

## Monoclonal Antibodies Recognizing Protease-generated Neopeptides from Cartilage Proteoglycan Degradation

APPLICATION TO STUDIES OF HUMAN LINK PROTEIN CLEAVAGE BY STROMELYSIN\*

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Clare E. Hughes<sup>‡</sup>, Bruce Caterson<sup>‡§</sup>,  
Robert J. White<sup>¶</sup>, Peter J. Roughley<sup>¶||</sup>, and  
John S. Mort<sup>¶||</sup>

From the <sup>‡</sup>Division of Orthopedic Surgery, University of North Carolina, Chapel Hill, North Carolina 27599 and <sup>¶</sup>Department of Surgery, McGill University and <sup>||</sup>Shriners Hospital for Crippled Children, Montreal, Quebec H3G 1A9, Canada

Monoclonal antibodies were raised that specifically recognize the NH<sub>2</sub>-terminal neopeptide sequence present in link protein cleavage products derived from stromelysin-degraded proteoglycan aggregate. Competitive enzyme-linked immunosorbent assay, using synthetic peptides as inhibitors, showed that one of these antibodies (CH-3) required, for antibody recognition, the free NH<sub>2</sub>-terminal amino acid isoleucine (residue 17 of the intact protein) in the sequence NH<sub>2</sub>-IQAENG at the stromelysin cleavage site of link protein 3. Human proteoglycan aggregate was digested with recombinant human stromelysin, bovine chymotrypsin, bovine trypsin, and porcine elastase, and their respective link protein degradation products were tested for immunoreactivity with antibody CH-3. Only stromelysin- and chymotrypsin-generated link protein 3 were recognized by antibody CH-3. Both of these enzymes generate link protein NH<sub>2</sub> termini with the sequence <sup>17</sup>IQAENG...; hence these studies indicated that monoclonal antibody CH-3 recognized this neopeptide sequence in only specific proteolytically modified link protein molecules. Since the occurrence of link protein 3 increases with aging, the incidence of CH-3 epitope in proteoglycans isolated from human knee articular cartilage of individuals of different ages was investigated. The prevalence of CH-3 epitope was found to be highest in newborn and adolescent articular cartilage samples. However, little CH-3 epitope was detected in older adult cartilage, although considerably more link protein 3 was present in these samples. These results suggest that additional proteolytic agents are responsible for the increased occurrence of link protein degradation products with aging.

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§ To whom correspondence should be addressed: CB 7055, Division of Orthopedics, Dept. of Surgery, University of North Carolina, Chapel Hill, NC 27599-7055. Tel.: 919-966-1212; Fax: 919-966-6730.

Mechanisms of proteoglycan breakdown in connective tissues are complex and involve multiple agents and pathways (1). This is indicated by several studies examining the turnover of matrix macromolecules in both articular cartilage and intervertebral disc (2-6). Recently, amino acid sequence analysis of cartilage proteoglycan breakdown products (4-6) has contributed to the identification of putative sites of catabolism within these macromolecules. The occurrence of the different sequences at these cleavage sites indicates that several different agents must be involved in proteoglycan turnover. At present the identity and the source of the agents responsible for this degradation and the extent to which each agent is contributing to the turnover of these molecules have not been definitively established. However, since many of the proteolytic cleavage sites in proteoglycan are now known, we hypothesized that it should be possible to use immunological procedures to both identify and quantitate the occurrence of an agent-specific cleavage site in these molecules. It has been demonstrated previously in the case of fibrinogen (7) that on cleavage of a protein, the new NH<sub>2</sub> and COOH termini produced have different properties, in terms of antibody recognition, than the same sequences present in the intact protein. These findings suggest that it should be possible, therefore, to produce antibodies specific for the products of proteoglycan aggregate components that have been cleaved by specific proteinases. Proteinases can cleave all susceptible peptide bonds without bias for the protein in which they occur. Thus, in principle, any matrix protein may be used as a marker for proteinase action. However, in practice, it is necessary that the protein be cleaved by physiologically relevant proteinases, and at least one recognizable degradation product must remain localized within the tissue. Link protein from cartilage proteoglycan aggregate satisfies these criteria (8).

