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Review

Articular cartilage and osteoarthrosis. The role of molecular markers to monitor breakdown, repair and disease*

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INTRODUCTION

Osteoarthrosis (osteoarthritis, arthrosis, OA) represents a considerable burden both to the individual patient and society: in 1988 in the USA the total cost of arthritis was estimated at $54.6 billion, most of which was due to OA (Kramer et al. 1983). More than 500000 arthroplasty procedures are performed in the USA annually. Arthritis is the leading chronic condition reported by the elderly; it is reported by almost 50% of persons aged 65 y and older (Praemer et al. 1992) and more than one third are limited in 5 or more physical activities (Yelin, 1992). Since reports on OA epidemiology consistently show an almost exponential increase of prevalence with increasing age (Danielsson et al. 1984; Felson et al. 1987, 1988; Lawrence et al. 1989; Badley & Tennant, 1992; Steven, 1992; Yelin, 1992), the rapidly changing demographic structure in many countries will in the future only emphasise the magnitude of this problem.

OA is not a single disease entity but a common end-stage of cartilage and joint failure (Fig. 1). Inherent in this definition is a multifactorial pathogenesis with many recognised risk factors. Of the endogenous risk factors, age, sex, race and inherited susceptibility have all been shown to play a role, but with different weights at different joint sites. The rare single base mutation of 519 arg→cys in the human type II procollagen gene, which has been shown to cause mild chondrodysplasia and early-onset OA, represents one example of a defined inherited susceptibility (Ala-Kokko et al. 1990; Katzenstein et al. 1990). Exogenous factors such as obesity, use and abuse of joints and abnormal joint shape are also proven risks, again with different weighting factors for different sites, sex and age (Felson 1988, 1993; Felson et al. 1988, 1992). Risk factors are probably additive; for example, patients with a presumed systemic risk factor shown by the development of distal interphalangeal OA had an increased risk of developing OA in the knee after meniscectomy (Doherty & Dieppe, 1983). There is good evidence for the association of occupational risk factors with OA. For example, farmers and soccer players have an increased relative risk of hip OA, and excessive knee loading is

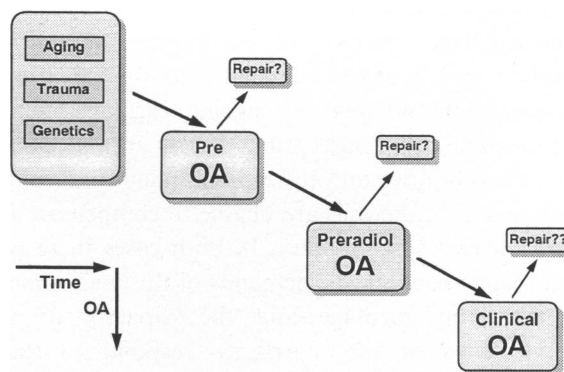

Fig. 1. The progress of osteoarthritis takes place over a time period of years and sometimes decades. In the population, risk factors such as ageing, trauma, genetics may in some individuals initiate a process which leads to changes in the metabolism and composition of the joint cartilage, 'PreOA'. Continued exposure to the same or other risk factors may in some cause a progression of these changes, leading to further abnormalities of the metabolism, composition, biomechanics and morphology of the cartilage, 'Preradiological OA'. Progress may eventually reach the final, 'Clinical OA' end-stage with classical symptoms, radiological changes and loss of joint cartilage substance. Progress from one stage to the next is not inevitable; some patients remain at the same level for long periods of time or even show evidence of spontaneous repair. The response of the individual joint to an insult is variable. Adapted from Lohmander (1994*a*), with permission.

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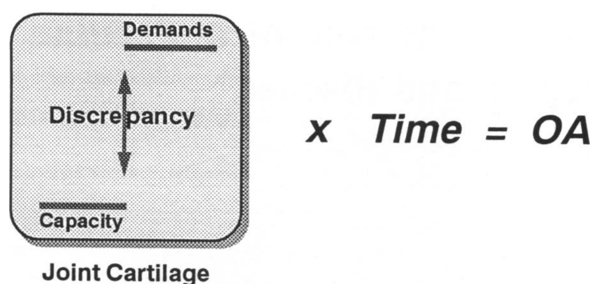

Fig. 2. The pathogenesis of OA. The pathogenesis of OA may be regarded as the result of a discrepancy between the *demands* on the matrix and cells (e.g. mechanical load, rate of replenishment of matrix components, proper assembly of matrix components, repair) and the *capacity* of the matrix and cells of the cartilage to answer to these demands. Both demands and capacity may change as a result of injury or ageing, etc. With time, such a discrepancy may lead to irreversible matrix damage and OA. From Lohmander (1994*b*), with permission.

associated with an increased relative risk of knee OA (Felson et al. 1991; Vingård et al. 1991; Axmacher & Lindberg, 1993; Lindberg et al. 1993; Roos et al. 1993).

The reasons why age is one of the strongest risk factors for the development of OA are not clear. It may be that the chondrocytes with increasing senescence lose the ability to replenish and repair the joint cartilage matrix lost by injuries or normal turnover and a deficit eventually results (cf. osteoporosis). Alternatively, the ageing cartilage matrix may become more susceptible to the normal cumulated microinjuries and the replenishment and repair mechanisms of the cells are unable to compensate for this increased susceptibility. In both cases there is a discrepancy between the demands of the environment on the joint cartilage and the capacity of the chondrocytes or the matrix to respond to these demands (Lohmander, 1994*a*) (Fig. 2). Although the lag time from the initiating event to the appearance of symptoms or radiographic signs of osteoarthrosis is variable, it is usually measured in years or decades. This would also tend to bias symptomatic OA towards the elderly.

