Expression of α and β subunits of the integrin superfamily in articular cartilage from macroscopically normal and osteoarthritic human femoral heads

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Abstract

Objective—The objective of this study was to detail the topographical and zonal distribution of α and β subunits of the integrin superfamily in normal and osteoarthritic cartilage.

Methods—Immunohistochemistry utilising antibodies towards α and β subunits was performed on cryostat sections of human articular cartilage from macroscopically normal ($n = 6$) and osteoarthritic ($n = 6$) femoral heads. Samples of articular cartilage were obtained from 12 topographically distinct sites from each femoral head. Each section was divided into zones (superficial, middle, deep) and staining scores were recorded.

Results—Normal cartilage stained for integrin subunits $\alpha 1$, $\alpha 5$, αV , $\beta 1$, $\beta 4$, and $\beta 5$, but not for $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 6$, $\beta 2$, $\beta 3$, and $\beta 6$. Intact and non-intact residual cartilage from osteoarthritic femoral heads stained for $\alpha 1$, $\alpha 2$, $\alpha 5$, αV , $\beta 1$, $\beta 4$, and $\beta 5$. Staining was occasionally seen for $\alpha 4$ and $\beta 2$, but not for $\alpha 3$, $\alpha 6$, $\beta 3$, and $\beta 6$. There was no topographical variation in the staining for any of the subunits in either normal or osteoarthritic cartilage. The only subunit that displayed a zonal variation was αV ; staining for this subunit was most pronounced in the superficial zone compared with the middle and deep zones.

Conclusion—Chondrocytes in normal and osteoarthritic cartilage express the integrin subunits $\alpha 1$, $\alpha 5$, αV , $\beta 1$, $\beta 4$, and $\beta 5$. Chondrocytes in osteoarthritic cartilage, in addition, express the $\alpha 2$, $\alpha 4$, and $\beta 2$ subunits. The αV subunit is expressed by more chondrocytes in the superficial zone in comparison with cells in the deeper zones. None of the subunits display topographical variation in expression.

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differentiation (CD) 44 of the hyaluronan binding protein family,^{5,6} intercellular adhesion molecule (ICAM) -1 of the immunoglobulin superfamily,^{7,8} and members of the integrin superfamily.^{9–11} In normal articular cartilage from femoral condyles, tibial plateaus, and taluses the integrin heterodimers $\alpha 1\beta 1$, $\alpha 5\beta 1$, and $\alpha V\beta 5$ are expressed strongly and the heterodimers $\alpha 3\beta 1$ and $\alpha V\beta 3$ weakly.^{9–11} The subunits $\alpha 2$ and $\alpha 6$ have been demonstrated on fetal articular chondrocytes.^{10,12} Potential ligands of integrins expressed by articular cartilage chondrocytes such as collagens, fibronectin, vitronectin, thrombospondin, and laminin are prominent components of the pericellular matrix.^{13,14}

In normal human femoral head articular cartilage, thickness and composition vary with topography.^{15,16} In addition, the morphology of chondrocytes, cellularity, and distribution of extracellular matrix components vary with depth.^{17–20} In osteoarthritic cartilage, qualitative alteration of the collagen content,^{21,22} pericellular loss of collagen type II,²³ neoexpression of collagen types I and III,^{24–26} increased expression of collagen type VI,²⁷ IX,²⁸ and X,^{23,29} and increased levels of fibronectin^{30,31} have been observed. The αV subunit containing integrins have been immunolocalised preferentially to the superficial zone of normal articular cartilage.⁹ However, it has not been reported whether the expression of integrins also reflects the topographical heterogeneity and variation in composition of normal articular cartilage, and the expression of integrins in osteoarthritic cartilage has not been detailed.

The objective of this study was, therefore, to detail the topographical and zonal distribution of α and β subunits of the integrin superfamily in normal and osteoarthritic cartilage from human femoral heads.

Methods

NORMAL CARTILAGE

Samples of macroscopically normal articular cartilage (Collins/McElligott grade 0)³² were obtained at necropsy within 24 hours of death from two women (age 66 and 82 years) and four men (median age 73 years, range 45–88). None of the subjects had a clinical history of arthritis or chronic systemic inflammatory disease.

