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REVIEW

Articular cartilage destruction in experimental inflammatory arthritis: insulin-like growth factor-1 regulation of proteoglycan metabolism in chondrocytes

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Summary

Rheumatoid arthritis, a disease of unknown aetiology, is characterized by joint inflammation and, in its later stages, cartilage destruction. Inflammatory mediators may exert not only suppression of matrix synthesis but also cartilage degradation, which eventually leads to severe cartilage depletion. Systemically and locally produced growth factors and hormones regulate cartilage metabolism. Alterations in levels of these factors or in their activity can influence the pathogenesis of articular cartilage destruction in arthritic joints. The main topic of the present review is the role of the anabolic factor insulin-like growth factor-1 in the regulation of chondrocyte metabolic functions in normal and in diseased cartilage. This is the most important growth factor that balances chondrocyte proteoglycan synthesis and catabolism to maintain a functional cartilage matrix. A brief overview of how chondrocytes keep the cartilage matrix intact, and how catabolic and anabolic factors are thought to be involved in pathological cartilage destruction precedes the review of the role of this growth factor in proteoglycan metabolism in cartilage.

Articular cartilage

Structure and function of articular cartilage

Articular cartilage forms the weight-bearing surface of the articular joint, providing it with a resilient, smoothly gliding surface. Articular cartilage consists of a highly organized extracellular matrix in which specialized cells, chondrocytes, are embedded. Aggregated proteoglycans and collagen fibrils are the two major structural components of the matrix, and form a three-dimensional framework giving the tissue a unique architecture.

Cartilage collagen has a very low basal rate of turnover, and the intact collagen network provides a permanent framework that gives the tissue tensile strength. Cartilage collagen consists mainly of type II collagen, but cartilage also contains small amounts of type VI, IX and X collagen; type X is restricted to the deep part of the cartilage and type XI is part of the collagen fibril. Moreover, type X collagen is mainly found in hypertrophic chondrocytes or in osteoarthritic cartilage (Reichenberger *et al.*, 1991; Nerlich *et al.*, 1992; Von der Mark *et al.*, 1992; Aigner *et al.*, 1993, 1995).

Proteoglycans are complex macromolecules with a high turnover rate; they consist of a core protein to which one or more glycosaminoglycan side chains or N-O-linked oligosaccharides are attached (Handley *et al.*, 1985). Glycosaminoglycan chains are polymers of repeating disaccharides that can vary in

number of chains, their length as well as their pattern of sulphation; they play a dominant role in the determination of physical properties of the protein to which they are attached. High negatively charged glycosaminoglycans entrap solvent, thus creating a high osmotic pressure which is restrained by collagen. The swelling pressure, together with changed hydraulic permeability, enables cartilage to resist compressive loads with minimal deformation.

The major proteoglycan found in articular cartilage is aggrecan (Hardingham & Fosang, 1992, 1995). Aggrecan molecules contain three globular domains (G1, G2 and G3), of which the G1 domain can bind noncovalently to a single chain of hyaluronan, forming large macromolecular aggregates. This interaction is promoted and stabilized by link protein (Hardingham *et al.*, 1992a) (Fig. 1). Furthermore, cartilage contains nonaggregated small proteoglycans, such as biglycan, decorin and fibromodulin (Witsch-Prehm *et al.*, 1992). These smaller proteoglycans are studied intensively these days, but so far clear information on their function is scant.

An important aspect of the structural integrity of articular cartilage is the ability of chondrocytes to synthesize and degrade proteoglycans to maintain a functionally intact cartilage. Small perturbations in cartilage metabolism may lead to increased or decreased local concentrations of matrix molecules which can alter physiological properties of the tissue. This may lead to rapid loss of tissue function and deterioration of the joint.

Three strikingly heterogeneous cartilage layers are generally recognized between the articular surface and the subchondral bone, i.e. surface, middle and deeper zones (Fig. 2) (Sampson & Cannon, 1986). Zonal subdivision is based on morphology, differences in cell density, the orientation, nature, content and distribution of proteoglycans, and the organization of the collagen fibrillar network (Maroudas *et al.*, 1990; Siczkowski & Watt, 1990; Aydelotte & Keuttner, 1991; Marles *et al.*, 1991). Chondrocytes in the surface zone are small, flattened, and oriented parallel to the articular surface, and they are rather inert with respect to matrix synthesis. Chondrocytes in the middle zone are larger and round, whereas chondrocytes in the deeper zones have the largest size and occur usually in groups. Chondrocytes in the middle and deeper zones of cartilage exhibit the highest metabolic activity and are responsible for cartilage homeostasis.