The rate of progress of OA is variable from patient to patient, even within the same age group and with the same type of joint injury (Lohmander, 1991). This suggests that factors such as genetic susceptibility, activity level, differences between joints etc. influence OA development. The individual response to joint insult is thus variable and progress of OA is not inevitable (Spector et al. 1992*a*). Perhaps this reflects a variable capacity for repair as much as a variable degradative response after a joint insult.

We are currently unable to slow or reverse the process of cartilage failure in the patient. The purpose of this review is, however, to demonstrate that the basic science of joint cartilage biology is now beginning to answer our specific questions on disease mechanisms on the cell and tissue level. By providing these answers and critically testing them in a controlled clinical setting, we can develop a rational treatment for OA.

CHANGES IN CARTILAGE BIOCHEMISTRY AND MARKERS AFTER JOINT INJURY AND IN OA

Early-stage OA is associated with increases in both degradative and reparative processes. The interaction of metabolic, biochemical and biomechanical changes in the tissue with 'exogenous' factors such as mechanical loading may be important to initiate the cascade of events which later leads to overt OA. In the initial phases, an increase of anabolic processes may be able to counterbalance an increased catabolism. Later, reparative efforts may fail due to quantitative or qualitative deficiencies in the synthesis and assembly of a matrix that is no longer able to withstand physiological loading. In the end, the defective cartilage matrix fails and overt, clinical OA is present (Lohmander, 1994*b*). Studies on changes in cartilage in OA are confounded by the profound regional and age differences noted in joint cartilage biochemistry and metabolism both within and between joints (Bullough et al. 1985; Aydelotte et al. 1988; Gruschko et al. 1989; Häuselmann et al. 1993).

Collagens

Type II collagen is the major component of joint cartilage matrix, with minor amounts of types VI, IX, X and XI collagens normally present (Heinegård & Oldberg, 1989; Hardingham, 1990; Eyre et al. 1992). It is apparent that at least collagen types II, IX and XI are present in copolymeric structures within the matrix and that types IX and XI, and other components of the matrix, may to some extent regulate type II fibril formation (Heinegård & Oldberg, 1989; Eyre et al., 1991*a*, 1992). We may presume, therefore, that degradation, repair and failure of joint cartilage in OA must involve changes in all of these collagens. Studies on changes of collagens in OA have been hampered by the very slow rate of turnover of collagen II in vivo in the adult (Maroudas, 1980), and the small amounts of the 'minor' collagens present in articular cartilage matrix.

A significant increase in the synthesis, deposition and turnover of collagen II in articular cartilage was shown to occur in the early phases of animal models of posttraumatic OA (Eyre et al. 1980; Friedman et al. 1990). Results also suggested an increased in vitro incorporation of radioactive precursor into type IX and XI collagens. However, both endogenous and newly synthesised type IX collagen appeared to have been depleted from the OA cartilage, perhaps due to proteolytic destruction during the early phases of response to joint injury. The increased content of collagen II C-propeptide observed in human OA cartilage would also be consistent with an increased synthesis of collagen II (Poole et al. 1991). An increase in collagen II mRNA levels which coincided with an increased pericellular immunostaining for collagen II in human OA cartilage would again strongly point to at least a transient, regional activation of collagen II synthesis in OA (Aigner et al. 1992). A shift in the phenotypic expression of the chondrocytes in more advanced stages of OA is suggested by the expression of 'noncartilage' collagen types I and III (Aigner et al. 1993*a*).

Type X collagen is normally only present in sites near cartilage mineralisation where chondrocytes hypertrophy (Grant et al. 1985; Schmidt & Linsenmayer, 1985). In OA, several investigators have reported an increased expression of this molecule both at sites of reinitiation of endochondral bone formation (osteophytes, tide mark) and in clusters of chondrocytes in fibrillated cartilage (Hoyland et al. 1991; von der Mark et al. 1992*a*, *b*; Aigner et al. 1993*b*). The fact that cartilage from experimental OA is enriched in type VI collagen, normally localised in the pericellular domain of the chondrocyte, suggests that synthesis of this collagen is also activated in OA (McDevitt et al. 1988; Ronzière et al. 1990; Brierley et al. 1991; Poole 1992).

A characterisation of the collagens in human OA cartilage, compared with normal, showed an increased extractability of types II and VI collagen, while no intact form of type IX collagen was detected (Brierley et al. 1991). Additionally, immunohistochemical analysis of human OA cartilage shows the matrix deposition of collagen types I and III, in addition to type II, in concordance with their mRNA expression (Aigner et al. 1993*a*; Nerlich et al. 1993). The changes found in human OA samples are thus consistent with those found in the animal models of OA. A critical role for changes in collagen integrity in the development of OA is suggested also by reports of early-onset OA in humans and in transgenic mice associated with molecular defects in collagen II and collagen IX, respectively (Ala-Kokko et al. 1990; Eyre et al. 1991*b*; Nakata et al. 1993; Vikkula et al. 1993; Williams & Jimenez, 1993).