Link protein (LP)<sup>1</sup> is a small globular protein which stabilizes the interaction between the cartilage proteoglycan monomer (aggrecan) and hyaluronan (9, 10). This interaction results in the formation of the large proteoglycan aggregates that endow cartilage with its property of resistance to compression under load (11, 12). Link protein isolated from human articular cartilage can be separated into three components with estimated molecular masses of 48, 44, and 41 kDa, which are referred to as LP1, LP2, and LP3, respectively (13-15). Peptide mapping and amino acid sequence analysis indicated that the different forms of link protein are derived from the same protein core (16-19). The difference in electrophoretic migration of LP1 and LP2 has been shown to be due to the differential substitution of *N*-linked oligosaccharides on the two different link protein components (15-17, 19, 20). Nguyen *et al.* (18) have demonstrated that human LP1 and LP2 differ only by the presence of an *N*-linked oligosaccharide at residue 6 in LP1. LP3 is a proteolytic cleavage product of either LP1 or LP2, which appears to have similar functional properties to those of the intact molecule (8, 15). The NH<sub>2</sub>-terminal region of LP1 and LP2, between residues 10 and 30, appears to be extremely susceptible to proteolytic attack, and

<sup>1</sup> The abbreviations used are: LP, link protein;  $\beta$ A,  $\beta$ -alanine; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.

the cleavage positions for many proteinases have been determined (8). When LP3 preparations isolated from normal and diseased human articular cartilage were analyzed, three distinct NH<sub>2</sub> termini were found. By comparison with the *in vitro* cleavage data, the proteinases of physiological relevance thought to be responsible for generating these new NH<sub>2</sub> termini were postulated to be stromelysin, cathepsin G, or cathepsin B (8). Human cartilage LP3 is therefore present as a family of molecules, the identity of which depends on the proteinase responsible for its generation. Since the generation of each LP3 produces a unique NH<sub>2</sub> terminus, we propose the nomenclature LP3(*n*) for each defined LP3, where *n* represents the sequence position of the NH<sub>2</sub> terminus of the molecule relative to that in the native protein. Thus, LP3 generated by the action of stromelysin is denoted as LP3(17).

In this paper we describe a novel approach, using monoclonal antibody technology, to identify proteolytic agents responsible for the degradation of matrix macromolecules, in normal turnover and in disease, by preparing antibodies specific for the cleavage sites produced by different proteinases in matrix molecules. As an example, this study was directed toward examining the role that stromelysin plays in the degradation of link protein during cartilage proteoglycan catabolism.

#### EXPERIMENTAL PROCEDURES

**Materials**—Pristane (2,6,10,14-tetramethylpentadecane) was obtained from Aldrich and 1,10-phenanthroline from Mallinckrodt Chemical Works. Titertek EIA plates were purchased from Flow Laboratories. Alkaline phosphatase-conjugated rabbit anti-mouse Fab<sub>2</sub> used in ELISA was obtained from Southern Biotechnology Inc., Birmingham, AL. Alkaline phosphatase-conjugated second antibody and substrate used in the Western blot analysis were obtained from Promega as the Protoblot Western blot AP system (catalog no. W3920). Nitrocellulose (0.2- $\mu$ m pore size) was obtained from Bio-Rad and polyvinylidene difluoride membrane (Immobilon) from Millipore. Human recombinant prostromelysin was a generous gift from Dr. Gillian Murphy, Strangeways Research Laboratories, Cambridge, U.K. Bovine chymotrypsin (tosyllysine chloromethyl ketone-treated), bovine trypsin (tosylphenylalanine chloromethyl ketone-treated), and porcine pancreatic elastase were all purchased from Sigma. Monoclonal antibody 8-A-4 was prepared as ascitic fluid and also purchased from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). This antibody recognizes a linear amino acid sequence epitope in the paired tandem repeat domains of link proteins isolated from many animal species (16). All other chemical reagents used that are not specifically mentioned above were of analytical grade and obtained from either Sigma or Fisher Scientific.

