Role of Anchorin CII, a 31,000-mol-wt Membrane Protein, in the Interaction of Chondrocytes with Type II Collagen

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ABSTRACT We have previously reported the isolation of a hydrophobic, type-II collagen-binding glycoprotein of molecular weight 31,000 (31,000-mol-wt protein) from chick chondrocyte membranes (Mollenhauer, J., and K. von der Mark, *EMBO Eur. Mol. Biol. Organ. J.*, 2:45–50). The function of this protein in anchoring pericellular type II collagen to the chondrocyte surface was inferred from its ability to bind native type-II collagen either when detergent solubilized or when inserted into liposomes. In the present study we have used specific antibodies to localize this protein, which we now call anchorin CII, to the surface of chondrocytes in both cartilage sections, and in cell culture. In immunofluorescence studies of isolated chondrocytes we observed a dense, punctate distribution of anchorin CII on the cell surface when chondrocytes were enclosed by a pericellular type II collagen matrix. Removal of the pericellular matrix with trypsin also removed anchorin CII. The membrane protein character of anchorin CII was indicated by the demonstration of antibody-induced patching and capping on the chondrocyte surface at 22°C and 37°C, respectively. In monolayer culture, the amount of anchorin CII appeared reduced on flattened chondrocytes lacking a pericellular type II collagen matrix but was prominent upon intercellular cell processes. Fab′ fragments prepared from either anchorin CII antiserum or an antiserum directed against the entire chondrocyte membrane inhibited the attachment of chondrocytes to a type II collagen substrate. In each case, the inhibition of attachment was neutralized by preincubation of Fab′ fragments with purified anchorin CII.

In hyaline cartilage, chondrocytes are embedded in an extracellular matrix consisting predominantly of chondroitin sulfate proteoglycan, type II collagen, several minor collagens, and glycoproteins. Type II collagen represents about 80–90% of the total cartilage collagen (1, 2). Minor cartilage collagens include molecules consisting of 1α, 2α, and 3α chains (3) and a class of collagen fragments characterized by intramolecular cystine bridges, M-collagens (4–7); they represent about 10–15% of the cartilage collagen (for review see reference 8).

Collagen provides the three-dimensional framework of the cartilage matrix in which the proteoglycans are imbedded and contributes essentially to the mechanical and physiological properties of cartilaginous tissues. However, type II collagen has also been shown to be influential in chondrogenic differentiation (9–12), and in the regulation of the chondrocyte phenotype (for review see 13, 14).

Immunohistochemical and electronmicroscopical studies on isolated chondrocytes (15–19) have demonstrated a close association of type II collagen with the chondrocyte surface. The precise nature of this cell:matrix association, however, is still unclear. Chondronectin, an adhesion factor that enhances the binding of chondrocytes to a collagen substrate, has been isolated from serum (20, 21). Whether this component is intimately associated with the chondrocyte membrane remains unclear.

Recently we have described the isolation of a collagen-binding glycoprotein of molecular weight 31,000 (31,000-mol-wt protein) from chondrocyte membranes by affinity chromatography on type II collagen-Sepharose (22). When solubilized in Nonidet P40 or inserted into synthetic lecithin vesicles, this hydrophobic protein reveals a specific affinity for native type II collagen (39). These characteristics suggested this protein to be an integral membrane protein responsible for the direct interaction of the chondrocyte surface with type II collagen. We now will refer to this protein as anchorin CII[1] (*anchorin*g of *c*hondrocytes to type *II* collagen).

In the present study the occurrence and distribution of anchorin CII on the chondrocyte surface and its role in

[1] *Abbreviations used in this paper:* anchorin CII, anchoring of chondrocytes to type II collagen; ELISA, enzyme-linked immunosorbent assay.

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chondrocyte/collagen interactions has been examined by using specific antibodies. These antibodies patched and capped, indicating the surface localization of anchorin CII on chondrocytes. The amount of surface-bound anchorin CII was reduced on flattened chondrocytes which are devoid of a pericellular type II collagen matrix. We also demonstrate the participation of anchorin CII in the chondrocyte/collagen interaction by inhibiting chondrocyte attachment to type II collagen substrates with Fab' fragments prepared from anchorin CII antiserum.

MATERIALS AND METHODS

Preparation of Anti-anchorin CII Antiserum: Anchorin CII was purified by affinity chromatography on type II collagen Sepharose of Nonidet-P40-solubilized chondrocyte membranes as described previously (22). The resultant protein is pure by criteria of SDS gel electrophoresis after silver staining (23) (Fig. 1 *b*). A rabbit was immunized by three subcutaneous injections of 0.1 mg anchorin CII initially emulsified in complete, and thereafter in incomplete, Freund's adjuvant. Serum was taken 2 wk after the last injection and affinity purified by chromatography on 1 mg anchorin CII coupled to 2 ml of Sepharose 4B. Bound immunoglobulins were eluted with 3 M KSCN in 0.05 M phosphate buffer, pH 6.0 (24). Antisera against whole chondrocyte membranes were prepared in rabbits by immunization with 0.5 mg purified chick chondrocyte membranes (22) according to the regimen described above.