Several reports thus strongly point to a transient increase of expression, during OA progression, of collagen II and some of the minor collagens. The changes in tissue content of the different collagens found both in animal models of OA and in samples of human OA cartilage to some extent seem to reflect these shifts in chondrocyte synthetic patterns. However, it is also evident that major modifications must occur in the interactions between the collagens themselves and between the collagens and other matrix components, as reflected, for example, by the changes in extractability.

It is generally assumed that the destruction of joint cartilage matrix in OA is caused by the action of proteases released by the chondrocytes themselves, by cells in the synovial fluid such as leucocytes and macrophages, or by cells in the synovium. Conditioned culture medium derived from interleukin-1-activated chondrocytes contains protease activity with the potential to degrade cartilage collagen types II, IX, X, and XI (Gadher et al. 1990). Moreover, stromelysin has the capacity to cleave collagen types II, IX, X, and XI (Wu et al. 1991; Eyre et al. 1992). This metalloprotease is synthesised both by chondrocytes and by synovial cells (Woessner, 1991) and could thus be involved in the degradation of cartilage collagens during the development of OA. Immunohistochemical evidence for localised collagen II degradation in cartilage matrix in OA has been presented (Dodge & Poole, 1989). In normal adult joint cartilage, only slight matrix staining was observed, consistent with the slow turnover of collagen in adult joint cartilage and the intactness of the collagen network. The consequences of the changes in collagen composition, synthesis, interaction and degradation that occur in OA, in terms of cartilage matrix function and survival, are not well understood, however.

We lack specific assays for body fluid markers of cartilage collagen turnover, but ongoing development in several laboratories of immunoassays for alpha chain fragments and peptide-bound crosslinks of collagen II and the minor cartilage collagens may lead to methods which monitor collagen degradation. A recently developed immunoassay for carboxy-terminal type II procollagen peptide could reflect the synthesis of collagen II in joint cartilage (Shinmei et al. 1993). The assay of nonpeptide bound collagen crosslinks unfortunately lacks specificity in that both cartilage and bone collagens contain the pyridinoline crosslink (Eyre et al. 1988; Seibel et al. 1989). Nevertheless, it

was shown that the failure of cartilage in rabbits injected intra-articularly with chymopapain was associated with a marked increase in the urinary excretion of both pyridinoline and deoxypyridinoline crosslinks (Uebelhart et al. 1993).

Matrix proteins and small proteoglycans

Articular cartilage contains a rich assortment of matrix proteins and small proteoglycans (Heinegård & Oldberg, 1989; Heinegård & Pimentel, 1992). The precise functions of these components of the matrix are mostly unknown, although for example the small proteoglycans decorin and fibromodulin bind to the surface of collagen fibrils and may regulate collagen fibre formation (Heinegård & Pimentel, 1992; Rosenberg, 1992). Another interesting aspect of the function of these molecules is that TGF-β can bind to biglycan and decorin (Yamaguchi et al. 1990). Very little is known about changes in the synthesis or structure of these molecules in OA.

Fibronectin is present in low concentrations in normal articular cartilage, and its concentration increases in OA cartilage (Lust & Burton-Wurster, 1992). Notably, fibronectin fragments can cause chondrolysis of cartilage in vitro, probably by enhancing metalloproteinase release from the chondrocytes (Homandberg et al. 1992). Such fragments have been found in OA joint fluid (Xie et al. 1992).

As noted, the destruction of joint cartilage matrix in OA is thought to involve proteases released by the chondrocytes, or by cells in the synovial fluid or synovium. Little information is available as to how this protease activity affects the matrix proteins or small proteoglycans. However, articular cartilage from patients with chronic polyarthritis contains increased amounts of fragments of decorin and possibly of biglycan (Witsch-Prehm et al. 1992).

Only a few reports are available which concern the release of fragments of cartilage matrix proteins to body fluids on OA (Fife, 1988; Fife et al. 1991). However, patients with OA or joint injury present with increased levels of fragments of cartilage matrix protein (COMP) in their joint fluid (Saxne & Heinegård, 1992*a*; Lohmander et al. 1993*e*). This suggests that cartilage matrix proteins are susceptible to activated proteases in vivo in OA. The fact that the ratios of aggrecan to COMP fragments vary with time after joint injury and OA disease stage, suggests that differences may exist in release and replenishment mechanisms of these 2 matrix molecules (Lohmander et al. 1993*e*).

Aggrecan

Major shifts are found in the turnover and structure of aggrecan in both experimental and human OA (Hardingham & Bayliss, 1990). In a series of studies on dogs developing OA after an induced lesion to the anterior cruciate ligament, early-phase stimulation of aggrecan synthesis has been noted both in vitro and in vivo (Palmoski & Brandt, 1982; Carney et al. 1984, 1985; Sandy et al. 1984). This increase, together with chondrocyte proliferation, may be responsible for the observed early cartilage hypertrophy in experimental OA (Adams, 1989; Adams & Brandt, 1991; Brandt et al. 1991 *a*). Stimulation of aggrecan synthesis was site-specific in that the directly loaded cartilage of the medial tibial plateau was more stimulated than the cartilage covered by the meniscus (Sandy et al. 1984). Further, it was noted that the stimulation was particularly marked for chondrocytes of the middle and deep zones of the tissue, in analogy with the increased collagen II synthesis observed in human OA (Aigner et al. 1992). Modifications in chondrocyte metabolism have also been observed in samples obtained from human OA joints. Thus an increased aggrecan synthesis was noted with advancing OA, while in advanced disease incorporation rates decreased again (Mankin et al. 1971; Thompson & Oegema, 1979; Bulstra et al. 1989).