**Preparation of Antigens for Immunization and Screening**—Peptides were synthesized, at a 0.5-mmol scale using standard Fmoc (9-fluorenylmethoxycarbonyl) chemistry, on an Applied Biosystems model 431A solid phase peptide synthesizer. Crude peptides were purified by reverse phase chromatography (Prep-10 Aquapore C8 column, Applied Biosystems) using an acetonitrile gradient in 0.1% trifluoroacetic acid. Peptides synthesized were IQAENGGC, IQAENG( $\beta$ A)C, Ac-IQAENG( $\beta$ A)C, IHQAENGGC, HIQAENGGC, QAENG( $\beta$ A)C, AENGGC, and DHLSDNYTGCC. Most peptides contained a COOH-terminal spacer amino acid, glycine, followed by a cysteine residue that was used as the coupling site for preparation of peptide-protein conjugates. In some peptides used to probe antibody specificity,  $\beta$ -alanine ( $\beta$ A) was used as a substitute for the glycine spacer residue.

The bifunctional reagent, *N*-hydroxysuccinimidylbromoacetate, was synthesized as described by Bernatowicz and Matsueda (21). For coupling, 0.2 ml of a 65 mg/ml solution of this bifunctional reagent in dimethylformamide was added, dropwise, with continuous stirring to 2.25 ml of ovalbumin (22 mg/ml) dissolved in 0.1 M potassium phosphate, pH 7.5, containing 1 mM EDTA at 4 °C. The mixture was then allowed to equilibrate to room temperature over a 30-min time period. The activated ovalbumin was separated from unreacted reagents by gel filtration using a Sephadex G-25 column (25  $\times$  1.5 cm) eluted with the EDTA-phosphate buffer described above. The synthetic peptide to be coupled to the carrier protein was dissolved in

water (0.4 ml) at a concentration of 6 mM and added to 0.4 ml of the Sephadex G-25 eluate peak fraction containing the activated ovalbumin. The mixture was initially shaken gently under nitrogen for 2 h at room temperature, followed by continued incubation overnight at 4 °C. Unreacted bromoacetate groups were blocked by the addition of mercaptoethanol (1  $\mu$ l), and the solution was dialyzed exhaustively against PBS. The success of the coupling reaction was determined by observation of a decrease in electrophoretic mobility of the peptide-ovalbumin conjugate, on SDS-PAGE, relative to a cysteine-ovalbumin conjugate prepared in the same manner. The identity of the peptide on the conjugate was confirmed by peptide sequencing, following SDS-PAGE and electroblotting onto a polyvinylidene difluoride membrane (22).

**Immunization and Fusion**—The antigen used for immunization was a peptide-ovalbumin conjugate, consisting of the synthetic peptide IQAENGGC coupled to the carrier protein. A single 4–6-week-old female BALB/c mouse was immunized with the ovalbumin-peptide conjugate. Procedures for immunization, cell fusion, and hybridoma selection were as described by Caterson *et al.* (23, 24). After 10–14 days in culture, the wells were visually inspected for the presence of viable hybridomas. Aliquots from each of the hybridoma-containing wells were tested in ELISA for the presence of mouse immunoglobulin with specificity directed against the original immunizing antigen. These hybridoma supernatants were also tested for antibody activity against ovalbumin-peptide conjugates containing an unrelated peptide sequence, in order to distinguish hybridomas that produced antibodies recognizing the synthetic peptide from those recognizing epitopes in either the coupling region of the conjugate or the carrier protein. Hybridomas from wells producing antibody directed against epitopes in the synthetic peptide component of the ovalbumin-peptide conjugate were then expanded and subcloned by limiting dilution. Media from the resultant monoclonal hybridomas were retested in ELISA for their production of immunoglobulin directed against the original antigen, then expanded in cell culture, and used for ascites production. Ascites were produced by the intraperitoneal injection of 10<sup>6</sup>–10<sup>7</sup> cells into female retired breeder BALB/c mice that had been previously primed with an intraperitoneal injection of 0.5 ml of Pristane 1–2 days earlier. Ascitic fluid was usually harvested 2–3 weeks after the hybridoma injection and stored in the presence of 0.02% NaN<sub>3</sub>. The antibody isotype was determined using an isotype screening kit supplied by Southern Biotechnology Inc., Birmingham, AL.