Destruction of articular cartilage

During chronic joint disease, such as rheumatoid arthritis, progressive joint inflammation results in irreversible cartilage destruction, leading to erosion

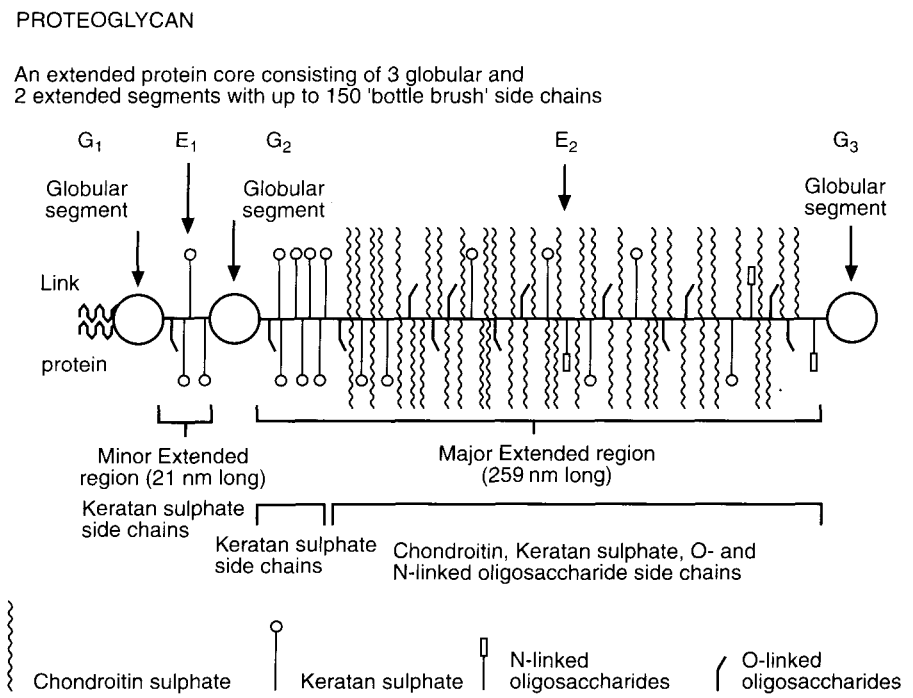

Fig. 1. The basic structural unit of aggrecan, consisting of a core protein to which highly negatively charged glycosaminoglycan chains or oligosaccharides are bound. The core protein is noncovalently attached to hyaluronic acid, and this binding is reinforced by link proteins.