Preparation of Fab' Fragments: Immunoglobulins from anchorin CII-, chondrocyte membrane-, and preimmune- rabbit sera were isolated by chromatography on DEAE Affi Gel Blue (Bio-Rad Laboratories, Richmond, CA) (25, 28). Fab' fragments were prepared by pepsin treatment of the immunoglobulins at pH 4.3, and by reduction and alkylation of $(Fab')_2$ as described elsewhere (26, 27). Incompletely digested immunoglobulins and Fc fragments were separated from free Fab' fragments by chromatography on protein A Sepharose (Pharmacia, Inc.). The unbound Fab' fraction was dialyzed against 0.02 M Tris HCl, 0.028 M NaCl, pH 8.0. An aliquot was shown to be homogeneously pure by SDS PAGE followed by silver staining and the remainder was dialyzed against F12 medium and stored frozen.

Enzyme-linked Immunosorbent Assay (ELISA): Titers of antisera and antibodies were determined by the ELISA test according to Engvall and Perlmann (28) with slight modification. 96-well microtiter plates were coated with 5 μg/well of anchorin CII or 50 μg of purified chondrocyte plasma membrane vesicles in 50 μl of coating buffer (28). After incubation for 24 h, plates were washed and saturated with 1% bovine serum albumin: 0.1% goat immunoglobulin. Bound antibody was detected with peroxidase-conjugated goat anti-rabbit IgG (Miles-Yeda, Rehovot, Israel) using diaminobenzidine as the substrate for the color reaction.

Immunoblotting: Chick chondrocyte plasma membranes were separated by SDS gel electrophoresis on 5–18% polyacrylamide gradient gels and transferred to nitrocellulose paper (29). Strips carrying transferred proteins were incubated with either antianchorin CII or preimmune sera diluted 1:50, and either antianchorin CII or preimmune Fab' fragments diluted 1:20. After washing, strips were incubated with peroxidase-conjugated goat anti-rabbit gamma-globulin (diluted 1:200), or goat anti-rabbit Fab' (1:200), followed by peroxidase-conjugated rabbit anti-goat IgG (1:200), (Miles-Yeda). Bound peroxidase-labeled antibody was visualized by the addition of 5 mg/ml Brenz catechol, 5 mg/ml Diphenylamine, H_2O_2 in 0.1 M sodium cacodylate, pH 6.0 (30).

Immunofluorescence: Frozen sections of sternal cartilage from 16-d chick embryos were double stained with rabbit antibodies to anchorin CII, followed by rhodamine-conjugated goat anti-rabbit IgG (Dynatech Laboratories, Inc., Alexandria, VA), and guinea pig antibodies to type II collagen, followed by fluorescein-conjugated rabbit anti-guinea pig (Behringwerke, Marburg, Federal Republic of Germany) as described previously (31). All sections were pretreated with 1 mg/ml testicular hyaluronidase (SERVA, Heidelberg, Federal Republic of Germany).

Chondrocytes were released from sternal cartilage of 16-d chick embryos with collagenase/trypsin as described (16, 18) and allowed to recover at 37°C in Ham's F12 medium containing 10% fetal calf serum (FCS). For the detection of anchorin CII on matrix-free chondrocytes, protein synthesis was blocked by adding cycloheximide (25 μg/ml) throughout both the enzyme treatment and recovery phase. Matrix reconstitution from intracellular pools was inhibited by the addition of 20 U/ml of purified bacterial collagenase (Advanced Biofactures) and 0.3 mg/ml testicular hyaluronidase (Sigma Chemical Co., St. Louis, MO) to 5 ml of the chondrocyte suspension during the recovery phase. For monolayer culture, chondrocytes were plated on glass coverslips in F12/10% fetal calf serum medium at 37°C in 5% CO_2 in air.

Cells in suspension or monolayer culture were stained without fixation at 0°C, room temperature, or 37°C after washing with F12 medium containing whole antiserum directed against anchorin CII or the corresponding purified antibodies. Since no difference was detected between either whole serum or purified antibodies, most experiments were performed with the antiserum diluted 1:10 with F12 medium. Chondrocytes in suspension were stained in plastic cups and washed by centrifugation. Type II collagen specific antibodies were from rabbit for single staining, and from guinea pig for double staining. Bound antibody was detected by appropriately diluted rhodamine-conjugated goat anti-rabbit IgG. Stained cells were viewed and photographed with a Zeiss ICM 405 inverted microscope, equipped with a HB50 lamp.

Attachment Assays: Freshly isolated chondrocytes from chick sternal cartilage (16, 18) were incubated for 1 h in F12/10% FCS medium containing 100 μCi/ml ^{75}seleno-methionine (Amersham Corp., Arlington Heights, VA). After extensive washing with F12 medium, cells were counted, and the incorporated radioactivity was determined in a γ counter.