**ELISA Screening Procedures**—Antigens were dissolved in PBS-azide (0.14 M NaCl, 0.01 M sodium phosphate, pH 7.2, containing 0.02% NaN<sub>3</sub>) and coated at a concentration of 3  $\mu$ g/ml on 96-well Titertek EIA plates by passive absorption either overnight at 4 °C or for 3 h at 37 °C. The plates were washed with PBS-azide, and the unreacted sites were blocked by the addition of EIA buffer (1% BSA in PBS-azide; 200  $\mu$ l/well) followed by incubation for 1 h at 37 °C. The plates were then washed three times with PBS-azide, and 200  $\mu$ l of each hybridoma culture supernatant was added to appropriate wells of the EIA plates and incubated for 1 h at 37 °C. The plates were washed four times with PBS-azide, followed by the addition of 200  $\mu$ l of a 1:500 dilution of enzyme-linked second antibody solution (alkaline phosphatase-conjugated rabbit anti-mouse Fab<sub>2</sub>) and incubation for 1 h at 37 °C. The plates were washed five times with Tris-saline buffer (0.2 M NaCl, 0.05 M Tris-HCl, pH 7.4) and then incubated at 37 °C with alkaline phosphatase substrate (*p*-nitrophenyl phosphate, 1 mg/ml in 0.126 M MgCl<sub>2</sub>, 0.86 M diethanolamine, pH 9.8) until optimal color development occurred (usually 30–90 min). The color was quantified by measuring the absorption at 405 nm on a Titertek Multiskan (Flow Laboratories).

**ELISA Inhibition Assays**—The immunizing peptide antigen was dissolved in PBS-azide and coated onto EIA plates at 3  $\mu$ g/ml (200  $\mu$ l/well), then blocked in EIA buffer, as described above. A panel of peptides, all at 1 mg/ml, was serially diluted in EIA buffer (concentration range from 0 up to 30  $\mu$ g/100  $\mu$ l) and incubated at room temperature for 1 h with monoclonal antibody CH-3 (final dilution of 1:10,000 in 200  $\mu$ l) prior to incubation for 1 h at 37 °C in the appropriate well of the antigen-coated and blocked EIA plate. The plates were washed and incubated with secondary antibody as described above in the direct ELISA. Substrate was then added, and the plates were incubated at 37 °C until the well that contained no competing antigen (0  $\mu$ g/ml) gave an absorbance reading of approximately 1.0 at 405 nm. This reading was taken as 100%.

**Proteinase Digestion of Cartilage Proteoglycan Aggregate**—Proteoglycan aggregate isolated from normal human knee articular cartilage was prepared by associative CsCl equilibrium density gradient cen-

trifugation (13). The proteoglycan aggregate fraction (A1)<sup>2</sup> was subjected to proteolytic digestion with either chymotrypsin, trypsin, or pancreatic elastase. Digestion mixtures (0.1 ml) contained 2 mg/ml proteoglycan aggregate in 0.1 M Tris-HCl, pH 7.5, and 20 µg/ml of either of the three proteinases listed above. Following incubation at 37 °C for 4 h, the enzymes were inactivated by addition of an equal volume of SDS-PAGE sample buffer (without mercaptoethanol) and heated immediately in a boiling water bath for 3 min. The proteoglycan aggregate was also subjected to digestion with recombinant prostromelysin, which had been preactivated by *p*-aminophenyl mercuric acetate treatment (25). Incubations contained 1 mg/ml proteoglycan aggregate in 0.1 M Tris-HCl, pH 7.5, containing 10 mM CaCl<sub>2</sub> and 10 µg/ml stromelysin and were allowed to proceed at 37 °C for 24 h and then terminated as above. Link proteins were separated by SDS-PAGE, electroblotted onto nitrocellulose, and immunolocalized with monoclonal antibodies using procedures described below.