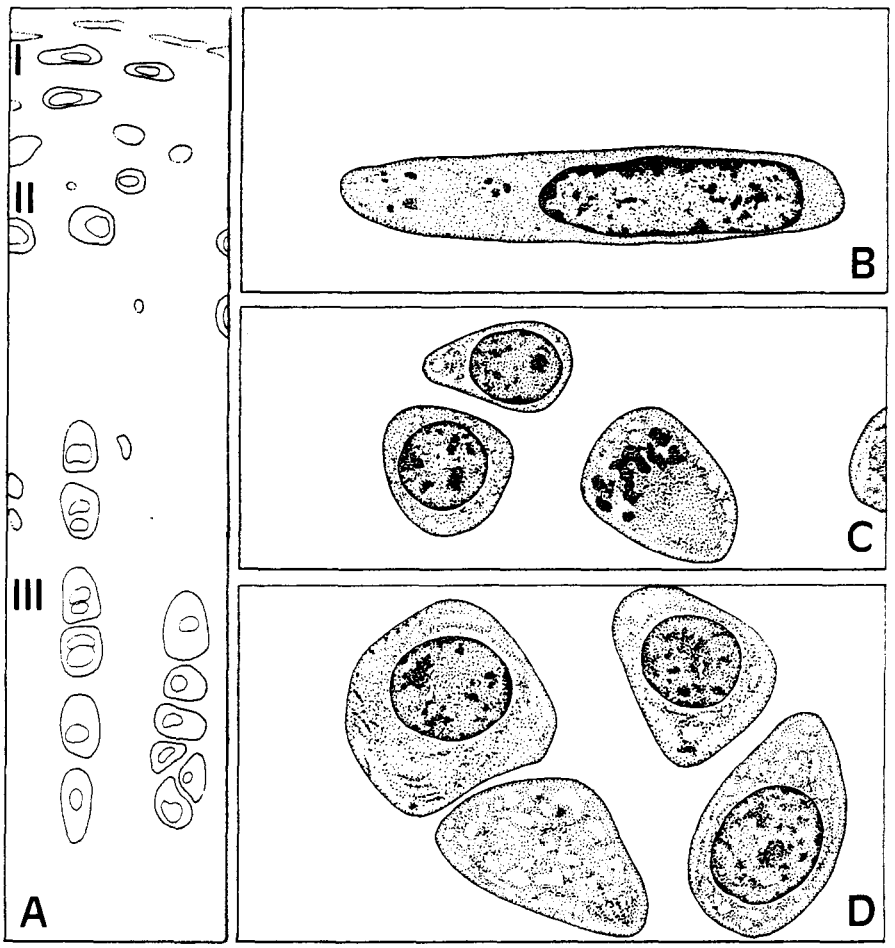

Fig. 2. (A) Schematic drawing of histologically stained human cartilage, showing the three striking heterogeneous cartilage layers that are generally recognized between articular surface and subchondral bone, namely surface (I), middle (II) and deeper zones (III). Zonal subdivision is based on morphology, differences in cell density and orientation, nature, content and distribution of proteoglycans as well as organization of the collagen fibrillar network. (B–D) Schematic drawings of chondrocytes in the three cartilage layers. (B) Chondrocytes in the surface zone are small, flattened, oriented parallel to the articular surface, and are rather inert with respect to matrix synthesis. (C) Chondrocytes in the middle zone are larger and round. (D) Chondrocytes in the deeper zones are largest and usually occur in groups. Chondrocytes in the middle and deeper zones of cartilage exhibit highest metabolic activity and are responsible for cartilage homeostasis. (A, ×390; B ×5226; C, ×4680; D, ×5616).

of articular cartilage, injury of surrounding tissues and eventually permanent loss of joint function (Fig. 3) (Kelly *et al.*, 1989; Harris, 1990).

For the study of processes that determine joint destruction in arthritic joints, animal models have been developed in which arthritis is induced experimentally. Studies performed by our group have revealed that the main aspects of pathological cartilage depletion are increased degradation of articular cartilage, reduced matrix synthesis, and chondrocyte death (Van den Berg *et al.*, 1981; Schalkwijk *et al.*, 1989b). Synovial cells, chondrocytes, and large numbers of macrophages and lymphocytes that infiltrate arthritic joints generate vast quantities of mediators during inflammation, causing invasion and erosion of cartilage and subsequent cartilage destruction. These mediators include cytokines, proteolytic enzymes and reactive oxygen species. In addition, chronic interference with factors that upregulate synthesis of matrix components in chondrocytes plays an important role in cartilage destruction during joint inflammation (Schalkwijk *et al.*, 1989a, b; Tyler, 1989; Verschure *et al.*, 1989). The importance of a balance between suppressive mediators, such as cytokines, proteolytic enzymes or reactive oxygen species, and anabolic factors, such as circulating hormones and locally produced growth factors for the structural integrity of articular cartilage is emphasized in the following paragraphs.

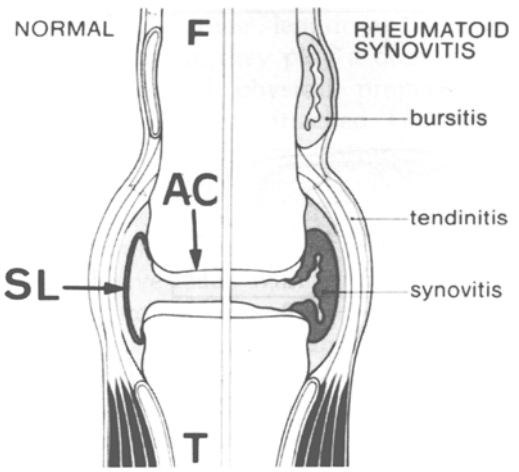

Fig. 3. Schematic representation of a normal and a rheumatoid arthritic knee joint. Joint inflammation is characterized by chronic synovitis leading to irreversible destruction of the articular cartilage. F = femur, T = tibia, SL = synovial lining, AC = articular cartilage.

Catabolic factors

Cytokines

Cytokines are thought to play an important role in the pathogenesis of rheumatoid arthritis. These signal molecules mediate cell–cell interactions and are able to induce, amplify and maintain inflammation. Cytokines have been implicated in the release of enzymes capable of cartilage destruction (Baici & Lang, 1990; Macnaul *et al.*, 1990; Shapiro *et al.*, 1990; Circolo *et al.*, 1991; Chandrasekhar & Harvey, 1992; Mort *et al.*, 1993; Wahl & Corcoran, 1993), but also have strong effects on metabolic functions of chondrocytes (Holt *et al.*, 1992). High levels of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumour necrosis factor α (TNFα), granulocyte-macrophage colony stimulating factor and leukocyte inhibitory factor respectively, have been detected in the synovial fluid of arthritic joints; interleukin-2 (IL-2), interleukin-4 (IL-4) and interferon γ are not elevated in arthritic joints (Nouri *et al.*, 1984; Di Giovanne *et al.*, 1988; Houssiau *et al.*, 1988; Saxne *et al.*, 1988; Xu *et al.*, 1989; Brennan *et al.*, 1990; Firestein & Zvaifler, 1990; Waring *et al.*, 1993). However, it must be realized that levels of cytokines in synovial fluid or plasma gives only limited information about their penetration and ultimate effects in the cartilage matrix. Moreover, the final effect of these cytokines is a balance between their concentrations and the concentrations of their specific inhibitors (Hopkins *et al.*, 1988; Dayer & Burger, 1994). Moreover, responses to cytokines are also dependent on the presence of receptors on their target cells.