Samples for cryostat sections were collected from anterior, posterior, lateral, and medial aspects of perifoveal, central, and peripheral

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Table 1 Primary antibodies

Specificity	Clone number	Source	Dilution
$\alpha 1$	TS2/7	Dr F Sanchez-Madrid, Universidad Aut6noma, Madrid, Spain	1:15
$\alpha 2$	P1E6	Life Technologies, Paisley, UK	1:1000
$\alpha 3$	P1B5	Becton Dickinson, San Jose, CA, USA	1:1000
$\alpha 4$	P4G9	Biogenesis, Poole, UK	1:1000
$\alpha 5$	BIIG2	Dr C Damsky, University of California, San Francisco, CA, USA	1:100
$\alpha 6$	GOH3	Serotec, Kidlington, Oxford, UK	1:1000
αV	VNR147	Chemicon International, Danvers, MA, USA	1:2000
$\beta 1$	TS2/16	Dr F Sanchez-Madrid, Universidad Aut6noma, Madrid, Spain	1:1000
$\beta 2$	MHM23	DAKO, Glostrup, Denmark	1:200
$\beta 3$	Y2/51	DAKO, Glostrup, Denmark	1:200
$\beta 4$	3E1	Chemicon International, Danvers, MA, USA	1:1000
$\alpha V\beta 5$	P1F6	Life Technologies, Paisley, UK	1:1000
$\beta 6$	E7P6G10P0	Dr D Sheppard, University of California, San Francisco, CA, USA	1:1000

areas of femoral heads. A total of 12 triangular ($5 \times 5 \times 5$ mm) full thickness samples of articular cartilage were collected from each femoral head, snap frozen in liquid nitrogen, and stored in cryovials at -80°C . The samples were later cut in $4\ \mu\text{m}$ thick cryostat sections and mounted on SuperFrost/Plus glass slides (Eire Scientific, Portsmouth, NH, USA) followed by fixation with acetone for 10 minutes.

OSTEOARTHRITIC CARTILAGE

Samples of femoral head articular cartilage were obtained from four women (median age 78 years, range 77–85) and two men (age 68 and 71 years) undergoing replacement surgery for osteoarthritis of the hip. All femoral heads contained areas of denuded bone. The overall Collins/McElligott³² grades of osteoarthritis were III and IV. The samples included a spectrum of histological changes; intact cartilage with all zones preserved, non-intact cartilage showing fibrillation and cluster formation, and non-intact cartilage in which the superficial and middle zones were absent and only part of the deep zone remained. A total of up to 12 samples of residual articular cartilage were collected and processed as above.

All samples from both normal and osteoarthritic femoral heads were obtained from white subjects.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was carried out using a Shandon Sequenza (Life Science International, Basingstoke, UK) to achieve consistency of staining quality and to prevent sections from floating off.

The immunohistochemical staining procedure has been described previously.^{6,11} In brief: non-specific background staining was blocked by incubating the sections for 20 minutes with $100\ \mu\text{l}$ of normal rabbit serum (DAKO, Glostrup, Denmark) in TRIS buffered saline (TBS) (1:5). The primary antibody ($100\ \mu\text{l}$) was diluted in normal rabbit serum in TBS and allowed to incubate for 30 minutes at room temperature. After washing twice in $2\ \text{ml}$ of TBS for five minutes, $100\ \mu\text{l}$ biotinylated rabbit antimouse immunoglobulins (DAKO) diluted 1:400 in normal rabbit serum in TBS was added for 30 minutes, followed by another wash. Antibody binding was visualised with an avidin and biotinylated horseradish peroxidase

complex ($100\ \mu\text{l}$) (DAKO), which was diluted according to the DAKO protocol and incubated for 30 minutes, followed by washing and $100\ \mu\text{l}$ of 3-amino-9-ethylcarbazole (Sigma, St Louis, MO, USA) for 20 minutes. After another wash, the sections were counterstained with Mayer's haematoxylin, washed again, and mounted with coverslips.

Table 1 shows the primary antibodies used in this study.