**Isolation and Analysis of Cartilage Matrix Proteins**—Macroscopically normal human articular cartilage was obtained from the distal femur at autopsy, within 16 h of death, from donors aged 6 weeks, 2 years, 10 years, 23 years, and 58 years, respectively. The tissue was finely diced and extracted with 4 M guanidinium chloride-containing proteinase inhibitors (26), and the protein-rich components of the cartilage extracts were isolated by direct dissociative CsCl equilibrium density gradient centrifugation using a starting density of 1.50 g/ml (14). After centrifugation, the tubes were divided into three fractions (D1–D3),<sup>2</sup> which were then dialyzed and freeze-dried. Cartilage matrix proteins in the low buoyant density D3 fraction were dissolved at 2 mg/ml in 4 M guanidinium chloride, 0.1 M sodium acetate, pH 6.3, and then dialyzed at room temperature against two changes of 0.125 M Tris-HCl, pH 6.8, containing 0.1% SDS. Samples (50 µg/well) were subjected to SDS-PAGE, followed by electroblotting onto nitrocellulose membranes. Link proteins were immunolocalized with monoclonal antibodies 8-A-4 and CH-3 using procedures described below.

**SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting**—Samples containing native and degraded link protein were electrophoresed on 10% polyacrylamide slab gels in SDS using procedures described by Laemmli (27). After electrophoresis, the fractionated proteins were electrophoretically transferred to nitrocellulose membranes. The transfer sheet was incubated in blocking solution (3% (w/v) bovine serum albumin in PBS-azide) overnight, then incubated with a 1:2000 dilution of monoclonal antibody 8A4 or a 1:100 dilution of CH3 in TBST (0.05% Tween 20 in 0.15 M NaCl, 0.01 M Tris-HCl, pH 8.0) for 30 min at 37 °C. After 3 washes with TBST buffer, the nitrocellulose sheets were incubated for 30 min at 37 °C with a 1:7500 dilution of an alkaline phosphatase-conjugated rabbit anti-mouse second antibody in TBST buffer. The nitrocellulose was then thoroughly washed in TBST buffer and then washed in one change of alkaline phosphatase buffer (100 mM NaCl, 5 mM MgCl<sub>2</sub>, 100 mM Tris-HCl, pH 9.5) before addition of freshly prepared alkaline phosphatase substrate solution (66 µl of nitro blue tetrazolium (50 mg/ml in 70% dimethylformamide) and 33 µl of 5-bromo-4-chloro-3-indoyl phosphate (50 mg/ml in dimethylformamide) in 10 ml of alkaline phosphatase buffer). In general, the immunoblots were incubated for 10–30 min at room temperature to achieve optimum color development.

RESULTS

**Characterization of a Monoclonal Antibody Specific for LP3(17)**—Mice were immunized with a synthetic peptide conjugate containing the first 6 residues of the NH<sub>2</sub>-terminal neopeptide produced from cleavage of native link protein by stromelysin. Preliminary screening of hybridoma supernatant specificity in direct ELISA identified six clones (denoted CH-3 to CH-8, respectively) that recognized the immunizing antigen. These antibodies showed no reactivity with unrelated synthetic peptide conjugates nor with the carrier protein. Hybridoma clone CH-3 showed the strongest reactivity in the preliminary screening and thus was processed further. The CH-3 hybridoma cell line was cloned by limiting dilution, and its antibody isotype determined as being an IgG<sub>1</sub>, heavy chain and κ light chain.