Despite the complexity of the cytokine network involved in joint inflammation, IL-1 and TNFα are identified as key mediators in the pathogenesis of rheumatoid arthritis (e.g. Bentzen *et al.*, 1985; Tyler, 1985; Benton & Tyler, 1988; Larrick & Kunkel, 1988; Verschure & Van Noorden, 1990; Henderson *et al.*, 1991; Van den Berg *et al.*, 1991; Elliot *et al.*, 1993; Arend & Dayer, 1995; Van de Loo *et al.*, 1995b; Joosten *et al.*, 1996). In our experimental models of arthritis, the arthritis-inducing stimulus determines whether TNFα drives IL-1 production, or whether IL-1 and TNFα are individually involved (Van den Berg, 1994; Joosten *et al.*, 1995). Moreover, IL-4 and IL-10 are able to interfere with TNFα and IL-1 production and they upregulate expression of their inhibitors, i.e. IL-1 receptor antagonist and soluble TNF receptor (Howard *et al.*, 1992; Wong *et al.*, 1993). IL-1 is a key mediator in the inhibition of chondrocyte proteoglycan synthesis in both antigen- and zymosan-induced models of rheumatoid arthritis (Van de Loo & Van den Berg, 1990; Van de Loo *et al.*, 1992, 1995a). Systemic treatment *in vivo* with anti-IL-1 antibodies or IL-1 receptor antagonist stimulates chondrocyte proteoglycan synthesis (Van de Loo *et al.*, 1992, 1995a; Lethwaite *et al.*, 1994). In the collagen-induced model of rheumatoid arthritis, systemic treatment *in vivo* with anti-IL-1 antibody or IL-1 receptor antagonist not only reduces inhibition of proteoglycan synthesis in chondrocytes, but also reduces cartilage destruction and joint inflammation (Wooley *et al.*, 1993; Van den Berg *et al.*, 1994). Evidence for a direct destructive role of TNFa *in vivo* is still lacking (Van de Loo *et al.*, 1995b; Joosten *et al.*, 1996), but it can induce cartilage damage *in vitro* (Saklatvala, 1986; Wilbrink *et al.*, 1991; Williams *et al.*, 1995). Its main effect in collagen-induced arthritis is probably induction of early joint swelling (Elliot *et al.*, 1993; Joosten *et al.*, 1996).

Proteolytic enzymes

High levels of degradative enzymes can cause breakdown of articular cartilage during joint inflammation. This topic is not discussed here because it has been reviewed extensively elsewhere (Evans, 1991; Firestein, 1992; Buttle, 1994; Henderson & Blake, 1994). Metalloproteinases such as collagenase and stromelysin are important proteolytic enzymes that may be involved in destructive processes (Nguyen *et al.*, 1989; Mort *et al.*, 1993). In addition, cysteine and serine proteinases seem to play a significant role as well (Van Noorden *et al.*, 1988; Maciewicz & Wotton, 1991; Ahmed *et al.*, 1992; Esser *et al.*, 1994). Recently, some doubt was raised about the relevance of the above mentioned proteinases, and it was hypothesized that a newly described enzyme, aggrecanase, could be of more importance (e.g., Flanary & Sandy, 1994; Fosang *et al.*, 1994; Hughes *et al.*, 1995; Lark *et al.*, 1995).

The activity of proteolytic enzymes is not only

determined by the presence of the enzyme molecules but also by post-translational activation processes and interactions of enzymes with their endogenous inhibitors (e.g. Roose & Van Noorden, 1995). In joint inflammation, post-translational activation may be increased, or an imbalance between the levels of matrix degrading enzymes and their inhibitors may occur (e.g. Ellis *et al.*, 1994; Martel-Pelletier *et al.*, 1994).

Reactive oxygen species

In addition to proteolytic breakdown, reactive oxygen species that are released in vast amounts by inflammatory and/or phagocytic cells can cause degradation of the articular cartilage matrix (Roberts *et al.*, 1989; Henrotin & Deby-Dupont, 1992). In previous studies, we and others have shown that *in vitro* exposure of articular cartilage to reactive oxygen causes severe inhibition of chondrocyte proteoglycan synthesis as well as degradation of the aggrecan proteoglycan (Bates *et al.*, 1985; Schalkwijk *et al.*, 1985a; Schalkwijk *et al.*, 1985b). However, the exact role of reactive oxygen species *in vivo* still has to be established. The ultimate effects of reactive oxygen species depends not only on their release but also on levels of endogenous scavenger enzymes and antioxidants (e.g. Schalkwijk *et al.*, 1985a).