PROCEDURE CONTROLS

Negative controls were provided by omitting the primary antibody and also by substituting non-immune mouse immunoglobulins (DAKO) for the primary antibody. No attempt was made to block for endogenous peroxidase as no endogenous peroxidase activity was seen in the negative controls. External positive tissue controls included sections of normal human tissue from skin and tonsils. Internal tissue controls were present in many of the sections; integrin positive cells include osteocytes ($\alpha 4$, $\alpha 5$, αV , $\beta 1$), osteoblasts ($\alpha 4$, $\alpha 5$, αV , $\beta 1$), osteoclasts ($\alpha 2$, $\alpha 5$, αV , $\beta 1$, $\beta 3$), fibroblasts ($\alpha 2$, $\alpha 5$, $\beta 1$), and endothelial cells ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, αV , $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\beta 5$).^{13,33}

To assess if the accessibility of integrin subunit epitopes to antibodies was hindered by extracellular matrix interactions, sections were treated with hyaluronidase ($10\ \text{mg/ml}$) (Sigma), collagenase ($10\ \text{mg/ml}$) (Sigma), or a combination thereof (hyaluronidase $2\ \text{mg/ml}$; collagenase $10\ \text{mg/ml}$) for 5–30 minutes at 37°C before the standard immunohistochemical staining.

MICROSCOPIC EVALUATION

As the samples for cryostat sections were all triangularly shaped in their horizontal dimension, a section of intact cartilage sampled and cut correctly would be rectangular in shape in the vertical dimension. Only sections of intact cartilage that clearly displayed the correct vertical orientation and thus contained an articular surface and calcified cartilage with a tidemark were considered for further examination. Also considered for examination were sections of non-intact residual cartilage from osteoarthritic joints that contained calcified cartilage with a tidemark. Only the deep zone or whatever part of the deep zone present (that is, the zone adjacent to the tidemark) was considered for examination in these sections.

The full section of intact cartilage from articular surface to tidemark was divided into quarters, as described previously.⁶ The quarter of the full section of cartilage that includes the surface was named the superficial zone. The half of the full section of cartilage below the superficial zone was named the middle zone. The quarter of the full section of cartilage below the middle zone and above the tidemark was named the deep zone. The calcified zone of articular cartilage was not included in the evaluation. Occasionally, the superficial zone was covered by a fibrous pannus, which was not included in the zonal division of the section.

Integrin staining for each zone was scored as: 4 = all chondrocytes positive; 3 = more positive

Table 2 Integrin subunits in articular cartilage from macroscopically normal femoral heads

Integrin subunit (number of sections stained)	Median score (range)
$\alpha 1$ (59)	3 (0–3)
$\alpha 2$ (71)	0 (–)
$\alpha 3$ (66)	0 (–)
$\alpha 4$ (71)	0 (–)
$\alpha 5$ (52)	2 (0–4)
$\alpha 6$ (72)	0 (–)
αV (58)	3 (1–4) superficial 2.5 (1–3) middle 2 (0–3) deep
$\beta 1$ (48)	2 (0–3)
$\beta 2$ (60)	0 (–)
$\beta 3$ (66)	0 (–)
$\beta 4$ (53)	0 (0–1)
$\alpha V\beta 5$ (65)	2 (1–3)
$\beta 6$ (72)	0 (–)

All topographical sites and zones of 4–6 subjects were scored together except for αV subunit where the score is given for the superficial, middle, and deep zone individually. Scores: 0 = no positive chondrocytes; 1 = more negative than positive chondrocytes; 2 = positive and negative chondrocytes of approximately equal number; 3 = more positive than negative chondrocytes; 4 all chondrocytes positive.

Table 3 Zonal distribution of αV subunit in articular cartilage from macroscopically normal femoral heads

Score	0	1	2	3	4
Superficial zone	0	5	11	39	3
Middle zone	0	6	20	32	0
Deep zone	2	13	19	24	0

Numbers for the three zones represent sections (58 in total from six subjects) which have received a score. Scores: see table 2.

than negative chondrocytes; 2 = positive and negative chondrocytes of approximately equal number; 1 = more negative than positive chondrocytes; 0 = all chondrocytes negative. Positive staining was recognised as a dark red colour associated with the cytoplasmic membrane or cytoplasm, or both. As chondrocytes were either strongly positive or exhibited no staining at all no grading of the positivity was made. Areas of sections that contained folding of the cartilage or exhibited non-specific background staining were not scored. Evaluation was carried out by two investigators and the scores given were agreed upon.

ETHICS

The study was approved by the regional ethics committee as part of a larger project involving articular cartilage and bone. Written information about the project was presented to patients scheduled for surgery and patient consent was obtained in these cases.