The structure of the newly synthesised aggrecan molecules in OA cartilage appears to differ from that of normal cartilage (Carney et al. 1985, 1992; Shuckett & Malemud, 1990; Ratcliffe et al. 1993). Molecules synthesised as a response to the early phases of OA were reported to be of larger hydrodynamic sizes than normal, with longer chondroitin sulphate chains (Bulstra et al. 1989). However, tissue extracts also contained increased amounts of smaller size proteoglycans unable to interact with hyaluronan (Bulstra et al. 1989; Shuckett & Malemud, 1990). Further evidence for the de novo synthesis of aggrecan molecules with altered composition as a response to OA has been found by analysis of the sulphation patterns of the chondroitin sulphate chains (Caterson et al. 1985; Carney et al. 1992; Pelletier et al. 1992, 1993*b*; Rizkalla et al., 1992; Shinmei et al. 1992; Ratcliffe et al. 1993). The functional significance of these modifications is unknown, but it is possible that they could alter the interaction of the glycosaminoglycan chains with, for example, growth factors or with other components of the matrix (Bayliss, 1992).

Some of the information in the literature on changes in turnover and structure of aggrecan in OA cartilage is conflicting, which could be explained by the

confounding influence of different disease models, ages, disease stages, joints and anatomical location within joints of the samples used. Another complicating issue is the fact that in some locations, degraded molecules may be retained in the tissue, while in other locations degraded fragments are quickly lost to the circulation, with consequences for the resulting tissue composition. The proteoglycan content of human OA cartilage shows no change in early stage disease, but undergoes a gradual loss with increasing histopathological progression (Mankin et al. 1971; Roberts et al. 1986). Evidence for a decreased proportion of aggregating proteoglycans was found in animal model OA as well as in human hip OA cartilage (Palmoski & Brandt, 1976; Sweet et al. 1977; Moskowitz et al. 1979; Shuckett & Malemud, 1989; Manicourt et al. 1991*a*). Importantly, changes induced by knee and hip OA have been compared in the same animal model, and the authors concluded that while the same degradative mechanisms could be active in both locations, aggrecan fragments were to some extent retained in the hip cartilage, while the corresponding fragments were quickly lost from knee joint OA cartilage (Inerot et al. 1991). Consequently, the average molecular composition of the retained aggrecan population differed between the 2 joints.

Information on the effects of OA on hyaluronan and link protein is sparse. The Pond–Nuki dog model was used to show a significant decrease in the content of hyaluronan, down to about one fifth of normal, in OA cartilage (Manicourt & Pita, 1988; Pita et al. 1992). Link protein undergoes age-related fragmentation, which confounds the interpretation of OA studies (Mort et al. 1983). Apparently, some of this fragmentation is generated by stromelysin (Nguyen et al. 1989).

The precise mechanisms responsible for the degradation of aggrecan, or the other components of the cartilage matrix, in OA are not known. A consistent finding in both induced and human OA cartilage is an increased release and extractability of both endogenous and newly synthesised aggrecan (McDevitt & Muir, 1976; Sweet et al. 1977; Moskowitz et al. 1979; Carney et al. 1984; Inerot et al. 1991). Like the increased collagen II and aggrecan synthesis, this is localised to defined anatomical areas in the early phases of OA but later spreads to all joint surfaces (Carney et al. 1984). The phenomenon may be related to the synthesis of molecules with defective aggregation properties, a defective extracellular maturation and assembly of proteoglycan aggregates, or to an increased proteolytic degradation of aggrecan, link protein or some other component critical to the retention of aggrecan in the matrix. The relative content of protein and keratan sulphate in these released molecules is decreased, suggesting that many of them have been affected by proteases (Inerot et al. 1991).

A large number of assays for aggrecan fragments in joint fluid and serum have been developed. These methods have been used to quantify epitopes located in different domains on the protein core (Fig. 3) (Heinegård et al. 1985; Saxne et al. 1986, 1992*b*; Witter et al. 1987; Ratcliffe et al. 1992), keratan sulphate and chondroitin sulphate chains (Thonar et al. 1985; Carroll, 1989; Björnsson, 1993), and specific sulphated structures in keratan sulphate and chondroitin sulphate (Thornston et al. 1989; Seibel et al. 1992; Shinmei et al. 1992; Ratcliffe et al. 1993).

With the use of these assays, increased concentrations were shown of keratan sulphate in serum and synovial fluid in human OA (Thonar et al. 1985; Sweet et al. 1988; Campion et al. 1991; Mehraban et al. 1991), while other reports have failed to confirm this observation in experimental or human OA (Cruz et al. 1989; Brandt & Thonar, 1989; Leipold et al. 1989; Spector et al. 1992*b*). Increased joint fluid concentrations of aggrecan fragments were found after joint injury and in experimental and primary and posttraumatic human OA (Heinegård et al. 1985, 1987; Ratcliffe et al. 1988; Lohmander et al. 1989). Similarly, increased levels of keratan sulphate in serum were demonstrated following experimental cartilage degradation in the rabbit knee joint and after cruciate ligament lesions in the dog (Williams et al. 1988; Manicourt et al. 1991*b*), but keratan sulphate levels in human OA serum and joint fluid did not correlate with each other (Campion et al. 1991). Analysis of specific sulphated sequences on the chondroitin sulphate chains released to joint fluid after injury and in OA suggests that glycosaminoglycan structure of the released molecular fragments is changed, compared with normal conditions (Shinmei et al. 1992; Ratcliffe et al. 1993). These changes are consistent with the de novo synthesis of altered chondroitin sulphate chains as shown by analysis of OA cartilage, as discussed above.