At present, nitric oxide (NO), a reactive oxygen species that is produced by NO synthase, is receiving a lot of attention in arthritis research. There is increasing evidence that nitric oxide production contributes to pathological features of joint inflammation (Evans *et al.*, 1995; Sakurai *et al.*, 1995). Nitric oxide production is regulated by cytokines and growth factors (Palmer *et al.*, 1993; Blanco *et al.*, 1995; Lo & Cruz, 1995). The role of nitric oxide has been studied by applying NO synthase antagonists and inhibitors. IL-1 is known to cause inhibition of chondrocyte proteoglycan synthesis through nitric oxide production (Stadler *et al.*, 1991; Charles *et al.*, 1993; Hauselman *et al.*, 1994; Taskiran *et al.*, 1994; Fukuda *et al.*, 1995; Jarvinen *et al.*, 1995). Moreover, nitric oxide is thought to play a regulatory role in activation of metalloproteinases in articular chondrocytes (Murell *et al.*, 1995). However, it is unclear whether nitric oxide is a mediator of cartilage catabolism (Hanglow *et al.*, 1995; Stefanofic-Racic *et al.*, 1996).

Anabolic factors

Rates of chondrocyte proteoglycan synthesis and degradation in articular cartilage are the result of the effects of a number of hormones and locally produced growth factors (Lebovitz & Eisenbarth, 1975; Franchimont & Bassleer, 1991; Hardingham *et al.*, 1992b; Trippel, 1995). Hormones such as steroids, and growth factors such as basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGFβ) and insulin-like growth factor (IGF-1), are known to affect cartilage metabolism. IGF-1 is the most important growth factor that stimulates chondrocyte proteoglycan synthesis and inhibits proteoglycan catabolism (Tyler, 1989). In the following paragraphs, the role of steroid hormones and growth factors in chondrocyte proteoglycan metabolism will be briefly described and subsequently, the role of IGF-1 in cartilage metabolism will be reviewed.

Effect of growth factors and glucocorticoids on chondrocyte proteoglycan metabolism

Growth factors, such as EGF, bFGF, PDGF, TGFβ and IGF-1, play a role in the regulation of chondrocyte proteoglycan synthesis and degradation. They are also important in repair processes during joint diseases (Cuevas *et al.*, 1988; Ogawa *et al.*, 1991; Harvey *et al.*, 1993). Controversy exists about their relative importance in modulating chondrocyte proteoglycan metabolism. It must be realized that most information has been gained from studies on isolated chondrocytes or from cartilage explants. Differences in experimental culture conditions, the origin and species from which chondrocytes or cartilage are obtained are all important factors that might determine the outcome of the experiments (Kato & Gospodarowicz, 1985b; Rosier *et al.*, 1988; Burton-Wuster & Lust, 1990; Hiraki *et al.*, 1990). Little is known of the role of growth factors in anatomically intact cartilage (Van der Kraan *et al.*, 1992a).

bFGF and EGF stimulate proteoglycan synthesis strongly in actively growing growth-plate chondrocytes, but only to a limited extent in chondrocytes that have become hypertrophic (Kato & Iwamoto, 1990; Iwamoto *et al.*, 1991; Trippel *et al.*, 1993; Sah *et al.*, 1994; Wroblewski & Edwall-Arvidsson, 1995). PDGF stimulates proteoglycan synthesis to a similar extent as IGF-1, whereas TGFβ exerts both stimulatory and inhibitory effects on chondrocyte proteoglycan synthesis (Van der Kraan *et al.*, 1992a, b; Morales, 1994). Furthermore, *in vivo* experiments showed that repeated intra-articular injections of TGFβ into mouse knee joints induce strong and long-lasting stimulation of chondrocyte proteoglycan synthesis (Van Beuningen *et al.*, 1993, 1994). Synergistic interactions between growth factors have been described as well in *in vitro* culture experiments (Inoue *et al.*, 1989; Nataf *et al.*, 1990; O'Keefe *et al.*, 1994). For example, IGF-1 synergistically enhances chondrocyte proteoglycan synthesis induced by bFGF or EGF in cultured chondrocytes (Kato *et al.*, 1983).