The reactivity of monoclonal antibody CH-3 against a series

of unconjugated peptides, related to the immunizing antigen but having modifications at both the NH<sub>2</sub>- and COOH-terminal amino acids, was tested in competitive ELISA (Fig. 1). These analyses showed that the unconjugated immunizing peptide and the same peptide with a modified COOH-terminal amino acid sequence both gave 50% of the maximum absorbance at a concentration of 25 µg/ml peptide (Fig. 1, *open symbols*). Acetylation of the peptide resulted in a marked reduction in inhibition, while removal of the NH<sub>2</sub>-terminal amino acid (isoleucine) resulted in a complete loss of this inhibition. Similarly, NH<sub>2</sub>-terminal addition of further residues from the link protein sequence (Fig. 2A) gave peptides that were unable to compete with the immunizing sequence, even when an alternative NH<sub>2</sub>-terminal isoleucine residue

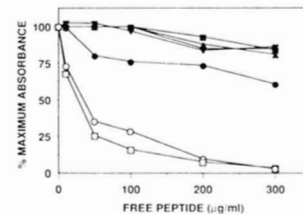


FIG. 1. Competitive inhibition ELISA assay using monoclonal antibody CH-3. EIA microtiter plates were coated with the ovalbumin-synthetic peptide (IQAENGGC) conjugate used as the immunizing antigen. Synthetic peptides used as competing antigens in the inhibition ELISA were: ○, IQAENGGC; □, IQAENG(βA)C; ●, Ac-IQAENG(βA)C; ■, IHIQAENGGC; ▲, HIQAENGGC; ▼, QAENG(βA)C; and ◆, AENGPHGCC.

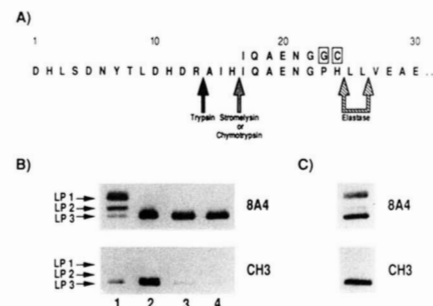


FIG. 2. Characterization of monoclonal antibody CH-3. A, diagrammatic representation of the NH<sub>2</sub>-terminal amino acid sequence (residues 1–30) of human link protein, illustrating (*below*) the cleavage sites of trypsin, chymotrypsin, stromelysin, and elastase, and (*above*) the peptide used as the immunogen. *Boxed residues* indicate the linker region of the synthetic peptide antigen. B, samples of human proteoglycan aggregate digested with proteinases (10 µg of proteinase/mg of proteoglycan aggregate, 4 h) were analyzed by SDS-PAGE and Western blotting. Link protein components were visualized using monoclonal antibodies 8-A-4 or CH-3 as indicated. Lane 1, control; lane 2, chymotrypsin; lane 3, elastase; lane 4, trypsin. C, human proteoglycan aggregate digested with *p*-aminophenyl mercuric acetate-activated recombinant prostromelysin (10 µg of proteinase/mg of proteoglycan aggregate, 24 h) and then analyzed as in B.

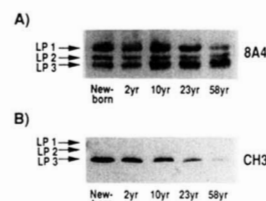


FIG. 3. Changes with age in the CH-3 epitope in human articular cartilage. Cartilage proteins from the D3 fraction of dissociative CsCl density gradients were subjected to SDS-PAGE and Western blotting. Link protein components were visualized using monoclonal antibody 8-A-4 (A) and monoclonal antibody CH-3 (B).

<sup>2</sup> A1 and D1–D3 as used in Ref. 12.

was present (Fig. 1, closed symbols). These results demonstrated the importance of the free NH<sub>2</sub>-terminal isoleucine residue and the dependence on the specific amino acid sequence adjacent to this residue in the epitope recognized by CH-3.