Microtiter plates with 96 wells (Falcon Labware, Oxnard, CA) were coated with 50 μl of either native type I or II collagen (1 mg/ml) in 0.1 M acetic acid and dried overnight. The plates were rinsed with F12 medium and seeded with labeled chondrocytes (50 μl/well, 30–50,000 cells, corresponding to ~5,000 cpm total). To assay the effect of antibodies, cells were preincubated with various concentrations of Fab' fragments in F12 medium for 1 h. To neutralize inhibition of attachment, Fab' fragments from antianchorin CII or chondrocyte membrane-antisera were absorbed with anchorin CII-sepharose before adding to the cells. Cells were allowed to attach to the substrate for various times; nonattached cells were removed with a multipipette, and the attached cells were washed twice with 0.1 ml F12 medium. Washings and nonbound cells were combined and counted. Attached cells were dissolved in 10% SDS, 0.01 M EDTA, and counted. All samples were prepared as duplicates. Attachment rates were calculated as:

$$\frac{\text{attached cells (cpm)} \times 100}{\text{attached cells (cpm)} + \text{supernatant cells (cpm)}}.$$

To correct for possible secretion of ^{75}seleno-methionine-labeled proteins during the attachment phase, aliquots of labeled washed cells were centrifuged at each time point, and radioactivity in the supernatant solution was counted.

RESULTS

Preparation of Antibodies Directed against Anchorin CII

Rabbits were immunized with anchorin CII purified from chick sternal cartilage (Fig.1; see also [22]). The titer of the antiserum was determined by ELISA on microtiter plates coated with either whole chondrocyte membranes or purified anchorin CII (Table I). The titer against whole membranes decreased after absorption of the antiserum by affinity chromatography on immobilized anchorin CII (Table I). Fab' fragments were prepared by pepsin treatment and reduction and alkylation of the antiserum. Antianchorin CII immunoglobulins were isolated by affinity chromatography on the immobilized antigen. Similarly, Fab' fragments were prepared from an antiserum against whole chondrocyte membranes.

The specificity of both antianchorin CII serum and Fab' fragments prepared from this antiserum was verified by immunoblotting experiments. Chondrocyte membranes were separated by SDS electrophoresis on polyacrylamide gels and transferred to nitrocellulose paper. Incubation of the resultant nitrocellulose paper strips with either whole antiserum (Fig. 2 *c*) or purified Fab' fragments (Fig. 2 *d*) directed against anchorin CII demonstrated only one band in the molecular weight range of 31,000. Incubation with an antiserum raised against total chondrocyte membranes lead to several reactive bands, and anchorin CII as one of the major components (Fig. 2 *b*), indicating that anchorin CII is a major antigen of the chondrocyte surface.

Localization of Anchorin CII in Cartilage Tissue

Frozen sections from either 16-d embryonic chick tibia or sternum were pretreated with testicular hyaluronidase to re-

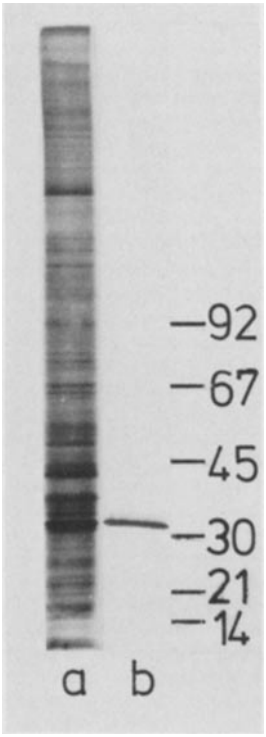

FIGURE 1 Silver stained SDS PAGE of chondrocyte membranes (*a*) and purified anchorin CII (*b*), isolated from chick chondrocyte plasma membranes by affinity chromatography on type II collagen sepharose (22). Values at right represent the molecular weight × 10^{-3}.

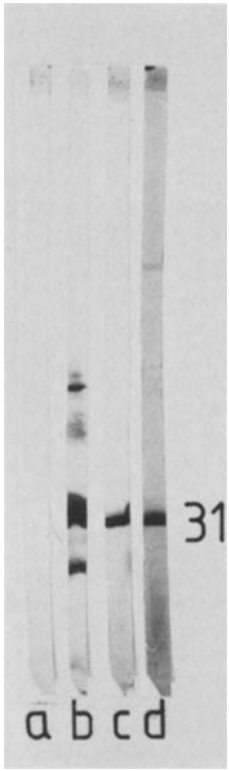

FIGURE 2 Immune blot of chick chondrocyte membrane proteins with anchorin CII antiserum. Chick chondrocyte plasma membranes were subjected to SDS electrophoresis on 5/18% polyacrylamide gradient gels, transferred to nitrocellulose paper, and incubated with Fab' fragments from rabbit preimmune serum (*a*), rabbit antiserum against chondrocyte membranes (*b*), rabbit antiserum against anchorin CII (*c*), and anti-anchorin CII Fab' fragments (*d*). Bound antibody was revealed by the peroxidase-staining procedure.

TABLE I

ELISA Test of Rabbit Antisera against Whole Chondrocyte Membranes and Purified Anchorin CII

Type of antiserum	Chondrocyte plasma membranes	Purified anchorin CII
Antichondrocyte membrane	11	4
Antianchorin CII	6	4
Affinity-purified antianchorin CII	4	5

Microtiter plates were coated with chondrocyte membrane protein or purified anchorin CII, and incubated with serial dilutions of antisera or antibodies. Bound antibody was determined with the peroxidase reaction as described in Materials and Methods and (28). Values are given as $-\log_2$ and represent 50% of the maximum color reaction in the ELISA.

move masking proteoglycans (31) and incubated with antisera to either anchorin CII or type II collagen. Whereas type II collagen was present throughout the entire cartilage matrix, anchorin CII was restricted to the chondrocyte-matrix interface (Fig. 3). Although the chondrocyte surface and the lacunar walls cannot be distinguished from each other at the level of the light microscope, these observations support the contention that anchorin CII is associated with the cell surface rather than the matrix.