Glucocorticoids are involved in the regulation of long-term metabolic functions of chondrocytes, but their effects on chondrocyte proteoglycan metabolism remain controversial. Intra-articular injections of glucocorticoids suppress joint inflammation and result in pain relief and marked preservation of joint function (Hollander *et al.*, 1951; Holden & Hume, 1962; Standberg, 1964; Gray & Gottlieb, 1983; Weiss *et al.*, 1990). Low levels of glucocorticoids stimulate chondrocyte proteoglycan synthesis *in vitro* (Kato & Gospodarowicz, 1985a; Takano *et al.*, 1985; Takigawa *et al.*, 1988; Bellows *et al.*, 1989). They also have chondroprotective effects on proteoglycan-depleted cartilage *in vivo*, by decreasing both inhibition of proteoglycan synthesis, and loss of chondrocyte proteoglycan and osteophyte formation (Williams & Brandt, 1985; Joosten *et al.*, 1990; Van den Berg, 1991, 1992). Glucocorticoids, such as cortisone, prednisolone, hydrocortisone, dexamethasone and triamcinolone acetonide also exert inhibitory effects on chondrocyte proteoglycan synthesis in normal cartilage *in vitro* as well as *in vivo* (Mankin & Conger, 1966; Anastassiades & Dziewiatkowski, 1970; Behrens *et al.*, 1975; Weiss *et al.*, 1988; Barrueco *et al.*, 1989; Chunekamrai *et al.*, 1989; Annefeld, 1992). IGF-1 and glucocorticoids have synergistic effects on chondrocytes (Itagane *et al.*, 1991). The effects of glucocorticoids on chondrocytes may be direct or via regulation of production of inflammatory mediators (Blondelon *et al.*, 1980; Sheppard *et al.*, 1982; Steinberg & Sledge, 1983; Silbermann & Maor, 1985; Shinmei *et al.*, 1988; Di Battista *et al.*, 1991; Itagane *et al.*, 1991; Price *et al.*, 1992; Schlaghecke *et al.*, 1992).

Insulin-like growth factor (IGF)

IGFs, which were originally defined as somatomedins, have been reported to be: a growth hormone-dependent serum factor able to stimulate ^{35}S-sulphate incorporation into cartilage *in vivo* ('sulphation factor'; Salmon & Daughaday, 1957), a cofactor extracted from serum able to stimulate replication of cells ('multiple stimulating activity'; Kato *et al.*, 1981), or a factor in serum exerting insulin-like activity on target tissues ('nonsuppressible insulin-like activity'; Zapf *et al.*, 1987).

Isolation and purification of IGF revealed that two forms exist: IGF-1 with a molecular mass of 7.5 kDa and pI of 8.5, and IGF-2 with a molecular mass of 7.4 kDa and pI of 7.0 (Clemmons & Shaw, 1986; Humbel, 1990). Structural analysis at biochemical and genetic levels revealed that IGF-1 and IGF-2 are both single polypeptides with significant sequence similarity with insulin (Daughaday & Rotwein, 1989; Rotwein, 1991; Adamo *et al.*, 1992; Van den Brande, 1992). IGF biosynthesis is regulated by various hormones from the pituitary gland, such as growth hormone, adrenocorticotropic hormone, luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone, and growth factors such as PDGF and bFGF. IGF is produced in significant amounts after tissue injury or during malnutrition (Smith *et al.*, 1989a; Isaksson *et al.*, 1991; Nissley & Lopaczynski, 1991). It was originally thought that this growth factor was produced by the liver and acted as a hormone (Hall & Tally, 1989). Now it is generally recognized that it is synthesized by numerous types of cells and tissues, suggesting that it acts via autocrine/paracrine as well as endocrine pathways (Daughaday, 1990; Phillips *et al.*, 1990; Le Roith & Roberts, 1991; Bang & Hall, 1992; Isgaard, 1992; Jennische *et al.*, 1992; Middleton & Tyler, 1992; Lazowski *et al.*, 1994). IGF levels in serum (20–80 nM) are higher than in tissues. However, IGF in serum is largely bound to specific IGF-binding proteins (IGFBPs) which prolong its half life (Adamo *et al.*, 1992). Therefore, the physiologically relevant levels of IGF may be tissue levels rather than serum levels. It has been suggested that IGF-1 is an important postnatal growth factor, affecting development and metabolism of many tissues other than cartilage (Demarquay *et al.*, 1990). IGF-2 plays a regulatory role in glucose metabolism and may be even more important as a fetal growth factor (Bhaumick & Bala, 1991).