The levels of aggrecan fragments in joint fluid correlate with the OA disease stage, and are higher in early-stage disease than in later, advanced stages, consistent with the decreasing cartilage and aggrecan content of the joint with progressing disease (Campion et al. 1991; Dahlberg et al. 1992). Concentrations of fragments of aggrecan and cartilage oligomeric protein are higher in the acute than in the chronic phases of joint injury (Lohmander et al. 1989, 1993*b*, *c*, *e*). In

Protease Cleavage of Aggrecan in Joint Cartilage

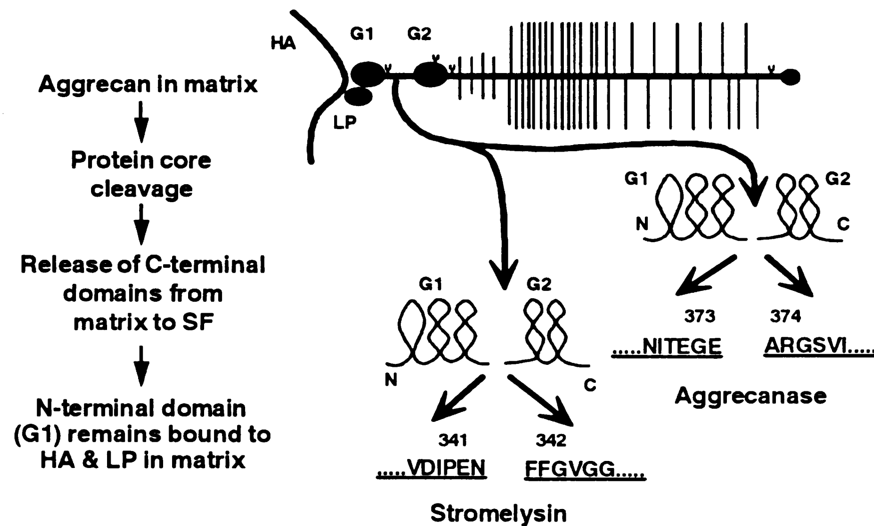

Fig. 3. Cleavage sites for stromelysin and aggrecanase in the interglobular domain of aggrecan core protein. The aggrecan is normally fixed in the cartilage matrix by interaction with hyaluronan and link protein. A proteolytic cleavage in the interglobular domain between the G1 and G2 domains will release the bulk of the aggrecan from this interaction and leave it free to diffuse from the matrix. The remaining part of the aggregate is functionally deficient, having lost the major part of its glycosaminoglycan chains. Evidence for the in vitro proteolytic cleavage of aggrecan in the interglobular domain both by metalloproteinase (stromelysin) and by the putative protease aggrecanase has been presented. See text for references and details. Adapted from Lohmander (1994*b*).

rheumatoid arthritis, the more C-terminal, chondroitin sulphate-bearing aggrecan domains are preferentially released in early disease stages, while the release of N-terminal, hyaluronan-binding domains increases in late disease (Saxne & Heinegård, 1992*b*). An increased release of link protein was shown in experimental canine osteoarthritis (Ratcliffe et al. 1992), consistent with the breakdown and release of all components of the proteoglycan aggregates under these conditions. The metabolic fate of the hyaluronan component of the proteoglycan aggregate is unknown, but the similar turnover rates of hyaluronan and aggrecan in cartilage explants in vitro suggests that degradation mechanisms exist for this component as well (Morales & Hascall, 1988; Ng et al. 1992). Hyaluronan receptors on the chondrocyte cell surface may be involved in this process (Knudson & Knudson, 1993).

These examples serve to exemplify the use of serum and joint fluid aggrecan markers to monitor OA. Longitudinal prospective studies based on hypotheses suggested by these results will be required to determine the clinical usefulness of assays such as these to monitor disease progress and clinical trials or to identify high-risk groups. Experience shows that patients in such studies will need to be carefully stratified with regard to diagnosis, disease stage, time after injury, therapy, age and gender.

PATHOGENESIS

The factors which induce the changes in expression of collagen and other matrix molecules in OA have not been identified, but may include changes of the chondrocyte environment, mechanical loading, cytokines, growth factors and perhaps molecular fragments produced by the matrix metabolism (Morales, 1992; Sah et al. 1992; Tyler et al. 1992; Urban & Hall, 1992).

A number of investigations on human and animal model OA have shown that changes in the metabolism and properties of joint cartilage and loss of molecular fragments from the matrix are early events in the disease process, while bulk loss of cartilage tissue is a late event (Hoch et al. 1983; Carney et al. 1984, 1985; Ratcliffe et al. 1992). It may thus be argued that increased degradation of cartilage matrix is a key event in the development of OA, However, since cartilage matrix is continuously turned over and held in a steady state under physiological conditions, loss of matrix may also be the result of defective mechanisms for replacement and repair of matrix normally turned over. The pathology of end-stage OA may thus be the result of both excess degradation and aberrant repair (Lohmander, 1994*b*).