Localization of Anchorin CII to the Surface of Isolated Chondrocytes

When released from cartilage with proteases and incubated in culture medium for 1 d, chondrocytes developed a dense pericellular matrix that stains intensely with antibodies to type II collagen (Fig. 4C). Such chondrocytes show a dense, punctate distribution of anchorin CII on their surface (Fig. 4, *A* and *B*), producing a peripheral halo of fluorescence typical of surface-associated antigens. Identical patterns were obtained by staining either with antiserum or affinity-purified antibodies to anchorin CII. The immunohistochemical demonstration of anchorin CII was enhanced by pretreatment of the cells with purified testicular hyaluronidase.

Removal of the pericellular matrix with trypsin results in a complete loss of surface-associated anchorin CII (not shown). It reappears on the cell surface after 1–2 h incubation in culture medium in a regular, punctate pattern (not shown). Recovery of anchorin CII on the cell surface was also observed when protein synthesis was blocked with cycloheximide during both enzymatic release from cartilage and recovery phase, suggesting the presence of intracellular pools of anchorin CII (Fig. 5*A*). When chondrocytes were stained with antibodies at 0°C, a punctate to regular distribution of anchorin CII was observed on the chondrocyte surface (Fig. 5*A*). Staining at 22°C or 37°C induced surface patching and capping of anchorin CII on matrix-free chondrocytes (Fig. 5, *B–F*) when reconstitution of a new pericellular matrix during antibody incubation was inhibited with collagenase and hyaluronidase. On matrix-enclosed chondrocytes, as shown in Fig. 4*C*, capping phenomena were not observed.

Distribution of Anchorin CII on Chondrocytes in Monolayer Culture

When plated into tissue culture dishes at medium to low cell density, chondrocytes spread on the substrate and shed their pericellular type II collagen matrix (Fig. 6). Flattened cells showed very little surface-associated type II collagen, although this collagen can be demonstrated intracellularly by staining of cells after fixation and drying (not shown; see 16–18). Extracellular staining of chondrocytes for anchorin CII after spreading demonstrated a reduction of the protein on the cell surface (Fig. 7, *A* and *B*). By focusing at different levels, anchorin CII could be located in a punctate fashion on both upper and lower surfaces of flattened cells (Fig. 7).

With progressive time in culture, the density of anchorin CII patches on the chondrocyte surface appeared to increase (Fig. 8). When cells regained a spherical morphology and reconstituted a pericellular matrix as indicated by the appearance of a highly refractile halo (Fig. 8, *D* and *F*), anchorin CII became nearly confluent at the cellular periphery (Fig. 8*E*), similar to that observed on chondrocytes in suspension culture (Fig. 4*A*). Anchorin CII was also prominent on cellular processes that interconnected chondrocytes within a colony (Figs. 7 and 8, arrows).

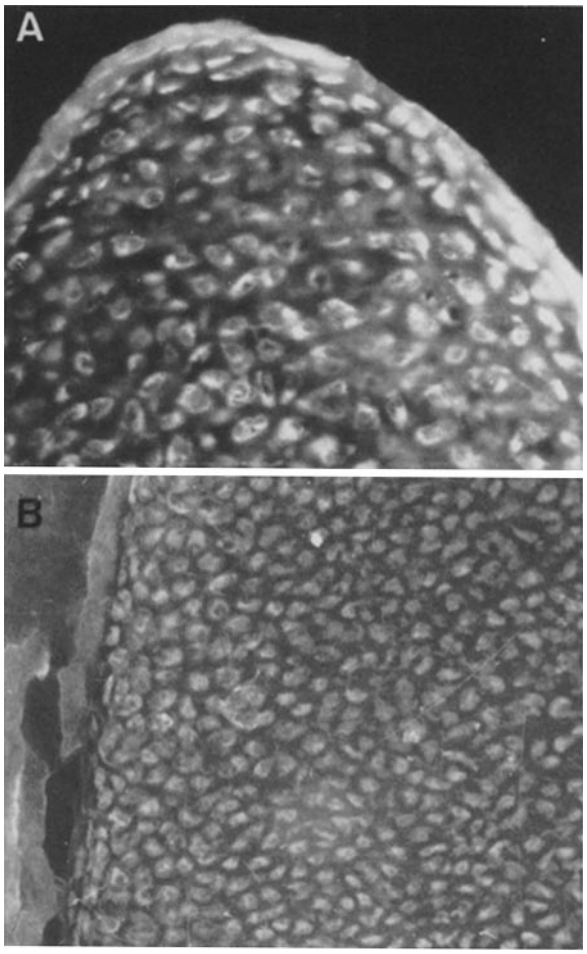

FIGURE 3 Immunofluorescent localization of anchorin CII in sections of 17-d embryonic chick sternal cartilage (*A*) and tibia (*B*). Stained with rabbit antiserum to anchorin CII, followed by rhodamin-conjugated goat anti-rabbit gamma globulin. Sections were pretreated with testicular hyaluronidase. (*A*) × 800; (*B*) × 600.

