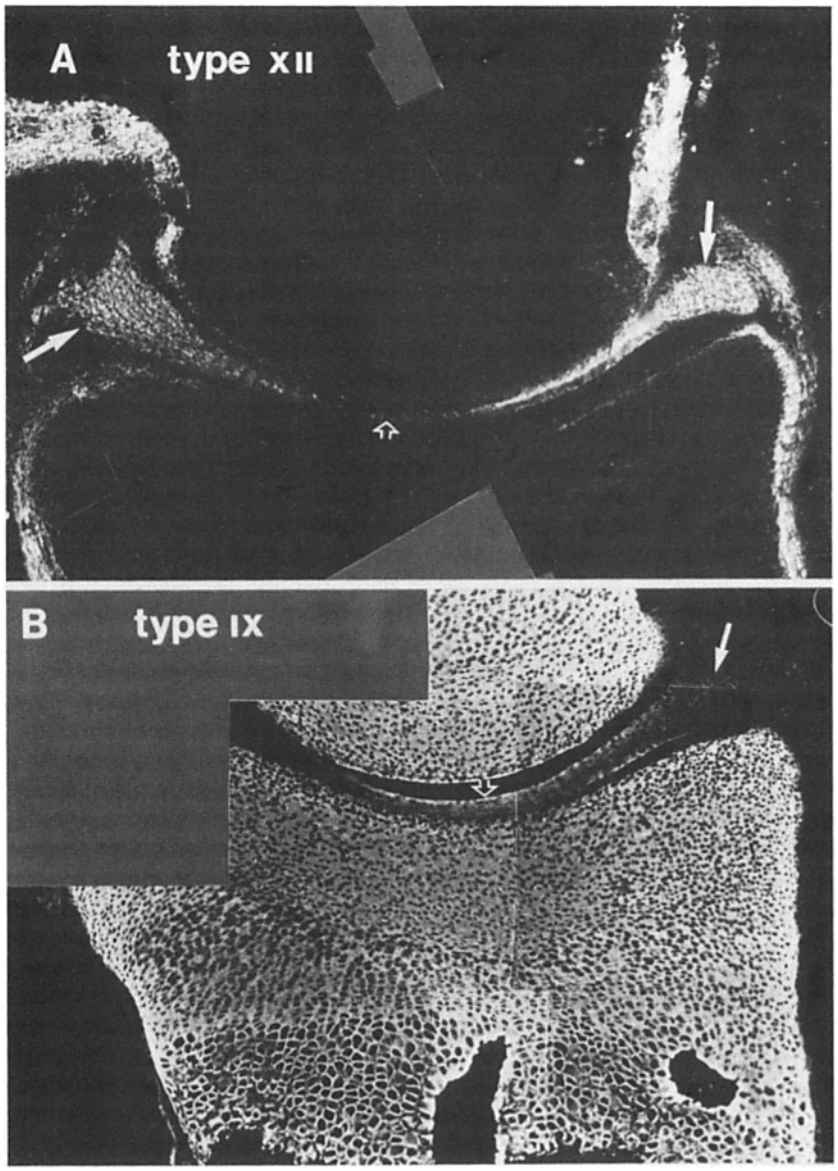

Figure 7. Cryostat sections cut longitudinally through two cervical vertebrae of a 15-d-old chick embryo. (*A*) Immunostaining with the 75d7 antibody. Note staining of the periphery of the intervertebral disk (*white arrows*), the faint staining of the center of the disk (*open arrow*) and the intense staining of the perichondrium and longitudinal ligaments. (*B*) Immunostaining with monoclonal antibody against type IX collagen. Note staining of the vertebral bodies and the center of the invertebral disk (*open arrow*). The periphery of the disk (*white arrow*) shows faint staining.

α1(XII) derived sequence (4) and all the bands also reacted with the antibody 75d7 (Fig. 4), microheterogeneities at the amino end are unlikely. However, there is the possibility of incomplete cyanogen bromide cleavage at the more carboxyl methionyl residues at positions 40, 24, and 15. Incomplete cleavage would result in a slightly larger immunoreactive peptide(s). In fact, a faint immunoreactive band at 14 kD can be seen in Fig. 4, lane *3*. Obviously, disulfide-bonded trimers composed of complete and incomplete CNBr-cleavage products could produce the cluster of peptide bands seen under nonreducing conditions.

The nucleotide sequence of the cDNA pMG377 showed that the carboxyl triple-helical domain of α1(XII) chains is quite similar to that of α1(IX) collagen chains. Sequence analysis of additional cDNA clones (unpublished data) demonstrates also that a portion of the amino terminal nontriple-helical domain of α1(XII) has a high degree of sequence similarity with the corresponding domain in the cartilage form of α1(IX). However, despite these similarities there are also clear differences between types IX and XII collagens. First, as demonstrated by the Western blotting data here, α1(XII) chains are about twice the size of α1(IX) chains. As shown elsewhere (4), this size difference is due to the presence of a much larger (190 kD) amino-terminal nontriple helical domain on α1(XII) than on α1(IX) chains (25 kD) (20). Second, while type IX collagen chains contain three triple-helical domains, the α1(XII) chain contains only two. Third, type IX collagen contains three genetically distinct subunits, and one of the chains contains a covalently attached chondroitin and/or dermatan sulfate chain (8, 10, 14). In contrast, type XII molecules are most likely homotrimers and do not appear to contain chondroitin or dermatan sulfate chains, since treatment with chondroitinase ABC does not change the electrophoretic migration of α1(XII) chains (4).

The Functional Role of Types IX and XII Collagen

Is this report on the presence of type XII collagen in type

I-containing collagen structures another example of mixed (heterotypic) collagen fibrils? There are several reports (1, 2, 9, 15) that indicate the existence of mixed fibrils containing types I and III collagen, types I and V collagen, and types II and XI collagen. However, whereas types I, III, V, and XI collagens are structurally similar, the multidomain collagen XII is structurally distinct from fibrillar collagens. The function of this collagen must therefore be different from that of fibrillar collagens. Obviously, the data presented here do not allow us to define this function. However, we hypothesize that type XII collagen may be associated with type I containing fibrils through the triple helical "tail", similar to the association between type IX collagen and type II containing fibrils in cartilage. Indeed it has been demonstrated that type IX collagen molecules are associated with the surface of cartilage fibrils (22), become covalently cross-linked to type II collagen (5, 21), and project a large globular domain attached to a triple-helical arm into the matrix (22). Our hypothesis is based on the genetic and structural similarities between α1(IX) and α1(XII). These similarities include a similar carboxy-terminal triple-helical domain with two imperfections in the Gly-X-Y structure (6, 17), and an exon structure at the 3′ end of the α1(XII) gene that is similar to that of the α1(IX) gene (6, 12). The monoclonal antibody we have developed against type XII collagen should prove useful in future studies to find out whether this hypothesis is correct.

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