The physiological rate of turnover of cartilage matrix is slower in the adult than in the young and is

variable for different matrix components (Maroudas, 1975; Lohmander, 1977). Available evidence suggests that the chondrocytes play a major role both in physiological turnover during development and growth and in the matrix degradation that occurs in osteoarthritis. Cytokines such as interleukin-1 and tumour necrosis factor both stimulate matrix catabolism by chondrocytes and strongly inhibit the synthesis of matrix molecules (Saklatvala, 1986; Dingle & Tyler, 1986; Lefebvre et al. 1990; Pelletier et al. 1993*a*). These cytokines therefore have the ability both to stimulate cartilage destruction and to inhibit repair activity. However, the significance of this signal pathway in OA has not yet been demonstrated. The complexity of the interaction between the chondrocytes, cytokines and growth factors is further illustrated by the fact that transforming growth factor-β and insulin-like growth factor-1 are effective antagonists of interleukin-1 action on cartilage and changes in the activity of these factors could be significant in OA pathogenesis (Fosang et al. 1991*b*; Hardingham et al. 1992; Middleton & Tyler, 1992; Malemud, 1993).

We assume that proteases active in the cartilage play a critical role in the degradation of the cartilage matrix in OA. The enzymes of the metalloproteinase family have the capacity to degrade all the components of the cartilage matrix (Docherty & Murphy, 1990; Woessner, 1991). These endopeptidases are secreted in a latent proenzyme form, are activated extracellularly and the active forms are inhibited by strong binding to tissue inhibitors of metalloproteinases (TIMPs) or to α-2-macroglobulin. An increase in metalloproteinase activity may therefore be caused either by an increase in the amount of activated enzyme or by a decrease in the amount of available inhibitor, or both.

Several investigators have pointed to an imbalance between the activity of metalloproteinases and their inhibitors in early OA lesions (Dean et al. 1989; Pelletier et al. 1990). The synthesis of stromelysin in OA by chondrocytes and by synovial cells has been established (Case et al. 1989; McCachren et al. 1990; Firestein et al. 1991; Gravallese et al. 1991; McCachren, 1991; Pelletier et al. 1993*a*; Zafarullah et al. 1993) and significant increases of both stromelysin and collagenase concentrations in human OA and posttraumatic joint fluids shown (Walakovits et al. 1991; Clark et al. 1993; Lohmander et al. 1993*a*). Concomitantly, the ratio between prostromelysin-1 and tissue inhibitor of metalloproteinase-1 in joint fluid was shifted from a normal molar excess of inhibitor to an excess of enzyme after injury and in OA (Lohmander et al. 1993*a*, *b*, *c*). The stage would thus seem to be set for a lead role for stromelysin in cartilage matrix degradation in OA. Accordingly, human articular cartilage contains hyaluronan-binding, amino terminal globular domains (G1) of the aggrecan which have a C-terminal identical to that produced by stromelysin in vivo (Flannery et al. 1992), suggesting that the core protein has been cleaved by stromelysin and the bulk of the molecule thereby released from the aggregate (Fig. 3). However, characterisation of aggrecan fragments released into the medium of bovine cartilage explants cultured in the presence or absence of interleukin-1 or retinoic acid shows a limited number of fragment sizes with a major amino terminal sequence of ARGSVIL, corresponding to a clip between positions Glu 373–Ala 374 in the interglobular domain of the human aggrecan core protein (Sandy et al. 1991; Ilic et al. 1992; Loulakis et al. 1992) (Fig. 3). No amino terminals corresponding to metalloprotease activity were identified. Significantly, the same aggrecan fragmentation patterns with the same major ARGSVIL amino terminals were observed in joint fluids obtained from patients with OA, joint injury or inflammatory arthritides (Sandy et al. 1992; Lohmander et al. 1993*d*) (Fig. 3). The putative cartilage protease (aggrecanase) which cleaves the NITEGE–ARGSVIL sequence of the interglobular domain of aggrecan and thereby releases the bulk of the molecule from the matrix, may thus be of considerable significance for cartilage degradation in OA. However, it has not yet been identified (Fosang et al. 1991*a*, 1992; Flannery & Sandy, 1993). The results obtained by structural analysis of aggrecan fragments isolated from human joint fluids illustrate that cartilage markers may be useful not only to monitor disease and treatment, but also to elucidate pathogenetic mechanisms in joint disease (Sandy et al. 1992; Lohmander et al. 1993*d*). Knowledge of the major N and C-terminal structures of the released molecular fragments now allows the development of antibodies towards these terminal structures. Such antibodies may be useful for immunohistochemical detection of sites of specific protease activity in OA cartilage, and for quantification in body fluids of fragments released as a result of the activity of these proteases (Fig. 3). These reagents should also be eminently suitable to monitor future clinical trials aimed at inhibiting protease activity in joint disease.

Fragments of cartilage matrix molecules that result from degradation are either taken up by the chondrocytes and further degraded by lysosomal enzymes, or are lost to the joint fluid by diffusion (Fig. 4).

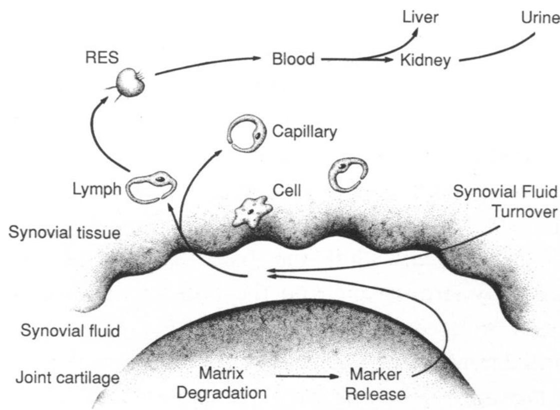

Fig. 4. Turnover of joint cartilage matrix fragments released into the joint fluid and other body fluid compartments. Fragments released into joint fluid from cartilage are removed mainly by lymphatic flow. Fragments which reach the blood circulation and which are not eliminated in the regional lymph nodes are largely metabolized by the liver. From Lohmander (1991), with permission.