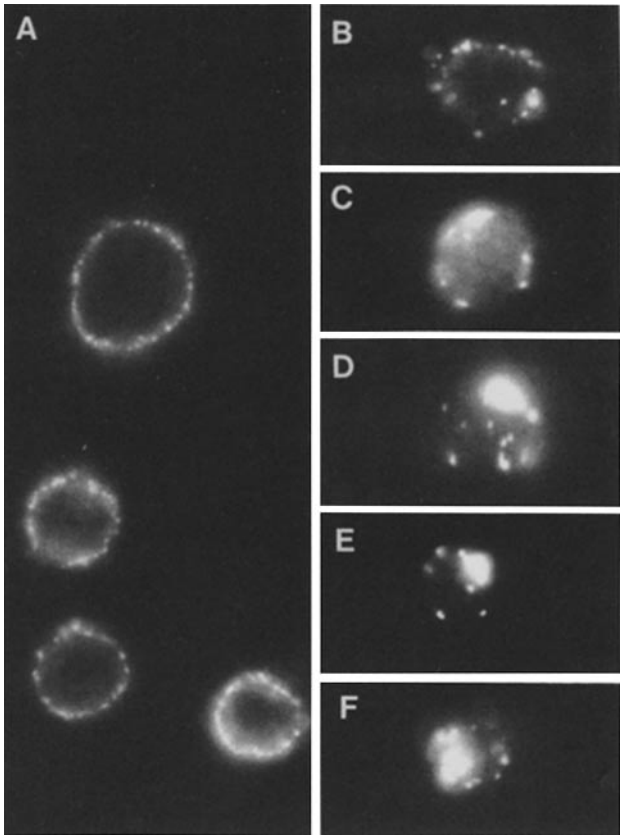

FIGURE 5 Capping and patching of anchorin CII on the surface of matrix free chondrocytes. Freshly isolated chondrocytes were incubated for 2 h with cycloheximide (25 μg/ml), purified bacterial collagenase (20 U/ml), and hyaluronidase (0.3 mg/ml), in serum-free F12 medium and stained unfixed with antiserum to anchorin CII at 0°C (*A*) or at 22°C (*B–F*). At 0°C anchorin is found in clusters spread regularly over the cell surface (*A*), whereas at 22°C capping (*C–F*) and irregular assembly (*B*) occurs on the cell surface. × 1,200.

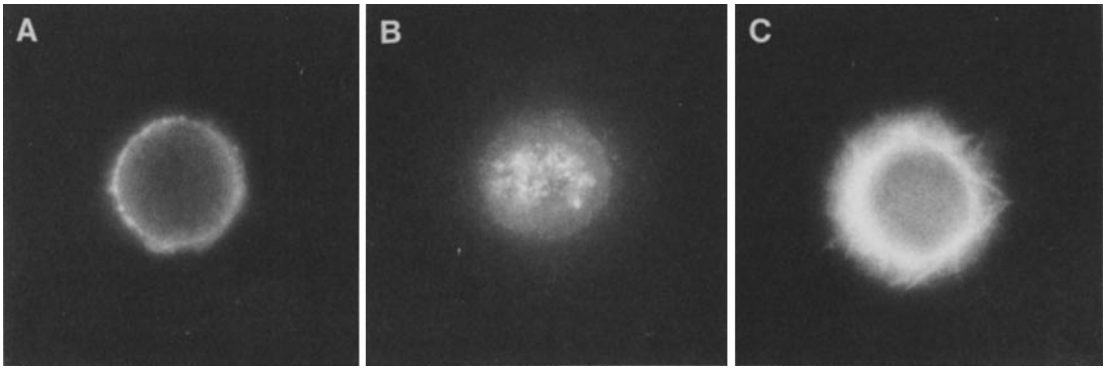

FIGURE 4 Immunofluorescent localization of anchorin CII and type II collagen on the chondrocyte surface. Chondrocytes were obtained from embryonic chick sternal cartilage by trypsin and collagenase digestion and allowed to reconstitute their pericellular matrix for 24 h in F12 medium with 10% fetal calf serum. Cells were stained unfixed at 0°C with anchorin CII antiserum (*A* and *B*) or rabbit antibodies to type II collagen (*C*), followed by rhodamin-conjugated goat anti-rabbit gamma globulin. (*A* and *B* are the same cell, focused at the periphery [*A*] and the top [*B*]). × 1,500.