The specificity of monoclonal antibody CH-3 for human LP3(17) was demonstrated by immunolocalization studies on specific proteinase digestion products of human articular cartilage proteoglycan aggregate (Fig. 2). Following SDS-PAGE and electroblotting, immunolocalization with monoclonal antibody 8-A-4 (which recognizes internal epitopes in link protein) visualized LP1, LP2, and LP3 in the undigested preparation, with LP1 and LP2 being most abundant and LP3 being present as a minor component. However, digestion with chymotrypsin, elastase, or trypsin resulted in complete conversion of LP1 and LP2 to LP3 products, the sequences of which had been determined previously (Fig. 2A). In contrast to 8-A-4, monoclonal antibody CH-3 gave a weak LP3 band in the undigested sample and did not react with the trypsin or elastase digestion products. However, chymotrypsin digestion, which is known to produce LP3(17), gave a strong reaction (Fig. 2B). Similarly, digestion with recombinant stromelysin produced a strong CH-3 reactive band (Fig. 2C). NH<sub>2</sub>-terminal sequencing of this recombinant stromelysin-generated LP3 gave the expected sequence. These data demonstrate the specificity of antibody CH-3 for the sequence NH<sub>2</sub>-IQAENG... in the native LP3 preparations.

*Changes with Age in LP3(17) Abundance*—In order to evaluate the importance of stromelysin action in cartilage changes associated with aging, the occurrence of CH-3 neopeptide in link proteins from human articular cartilage extracts from various individuals of different ages was examined (Fig. 3). Immunolocalization analysis, using antibody 8-A-4, indicated that the relative abundance of LP3 was found to increase with age at the expense of the LP1 and LP2 components, in agreement with previous results (14). In contrast, however, the proportion of LP3(17), as visualized using the monoclonal antibody CH-3, was found to decrease with the age of the individual.

#### DISCUSSION

Results from several groups indicate that the mechanism of proteoglycan catabolism in connective tissues is complex. Protein sequencing studies have documented many of the cleavage sites that result from catabolism of the proteoglycan aggregate components, aggrecan (4–6) and link protein (8, 18). In recent years, reports in the literature have focused on the metalloproteinases stromelysin and collagenase as being the major agents involved in cartilage proteoglycan degradation (28). However, it is clear that many of the proteolytic agents responsible for this catabolism still remain to be identified (4–6, 8). Research in our laboratories has been directed toward using monoclonal antibody technology to identify specific *in vivo* cartilage proteoglycan breakdown products as a means of discriminating between the large number of proteolytic agents that are potentially involved in this process.

The present work demonstrates the feasibility of this approach. Monoclonal antibodies specific for the stromelysin cleavage site in the degraded link protein present in catabolized cartilage proteoglycan aggregate have been produced. One of these antibodies (CH-3) recognized only the neopeptide sequence containing an NH<sub>2</sub>-terminal isoleucine (residue

17 of the intact protein) and its adjacent amino acids but not the identical sequence present in the undegraded link protein. This finding suggests that monoclonal antibody CH-3 can be used to distinguish between the degradative action of stromelysin versus other proteinases in matrix turnover, since of the physiologically relevant proteinases studied so far only stromelysin cleaves at this position. Our studies using monoclonal antibody CH-3 also provided evidence suggesting that the major proportion of LP3 in the adult is not a final degradation product of stromelysin, even though during aging an increasing amount of the link protein in human articular cartilage is present in the LP3 form. This indicates that catabolic agents other than this metalloproteinase contribute to link protein degradation and presumably to the turnover of matrix molecules in general in normal adult cartilage. It still remains to be established whether or not multiple proteolytic agents (including stromelysin) are involved in this process.

These results indicate that neopeptide monoclonal antibody technology is a powerful tool for identifying the unique degradation products that result from the action of individual proteolytic agents in extracellular matrix degradation. In addition to their application in immunoblotting, these reagents can also be used for immunohistochemical localization and quantitative immunoassay. Thus, this methodology has great potential for discriminating between the different mechanisms of cartilage metabolism operating in health and disease.

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