Fragments in the joint fluid may be taken up by and further degraded in the synovial tissue cells, or be removed by bulk flow with the synovial fluid to the lymph circulation (Fraser et al. 1988). A substantial proportion of matrix molecule fragments removed by the lymphatic circulation are eliminated or at least further degraded in the regional lymph nodes (Fraser et al. 1988; Tzaicos et al. 1989). The majority of the remaining fragments that reach the blood stream are rapidly and within minutes removed from circulation, most likely by the liver cells (Maldonado et al. 1989; Engström-Laurent & Hellström, 1990; Smedsröd et al. 1990). The collagen crosslinks, however, apparently survive the circulation and are found enriched in urine (Eyre et al. 1988; Seibel et al. 1989).

The interpretation of findings on cartilage markers in body fluids will thus vary with their source. For joint fluids, it may be suggested that cartilage marker findings will be representative for that joint, while cartilage marker findings in serum and urine will reflect matrix turnover in all joints and perhaps all body cartilage. Further, molecular fragment metabolism in, for example, regional lymph nodes and liver will likely induce changes in both structure and amount of the marker in question. It is therefore not surprising that correlations between joint fluid and serum markers are often lacking, even when the same assay is used for both compartments (Campion et al. 1991). On the other hand, concentrations of aggrecan fragments in serum and synovial fluid correlate in juvenile chronic arthritis and concentrations of cartilage oligomeric protein fragments in serum and joint fluid correlate in monoarthrodial reactive arthritis and after joint injury (Saxne et al. 1989, 1992*a*; Lohmander et al. 1993*e*).

DIAGNOSIS

As noted, the OA process is a continuum from early initiating events on the cell and tissue level to overt clinical disease which can be diagnosed by classical criteria (Fig. 1). The criteria by which the condition is defined will by necessity differ between, for example, the cartilage biochemist, general practitioner, orthopaedic surgeon, radiologist and epidemiologist, depending on their different tools, needs and purposes. In the clinic OA is diagnosed by a combination of symptoms, clinical signs and radiological findings (Altman et al. 1986, 1987; Lequesne, 1991). These methods are associated with uncertainties and ways are therefore sought by which the diagnosis and assessment of disease progression could be made with greater precision and reproducibility. Promising techniques currently being evaluated are, for example, magnetic resonance imaging, bone scintigraphy, arthroscopy, and body fluid markers of joint cartilage turnover.

Although radiological changes are regarded as the standard in OA diagnosis, it may be argued whether they represent the 'gold standard' or 'fool's gold' in analogy with the radiographic grading of the rheumatoid arthritis process (Brower, 1990). Thus radiological changes as assessed by a score which takes osteophytes, sclerosis and joint space narrowing into account (Kellgren & Lawrence, 1957), or by a score which only grades the degree of joint space narrowing (Ahlbäck, 1968), show a limited correlation with arthroscopic grading of joint surface changes (Lysholm et al. 1987; Brandt et al. 1991*b*). Clinical signs, in turn, show only moderate correlation with radiographic changes (Hart et al. 1991). Recent reports suggest, however, that progressive joint space narrowing in knee or hip OA can be reproducibly measured over periods as short as 3 y (Dougados et al. 1992; Kirwan et al. 1992; Lequesne et al. 1992). A problem associated with the radiological grading of OA is that radiography will present only a record of past destructive events and not give information on current disease activity.

Bone scintigraphy with the use of ^{99}Technetium labelled diphosphonates provides information on bone remodelling activity and may predict surgery or joint space loss in knee OA (Dieppe et al. 1992). Magnetic resonance imaging is more sensitive than conventional radiography and computer tomography for assessing the extent and severity of osteoarthritic changes and chondral and subchondral lesions (Chan et al. 1991; Kaye, 1993; Spindler et al. 1993), and further progress in this area shows great promise.

Point scaling systems for the arthroscopic grading of articular cartilage lesions in hip and knee have been developed (Noyes & Stabler, 1989; Ike, 1993). These techniques are now available for validation in the OA research environment, but it will probably take some time before their value has been proven.

The assay of molecular fragments of cartilage matrix molecules released into joint fluid, serum or urine has been suggested as a means to monitor cartilage turnover in vivo and to serve as a tool for the diagnosis, monitoring and providing a prognosis for OA and other joint cartilage disease (Heinegård & Saxne, 1991; Lohmander, 1991; Lohmander et al. 1992; Thonar et al. 1992, 1993). This approach holds hope for the eventual development of process markers and a better understanding of the disease process in OA. Several problems remain to be solved, however. For example, if a marker is to serve as a *quantitative* measure of cartilage turnover, we need to know the metabolism and clearance of this marker in the compartment in question (Levick, 1992). The release of a specific molecular fragment from cartilage matrix into joint fluid may increase both as a result of increased net degradation and as a result of increased synthesis and turnover, with unchanged tissue net content. Ideally, markers which could specifically and separately monitor synthesis (e.g. procollagen II C-propeptide) or degradation of resident mature molecules (e.g. peptide-bound collagen II crosslinks) should be sought. Moreover, assays for degradation products should be specific for fragments generated by different proteases (Fig. 3). Although differences in average concentrations of body fluid markers between various joint diseases have been shown, data on inter- and intra-individual variations for these markers over time in representative control or diseased populations are usually not available. Until such data have been presented, calculations of statistical power, required number of patients, etc. in clinical trials cannot be made. The immunoassay of stromelysin-1 protein in joint fluid discriminates joints with pathology from healthy joints with a sensitivity of 84% and a specificity of 90% (Lohmander et al. 1993*a*). However, sufficient data are not yet available which allow discrimination between different joint diseases.