STATISTICS

The Friedman test was used to test the null hypothesis that there is no systematic topographical or zonal pattern of immunohistochemical scores in normal or osteoarthritic cartilage. Tests for systematic topographical pattern were performed for each zone for all normal femoral heads. Tests were not performed for osteoarthritic femoral heads because of insufficient numbers of available sections and, hence, data to compute. Tests for systematic zonal pattern were performed for the superficial zone versus the deep zone for each normal and osteoarthritic femoral head. The Mann-Whitney U test was used to test the

Figure 1 Articular cartilage stained for the αV integrin subunit. (a) The majority of chondrocytes are αV positive in the superficial zone of articular cartilage from a macroscopically normal femoral head. (b) Both αV positive and αV negative chondrocytes in the deep zone of articular cartilage from a macroscopically normal femoral head. (c) A cluster containing both αV positive and αV negative chondrocytes in non-intact articular cartilage from an osteoarthritic femoral head; the cartilage contains several clefts. Immunohistochemistry with an immunoperoxidase technique and haematoxylin counterstaining was used (bar marker: 25 μm).

null hypothesis that there is no systematic difference between immunohistochemical scores in normal and osteoarthritic cartilage. The limit of significance was chosen as 0.05.

Results

NORMAL CARTILAGE

Forty eight to 70 sections of the possible 72 sections of cartilage from the six macroscopically normal femoral heads stained for the various α and β subunits were available for evaluation (table 2). Sections were excluded for evaluation because the tidemark was not included or because of excessive non-specific background staining.

Table 2 presents a summary of results for all integrin subunits for articular cartilage from macroscopically normal femoral heads. There was no systematic pattern in the staining with respect to topographical site for any of the

Table 4 Integrin subunits in intact and non-intact residual articular cartilage from osteoarthritic femoral heads

Integrin subunit (number of sections stained)	Intact median score (range)	Non-intact median score (range)
$\alpha 1$ (41)	2 (0–3)	2 (1–3)
$\alpha 2$ (51)	1 (0–2)	1 (1–2)
$\alpha 3$ (47)	0 (–)	0 (–)
$\alpha 4$ (50)	0 (0–1)*	0 (0–1)*
$\alpha 5$ (48)	2 (0–3)	2 (1–3)
$\alpha 6$ (55)	0 (–)	0 (–)
αV (35)	3 (2–3) superficial 2 (1–3) middle 2 (1–3) deep	1 (1–3)
$\beta 1$ (47)	2 (1–3)	3 (1–3)
$\beta 2$ (52)	0 (0–1)*	0 (0–1)*
$\beta 3$ (54)	0 (–)	0 (–)
$\beta 4$ (48)	1 (0–3)	1 (0–2)
$\alpha V\beta 5$ (45)	2 (1–3)	1 (1–3)
$\beta 6$ (55)	0 (–)	0 (–)

All topographical sites and zones of 4–6 subjects were scored together except for αV subunit where the score is given for the superficial, middle, and deep zone individually. * = Cytoplasmic reaction in occasional chondrocytes. Scores: see table 2.

integrin subunits ($p > 0.05$). Only the αV subunit displayed a systematic pattern in the zonal staining (see below). Occasional $\beta 4$ positive chondrocytes were seen throughout the zones in approximately one third of the sections.

Table 3 presents the zonal distribution of αV in articular cartilage from macroscopically normal femoral heads. All 12 topographical sites had a decreased number of αV positive chondrocytes in the middle and deep zones compared with the superficial zone (fig 1). This zonal pattern was seen in 48% of sections and this represented a statistically significant difference for the superficial zone versus the deep zone for all femoral heads ($p \leq 0.05$). In only one section did chondrocytes in the superficial zone stain to a lesser degree for αV than cells in the middle and deep zones.

OSTEOARTHRITIC CARTILAGE

Thirty five to 55 sections of the possible 72 sections of cartilage from the six osteoarthritic femoral heads stained for the various α and β subunits were available for evaluation (table 4). Sections were excluded for evaluation because correct orientation could not be determined, no articular cartilage was present in the section, or because of excessive non-specific background staining.