IGF receptors

Biological action of IGF-1 and IGF-2 is initiated by binding to their specific cell surface receptors, which differ significantly in structure and peptide binding specificity (Fig. 4) (Czech *et al.*, 1989; Smith *et al.*, 1989b; Yokono *et al.*, 1989). IGF-1 binds preferentially to the type 1 receptor, and IGF-2 to the type 2 receptor, although both receptors can bind the other IGF with lower affinity (Le Roith *et al.*, 1991; Tollefsen *et al.*, 1991). Type 1 receptor is a heterotetrameric glycoprotein consisting of two extracellular α subunits with a molecular mass of 130 kDa, and two transmembrane β subunits with a molecular mass of 95 kDa. The α and β subunits are held together by disulphide bonds, thus forming a tetramer consisting of two αβ dimers (Ullrich *et al.*, 1986; Le Roith & Raizada, 1989; Neely *et al.*, 1991). Type 1 receptor is formed as a precursor protein that needs a number of post-transcriptional and post-translational modifications including glycosylation, dimerization and proteolytic processing to yield the mature complex consisting of two αβ subunits (Rechler & Nissley, 1990; Moxham & Jacobs, 1992). The ligand binding site is located in the extracellular α subunit, whereas the intracellular β subunit contains a tyrosine kinase domain responsible for signal transduction (Kjeldsen *et al.*, 1991; Zhang & Roth, 1991). Association of two αβ dimers is required

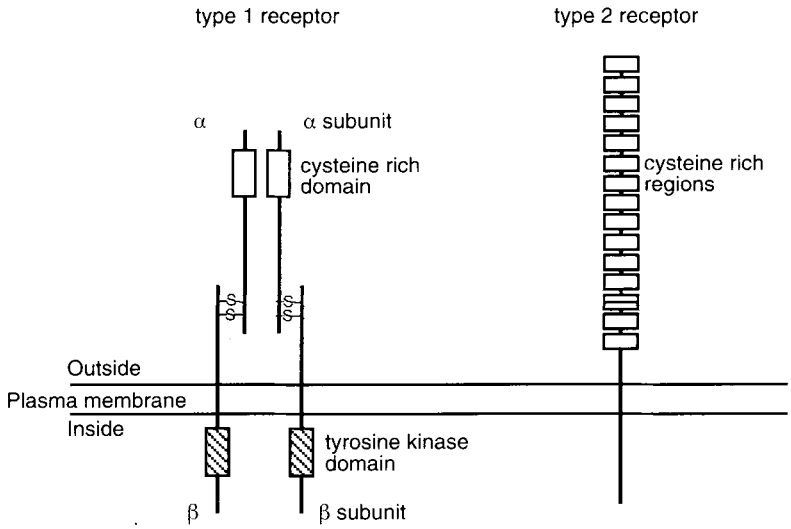

Fig. 4. Structure of the type 1 and type 2 IGF receptors. The type 1 receptor is a heterotetrameric complex composed of two extracellular α subunits and two transmembrane β subunits held together by disulphide bonds. The α subunits bind IGF-1 and the β subunits contain tyrosine kinase activity in their cytoplasmic domain. The type 2 receptor has a short cytoplasmic tail and a large extracellular domain, consisting of many cysteine-rich regions.

for receptor signal transduction after ligand binding (Ikari *et al.*, 1988; Tollefsen & Thompson, 1988; Wilden *et al.*, 1989; Soos *et al.*, 1990). IGF-1 binding to the α subunit results in intracellular signal transduction by autophosphorylation of the β subunit and activation of the receptor tyrosine kinase. Tyrosine kinase is responsible for intracellular signal transduction, initiating a cascade of phosphorylation steps including activation of serine and threonine kinases (Corps, 1990). Recently, signal transduction from the IGF-1 type 1 receptor via tyrosine kinase receptor has been linked with the G protein-associated phosphatidylinositol hydrolysis pathway, in a way similar to that described for other growth factor receptors (Cantley *et al.*, 1991; Malcolm & Wright, 1993). After IGF binding and signal transduction, the type 1 receptor is internalized and recycled to the cell surface. The number of type 1 receptors at the cell surface is downregulated by increasing concentrations of IGF-1 (Schalch *et al.*, 1986).

Type 2 receptor is a single chain polypeptide with a molecular mass of 250 kDa consisting of a single transmembrane region and a large extracellular domain (Dahms *et al.*, 1987). This receptor does not seem to have signalling functions similar to the type 1 receptor (Haig & Graham, 1991). Type 2 receptor is identical to the cation-independent mannose-6-phosphate receptor, which binds lysosomal proteins and mediates their transport to the lysosomes (Poiraudeau *et al.*, 1994). The mechanism of action of the type 2 receptor was also found to be mediated via the G protein-associated phosphatidylinositol hydrolysis pathway (Nishimoto *et al.*, 1989).