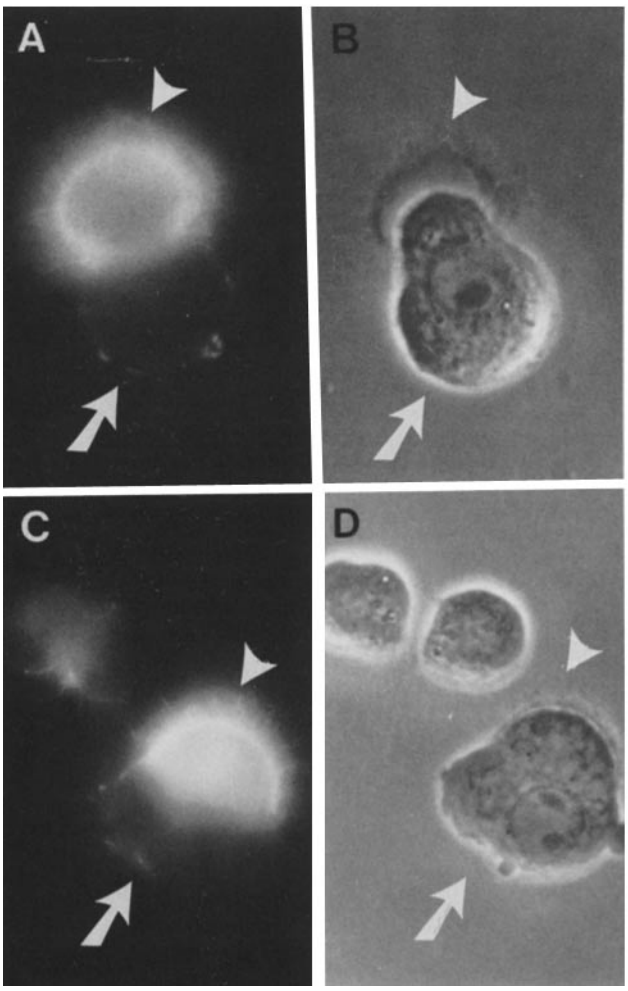

FIGURE 6 Shedding of the pericellular type II collagen matrix during spreading of chondrocytes in monolayer culture. Chondrocytes were plated onto tissue culture dishes in F12 medium with 10% fetal calf serum, and stained after 24 h unfixed for extracellular type II collagen as in Fig. 4C. The cell body itself which spreads on the substrate does not stain for type II collagen (arrows), while it leaves behind a cell "ghost" or glycocalyx (arrowheads) carrying the extracellular type II collagen matrix. *B* and *D* are phase contrasts of *A* and *C*, respectively. × 1,000.

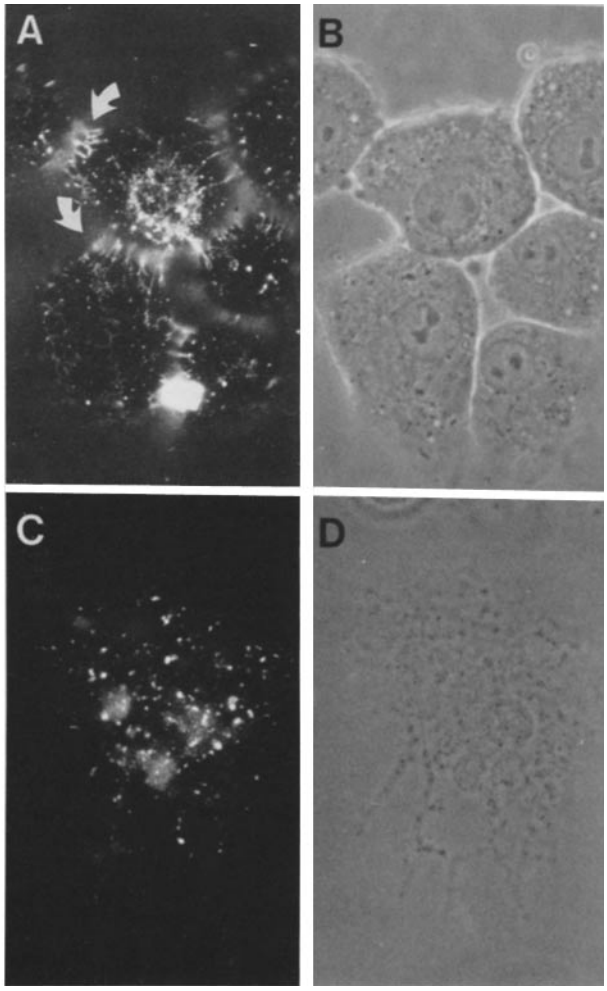

FIGURE 7 Distribution of anchorin CII on top (*A* and *B*) and bottom (*C* and *D*) of flattened chondrocytes. 6-d chondrocyte monolayer cultures were labeled unfixed with anti-anchorin CII at 0°C and photographed focusing on top or bottom of the cells. Note the pesence of anchorin CII also on intercellular processes (arrows). × 1,000.

Chondrocyte Attachment to Type II Collagen

The participation of anchorin CII in the interaction of chondrocytes with type II collagen was further investigated in cell attachment studies. Freshly isolated chondrocytes were labeled with ^{75}seleno-methionine, and allowed to attach to microtiter plates coated with type II collagen in the absence of serum. 1–2 h after plating, ~50% of the chondrocytes attached to the collagen substrate (Fig. 9). After achieving a near-plateau level after 1–2 h, the adhesion rate increased slowly, reaching levels of 90% after 24 h, perhaps due to either the synthesis of endogenous attachment factors or complete recovery of the chondrocyte surface from protease treatment.

Attachment to type II collagen could be inhibited with Fab' fragments prepared from anchorin CII antiserum in a concentration-dependent manner (Fig. 10). Inhibition >50% could not be achieved, even when an excess of antibodies was applied, indicating additional chondrocyte adhesion mechanisms. Similarly, chondrocyte attachment to type II collagen could be equally inhibited by using either an antiserum directed against whole chondrocyte membranes or Fab' fragments prepared from this (Fig. 11). The inhibition by both antianchorin CII and antichondrocyte membrane Fab' fragments was neutralized by preabsorption of the antibody fragments with anchorin CII coupled to Sepharose beads (Fig. 11).