Table 4 presents a summary of results for all integrin subunits for intact and non-intact residual articular cartilage from osteoarthritic femoral heads. The median score for all topographical sites and zones combined is shown with ranges. There was no overt variation in the staining pattern with respect to topographical site for any of the integrin subunits. Only the αV subunit displayed a systematic pattern in the staining with respect to zone (see below) (fig 1). In contrast with chondrocytes in normal articular cartilage, all sections except one contained occasionally $\alpha 2$ positive chondrocytes (fig 2). Occasional $\beta 4$ positive chondrocytes were seen throughout the zones in approximately half the sections. In approximately one third of sections occasional chondrocytes stained for $\alpha 4$. In approximately

Figure 2 Articular cartilage stained for the $\alpha 2$ integrin subunit. (a) $\alpha 2$ negative chondrocytes in the superficial zone of articular cartilage from a macroscopically normal femoral head. (b) A cluster containing both $\alpha 2$ positive and $\alpha 2$ negative chondrocytes in non-intact articular cartilage from an osteoarthritic femoral head. Immunohistochemistry with an immunoperoxidase technique and haematoxylin counterstaining was used (bar marker: 25 μm).

Table 5 Zonal distribution of αV in intact and non-intact residual articular cartilage from osteoarthritic femoral heads

Score	0	1	2	3	4
Intact residual cartilage					
Superficial zone	0	0	9	21	0
Middle zone	0	5	14	11	0
Deep zone	0	12	8	10	0
Non-intact residual cartilage					
Deep zone	0	4	0	1	0

Numbers for the three zones represent sections (30 for intact and five for non-intact residual articular cartilage from six patients) which have received a score. Scores: see table 2.

10% of sections occasional chondrocytes stained for $\beta 2$.

The differences in immunohistochemical staining scores between normal and osteoarthritic cartilage was not statistically significant for the various α and β subunits ($p > 0.05$) except for the $\alpha 2$ and $\alpha 4$ subunits ($p \leq 0.05$).

Table 5 presents the zonal distribution of αV in intact and non-intact residual articular cartilage from osteoarthritic femoral heads. The zonal pattern observed for normal cartilage was seen in 63% of sections and was statistically significant for the superficial zone compared with the deep zone for all femoral heads ($p \leq 0.05$). In the remaining sections, no zonal variation was observed.

Chondrocytes in clusters and in areas of excessive surface fibrillation showed a similar pattern of integrin subunit staining as isolated chondrocytes and chondrocytes in other areas of the same section.

The pre-treatment of sections with hyaluronidase, collagenase, or a combination thereof before the standard immunohistochemical

staining did not reveal additional integrin subunit staining.

Discussion

This study confirmed the presence of integrin subunits $\alpha 1$, $\alpha 5$, αV , $\beta 1$, $\beta 4$, and $\beta 5$ and the absence of subunits $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 6$, $\beta 2$, $\beta 3$, and $\beta 6$ in normal human articular cartilage.^{9–11} The $\alpha 3$ subunit was absent in this study, but Salter *et al.*¹¹ found occasional $\alpha 3$ expression in all zones in femoral condylar and tibial plateaus cartilage. Woods *et al.*⁹ could not detect the $\alpha 3$ subunit utilising immunohistochemistry, but these authors⁹ as well as Yonezawa *et al.*³⁴ confirmed the presence of this subunit by flow cytometric analysis. In this study, the $\beta 3$ subunit was also absent, confirming studies performed with immunohistochemistry^{9–11} and flow cytometric analysis.^{9–34} However, Woods *et al.*⁹ detected the heterodimer $\alpha V\beta 3$ on chondrocytes within the most superficial 30 μm of normal articular cartilage. Our detection of the $\beta 4$ subunit in occasional chondrocytes, but not the $\alpha 6$ subunit of the $\alpha 6\beta 4$ heterodimer may represent a similar instance.

To our knowledge, this is the first study detailing the topographical and zonal distribution of integrins in osteoarthritic cartilage. In residual articular cartilage from osteoarthritic human femoral heads, chondrocytes expressed $\alpha 1$, $\alpha 5$, αV , $\beta 1$, $\beta 4$, and $\beta 5$. In addition, we found neo-expression of $\alpha 2$, $\alpha 4$, and $\beta 2$ in osteoarthritic cartilage. No staining was noted for $\alpha 3$, $\alpha 6$, $\beta 3$, and $\beta 6$. Jobanputra *et al.*³⁵ did not detect the subunits $\alpha 2$ and $\beta 4$, but did detect the $\alpha 3$ subunit in articular cartilage from the medial tibial plateaus of patients with osteoarthritis secondary to rheumatoid arthritis. We believe these differences in results reflect the differences in the selected material. In extracted chondrocytes from OA knee joints, the subunits $\alpha 1$ – $\alpha 6$, αV , and $\beta 1$ were all detected by flow cytometric analysis.³⁶ The differences in results obtained in studies using immunohistochemistry or flow cytometric analysis may reflect either (1) masking of integrin subunits in cartilage sections eliminated in the process of chondrocyte extraction before flow cytometric analysis or (2) neoexpression of integrin subunits in response to the extraction process.