TREATMENT

The main problems associated with the progression of OA are pain, impairment and disability. A large proportion of both the working-age population and the elderly with OA are limited in their working ability and activities of daily living as a consequence of the condition (Yelin, 1992). Treatment seeks to decrease pain and preserve and restore function. An assortment of treatment modalities are in use and will vary with the patient, joint site and the stage of the disease: education, walking aids and braces, physiotherapy, drugs, abrasion arthroplasty, osteotomy and finally joint replacement. Irrespective of the method of treatment used and its success in decreasing, for example, pain, it rarely if ever is able to modify the disease process itself, but only the consequences of it. For any of the procedures, efficacy and outcome should be compared with the natural history of a control group with the appropriate age, disease stage, etc. This task is not trivial in the light of our limited documentation of the natural history of OA in many joint sites.

The use of nonsteroidal anti-inflammatory drugs for the treatment of OA is widespread and this family of drugs provides well-documented pain relief which, however, occurs at the price of significant side-effects, particularly in the elderly (Somerville et al. 1986; Brandt, 1993). The question of disease modification, 'chondroprotection', by drug treatment in OA is controversial (Burkhardt & Ghosh, 1987), and although several studies have been reported on the protective effects of injections of polysulphated polymers and/or growth factors in animal OA models (Altman et al. 1989; Dean et al. 1991; Moskowitz et al. 1991; Rogashefsky et al. 1993), well controlled studies in the human are still lacking.

A proposed use for assays of cartilage markers in body fluids is to monitor the effects of surgical or pharmacological treatment of, for instance, OA on joint cartilage metabolism. However, at this time only a few such studies have been presented and no conclusions on their usefulness for this purpose can yet be drawn. Treatment of OA patients led to decrease in the concentration of keratan sulphate in synovial fluid (Carroll et al. 1992). The prolonged decrease in serum concentrations of keratan sulphate after systemic treatment of asthma patients with steroids suggests effects on cartilage metabolism and/or elimination mechanisms of these fragments from the circulation (Campion et al. 1989). Conversely, patients treated with nonsteroidal anti-inflammatory drugs for OA did not differ in serum concentrations of keratan sulphate from untreated patients (Sweet et al. 1988). In rheumatoid arthritis, local or systemic treatment with steroids leads to marked changes in joint fluid and serum levels of aggrecan fragments (Saxne et al. 1986).

In spite of the immense success of arthroplasty as a treatment for OA, problems which merit attention remain, such as costs, implant loosening and joints for

which there are as yet no satisfactory implants. Joint replacement will no doubt remain the treatment of choice for advanced OA of the hip and knee, but only treats the consequences of the disease, not the disease itself. To develop the means to retard cartilage destruction and slow or reverse the progress of OA remains the ultimate target.

CONCLUDING REMARKS

Cartilage matrix metabolism undergoes pronounced shifts during the development of OA. These alterations, in interplay with mechanical loading and other exogenous and endogenous factors, over time lead to the changes observed in cartilage matrix biochemistry and function in OA. Experimental evidence points to a marked increase by the chondrocytes of both synthesis and degradation of matrix molecules in the early phases, with little or no net loss of the major matrix molecules. Later there may still be evidence for an increased synthesis of matrix components, but due to defects in the structure of new molecules or extracellular assembly, or shifts of degradative activity, net loss of matrix occurs. In the end, the compensatory efforts mounted by the chondrocytes collapse while degradation continues and the matrix and joint fail. The 'point of no return' on this pathway has not been defined and may indeed be different in different joints, individuals and experimental models. The influence of ageing may also cause a shift of the 'point of return' so that a lesser joint insult may initiate the OA process in the aged cartilage (Lohmander, 1994*a*).

Loss of significant amounts of aggrecan from joint cartilage matrix is a frequent finding at more advanced stages in many experimental OA models, and is presumably associated with the concomitant decrease in compressive stiffness (Hoch et al. 1983). It is also clear, however, that joint cartilage may suffer major losses in aggrecan content both in human and experimental joint disease and still be able to recover with no apparent sequelae (Saxne et al. 1993; Williams et al. 1993). What critical structure is affected at the 'point of no return' is still unknown, although collagen IX has been proposed as one possible candidate (Eyre et al. 1991*a*). It is also unknown what the other mandatory steps in this cascade of events may be. For example, is the removal of a significant amount of aggrecan obligatory before, for example, collagens can be attacked by proteases? What role does mechanical loading play as a driving force in the OA cascade? Are the factors which initiate OA and drive its progress the same? What role does the increased synthesis of matrix components in early-stage OA play for matrix function? What signals initiate this increase? What is the functional significance of the structural changes in chondroitin sulphate chains in OA? Why is reassembly of a functional matrix ineffective in OA? The elucidation of the OA disease process remains a focal point for joint cartilage research.

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