DISCUSSION

From the work of several laboratories it has become increasingly evident that specific receptors for extracellular collagen exist upon connective tissue cells. Specific binding of collagen and collagen peptides has been shown for both fibroblasts (32–34) and hepatocytes (35). However, so far collagen-binding membrane receptors have been isolated only from membranes of nonconnective tissue cells such as platelets (36), spermatozoa (37), or bacteria (38). With the isolation of the collagen-binding protein anchorin CII from chondrocyte membranes it has become possible for the first time to study the molecular basis of the interaction of connective tissue

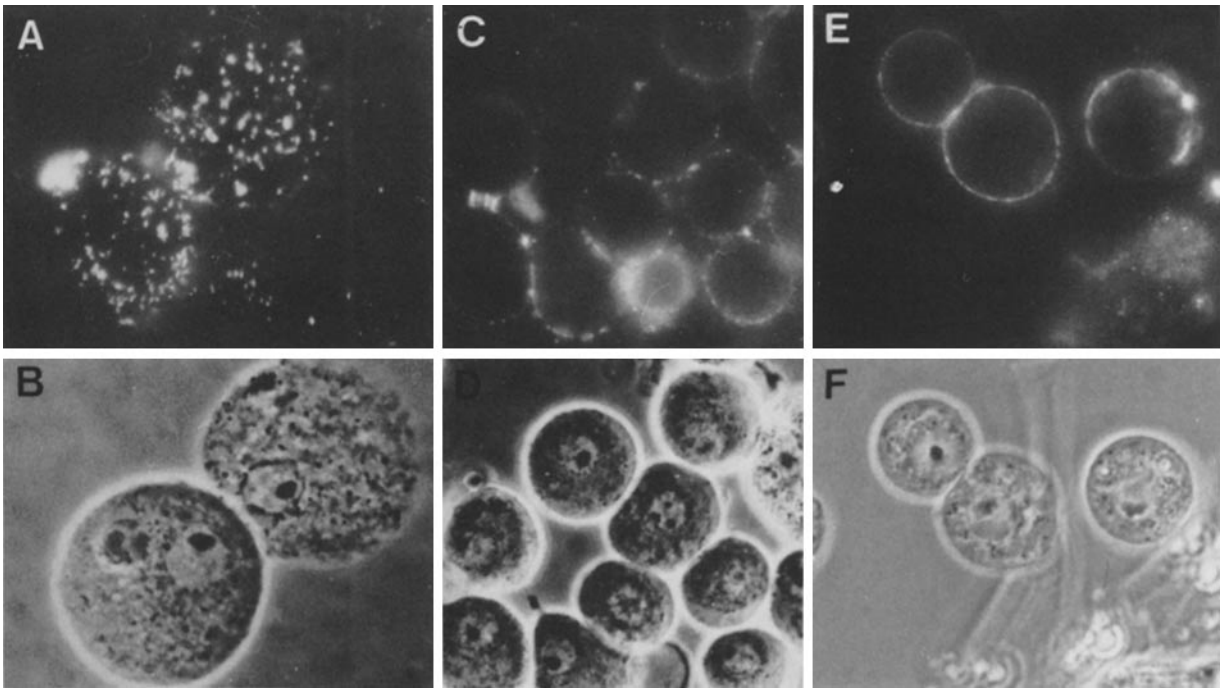

FIGURE 8 Increase of surface associated anchorin CII with reconstitution of the pericellular matrix in high density chondrocyte culture. 6-d high density chondrocyte cultures were stained with anti-anchorin CII at 0°C. Although anchorin CII occurs in patches on the cell surface (*A* and *C*), it may appear as a continuous layer at the periphery of cells that are surrounded by a refractile matrix (*E* and *F*). (*A* and *B*) focused on top (× 1,500); (*C–E*) focused at the periphery of cells. × 1,000.

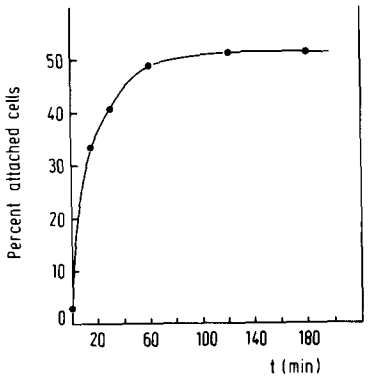

FIGURE 9 Time course of chondrocyte attachment to type II collagen. Freshly isolated chondrocytes were allowed to reconstitute cell surface protein for 1 h after enzymatic release from cartilage and labeled during this phase with ^{75}seleno-methionine. The labeled cells were washed and plated onto type II collagen-coated microtiter plates. Bound cells were counted in a gamma counter.