There was no topographical variation in the expression of subunits in either normal or osteoarthritic cartilage. The only subunit that displayed a zonal variation was αV . Minor variations in the expression of subunits with respect to topography or zones may have been missed because of the rather crude immunoscore system utilised in this study. Interobserver and intraobserver reproducibilities of immunoscore are rather low (unpublished data), however, the chosen immunoscore system was found to be reproducible.

We have not attempted to correlate the integrin staining in residual cartilage from osteoarthritic femoral heads with a histopathological score obtainable through a grading system such as the histological-histochemical grading system for osteoarthritic articular cartilage³⁷ or modifications thereof.^{38–39} Recently, we have demonstrated low intraobserver

and interobserver reproducibilities and questionable validity of the original histological-histochemical grading system for osteoarthritic articular cartilage.⁴⁰

The OA articular cartilage selected for this study has been obtained from femoral heads graded III or IV according to the Collins/McElligott system³² and, hence, represents end stage disease at least in some areas. The OA process in the non-intact residual cartilage of grade III and IV joints may thus be different from the OA process taking place in the non-intact residual cartilage of grade I and II joints. Unfortunately, without a reliable histopathological grading system it is difficult to obtain human articular cartilage representative of the different stages of the OA process. Animal studies may further elucidate the expression of α and β subunits of the integrin superfamily during the different OA stages.

From the integrin subunits detected in this and previous^{9–11} studies, it is possible to deduce the presence of the following integrin heterodimers in normal and OA human articular cartilage: $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 4\beta 1$, $\alpha 5\beta 1$, $\alpha V\beta 1$, $\alpha V\beta 3$, $\alpha 6\beta 4$, and $\alpha V\beta 5$. The $\beta 2$ subunit may associate with the αL , αM , and αX subunits. The heterodimers $\alpha 1\beta 1$ and $\alpha 2\beta 1$ are known receptors for collagens and laminin; $\alpha 3\beta 1$ for collagens, fibronectin, and laminin; $\alpha 4\beta 1$ for fibronectin, chondroitin sulphate, and VCAM (vascular cell adhesion molecule)-1; $\alpha 5\beta 1$ for fibronectin; $\alpha V\beta 1$ for vitronectin and fibrinectin; $\alpha V\beta 3$ for collagens, fibronectin, vitronectin, thrombospondin, and laminin; $\alpha 6\beta 4$ for laminin; $\alpha V\beta 5$ for vitronectin; $\alpha L\beta 2$ for ICAM-1/2/3; $\alpha M\beta 2$ for ICAM-1, fibrinogen, factor X, and complement factor iC3b; and $\alpha X\beta 2$ for fibrinogen, denatured albumin and complement factor iC3b.^{13–14–41}

It is as yet unclear which extracellular matrix molecules human articular chondrocytes adhere to. Studies of the distribution of possible ligands such as fibronectin³¹ would suggest potential interaction. Such functional relations based upon codistribution is at best indicative and additional, possibly in vitro, studies are needed to unravel the precise role of integrin subunits in articular cartilage.

The significance of the neoexpression of $\alpha 2$, $\alpha 4$, and $\beta 2$ in residual cartilage from osteoarthritic femoral heads remains to be elucidated. The $\alpha 2$ subunit is strongly expressed in fetal cartilage¹⁰ and the neoexpression in osteoarthritic cartilage of this subunit may represent an example of “fetal phenotype” expression in osteoarthritic cartilage potentially as a functional response to production of collagen type I and III.²⁶

In conclusion, our study demonstrates the expression of $\alpha 1$, $\alpha 5$, αV , $\beta 1$, $\beta 4$, and $\beta 5$ in normal and osteoarthritic cartilage. In osteoarthritic cartilage, neoexpression of $\alpha 2$, $\alpha 4$, and $\beta 2$ is observed. There is no topographical variation in the expression of subunits in either normal or osteoarthritic cartilage. The only subunit that displays a zonal variation is αV ; more chondrocytes express αV in the superficial zone compared with the deep zone.

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