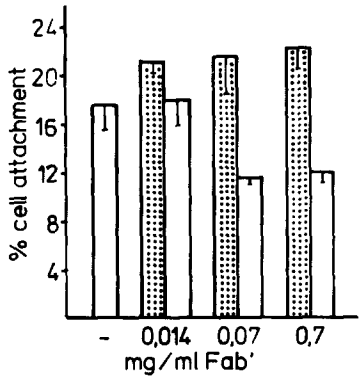

FIGURE 10 Inhibition of chondrocyte attachment to type II collagen. Prior to plating, chondrocytes were incubated with the indicated amounts of anti-anchorin CII Fab' fragments (open bars) or preimmune Fab' fragments (dotted bars). The first bar represents the control experiment in the absence of Fab' fragments.

cells with their extracellular matrix. The collagen-binding component of chondrocytes, anchorin CII, is a fucose-containing glycoprotein of 31,000-mol wt and exhibits properties of an integral membrane protein. This notion is supported by the observation of capping and patching on the surface of matrix-free chondrocytes, induced by antibodies at elevated temperatures. Anchorin CII binds to native type II collagen both in detergent-solubilized and liposome-intercalated form (22). However, only in the liposome-inserted form the binding is specific to type II collagen (39), whereas detergent-solubilized anchorin CII exhibited equal affinity to collagen types I, II, and III, (1α, 2α, 3α), V, and M collagen (J. Mollenhauer and K. von der Mark, in preparation). Furthermore, the binding of anchorin CII to type II collagen is resistant to higher salt concentrations in liposome-inserted form than in detergent-solubilized form (22).

On the surface of suspended chondrocytes surrounded by a pericellular type II collagen matrix, anchorin CII appeared in a punctate, dense-to-confluent distribution. Such chondrocytes did not exhibit patching and capping in the presence of antibodies to anchorin CII, probably due to immobilization of the collagen-binding sites by the pericellular matrix.

In monolayer culture chondrocytes shed their pericellular matrix during spreading. This phenomenon may be explained by the utilization of anchorin CII for nonspecific adhesion to the tissue culture dish. This may lead to a dilution of collagen-binding sites on the cell surface, insufficient for anchoring the pericellular matrix to the cell. Utilization of distinct cell surface proteins for attachment to tissue culture plastic has been demonstrated for a focal adhesion protein, FC1, from fibroblast membranes (41).

As chondrocytes reconstitute their pericellular matrix with progressive time in monolayer culture, the intensity of anchorin CII foci on the surface appeared to increase. Although

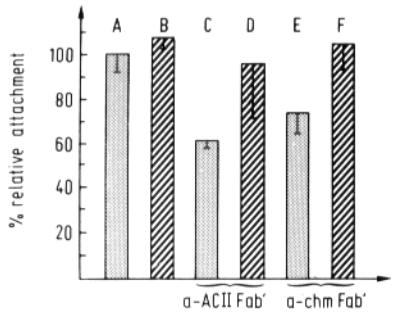

FIGURE 11 Neutralization of attachment inhibition by anti-anchorin CII Fab' fragments with purified anchorin CII: attachment conditions as in Fig. 10. Attachment rate to type II collagen for 1 h in the absence of Fab' fragments was set at 100% (*A*). (*B*) Attachment in the presence of preimmune Fab' fragments. (*C*) Attachment in the presence of 0.7 mg/ml anti-anchorin CII Fab' fragments. (*D*) As in *C*, with anti-anchorin CII Fab' fragments preabsorbed with anchorin CII sepharose. (*E*) Attachment in the presence of antichondrocyte membrane Fab' fragments (0.7 mg/ml). (*F*) As in *E*, with antichondrocyte membrane Fab' fragments preabsorbed on anchorin CII sepharose.

this observation will have to be confirmed by more quantitative methods, it suggests that the formation of the pericellular matrix of the chondrocytes may be influenced by the surface density of anchorin CII.

The role of anchorin CII as the major anchorage site for chondrocytes in their collagen matrix is substantiated by the ability of antianchorin CII Fab' fragments to reduce the rate of attachment of isolated chondrocytes to type II collagen. However, attachment could not be completely inhibited even by an excess of Fab' fragments. Interestingly, Fab' fragments from a whole chondrocyte membrane antiserum also inhibited attachment by a maximum of 50%.

This suggests that alternative mechanisms for the adhesion of chondrocytes to collagen other than mediated by anchorin CII seem to exist. In studies by Hewitt et al. (20, 21) the attachment of chondrocytes to collagen substrates was enhanced by the serum-derived protein chondronectin. Although immunohistochemical studies indicate the presence of chondronectin within chondrocytes (21), chondronectin appears to be a soluble, extracellular adhesion molecule rather than a component of the chondrocyte surface. Since anchorin CII enables attachment of chondrocytes to type II collagen in the absence of serum, and thus of exogenous chondronectin, we conclude that direct interaction between chondrocytes and native type II collagen occurs through anchorin CII.

Other adhesion mechanisms may include cell surface proteoglycans such as membrane-intercalated heparan sulfate (40), although this component has not yet been identified on chondrocytes. Nonspecific hydrophilic and hydrophobic interactions between the cell surface and artificial substrates should also be considered.

Evidence is available for the existence of membrane intercalated matrix receptors similar to anchorin CII on the surface of other cells. For instance, laminin-binding proteins of molecular weight 68,000–70,000 have been isolated from the membranes of tumor cells (42, 43) and skeletal muscle cells (44). A collagen-binding protein of the same molecular weight was found on the surface of platelets (36). Such matrix receptors may be utilized for the adhesion, migration, and/or anchorage of cells to the extracellular matrix.

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