IGF binding proteins (IGFBPs)

IGF binding proteins (IGFBPs) are proteins with high affinity for insulin-like growth factors. Their interaction plays an essential role in modulating biological functions of these growth factors (Baxter, 1993). Multiple binding proteins have been detected in plasma, amniotic fluid and spinal fluid and in a variety of tissues. Despite a large similarity in structure, the different binding proteins have distinctly different properties that might provide clues to elucidate their exact functions (Baxter, 1991; Lamson *et al.*, 1991). Six classes of these binding proteins, designated IGFBP-1 to 6, have been purified and cloned so far (Drop *et al.*, 1992; Bhaumick, 1993). In serum, they function as a storage depot allowing a ready supply of insulin-like growth factor to tissues. The proteins can bind the growth factor either to sequester it so as to prevent its binding to its receptors, or to present IGF to the receptor so as to improve ligand-receptor interactions, depending upon the structural and biochemical properties of the binding proteins. The ability of IGFBPs to adhere to the cell surface appears to be important in the latter process. IGFBP-1 and IGFBP-2 have RGD sequences (Arg-Gly-Asp) in their terminal region and are therefore structurally capable of binding to integrin receptors at the cell surface (Ruoslahti & Pierschbacher 1987; Brewer *et al.*, 1988). The agonistic or antagonistic nature of IGFBP

modulation of the biological functions of IGF depends on the cell or tissue types involved (Baxter & Martin, 1989; Sara & Hall, 1990).

Chondrocytes produce IGFBPs (Froger-Gaillard *et al.*, 1989; Tesch *et al.*, 1992; Olney *et al.*, 1993; Chevalier & Tyler, 1994). In osteoarthritis, production of IGFBP-3 and 5 by chondrocytes is increased (Dore *et al.*, 1994; Olney *et al.*, 1996; Tardif *et al.*, 1996; Chevalier & Tyler, 1996), which indicates that IGFBPs are involved in pathological cartilage destruction.

Effects of IGF on proteoglycan metabolism in cartilage

IGF-1 is the major growth factor that balances proteoglycan biosynthesis and breakdown in chondrocytes for the maintenance of the cartilage matrix (Schoenle *et al.*, 1982; Hascall *et al.*, 1983; Froesch *et al.*, 1985; McQuillan *et al.*, 1986; Luyten *et al.*, 1988; Schalkwijk *et al.*, 1989a; Curtis *et al.*, 1992; Middleton & Tyler, 1992). IGF-1 can stimulate chondrocyte proteoglycan synthesis in culture in the absence of foetal calf serum (Barone-Varelas *et al.*, 1991; Böhme *et al.*, 1992) and it can directly inhibit both basal and cytokine-induced degradation of proteoglycan in cartilage (Tyler, 1989). IGF-2 is less potent than IGF-1, requiring 50–100 times higher concentrations to achieve similar effects (Luyten *et al.*, 1988). Selective elimination of IGF-1 from serum or synovial fluid with neutralizing antibodies results in a distinct decrease in cartilage proteoglycan synthesis and enhanced proteoglycan breakdown (Schalkwijk *et al.*, 1989a). IGF-1 seems to inhibit production and release of proteases or protease activators, although evidence is not yet conclusive (Ballard *et al.*, 1986; Chandrasekhar *et al.*, 1992; Padayatty *et al.*, 1993).

IGF binding sites on chondrocytes in articular cartilage have been identified as specific IGF receptors (Postel-Vinay *et al.*, 1983; Trippel *et al.*, 1983, 1988; Watanabe *et al.*, 1985; Jansen *et al.*, 1989; Makower *et al.*, 1989; Yasunaga *et al.*, 1995). Trippel *et al.* (1988) calculated that an average of 3×10^4 type 1 receptors are present on the surface of each chondrocyte with a binding affinity to IGF-1 of 4×10^{-8} M.

The effects of IGF-1 and IL-1 are opposite: IGF-1 can speed up recovery from IL-1-induced inhibition of chondrocyte proteoglycan synthesis, while IL-1 promotes cartilage breakdown by inhibiting IGF-1-stimulated synthesis (Taylor *et al.*, 1988; Fosang *et al.*, 1991; Berenbaum *et al.*, 1994; Neidel *et al.*, 1994; Rayan & Hardingham, 1994). IL-1 downregulates IGF mRNA levels in chondrocytes and induces secretion of IGFBP and IGF-1 receptor, indicating that an autocrine loop regulates IGF-1 function (Lazarus *et al.*, 1993; Matsumoto *et al.*, 1994). These findings suggest that IL-1 and IGF may play significant roles in the pathogenesis of inflammatory joint diseases.

IGF-1 nonresponsiveness of chondrocytes

Despite the importance of IGF in the regulation of proteoglycan metabolism in chondrocytes, only a limited number of studies have been focused on the role of IGF in rheumatoid arthritis. Measurements of IGF levels in serum or synovial fluid of patients with various types of arthritis yielded conflicting results (Coates *et al.*, 1977, 1978; Bennett *et al.*, 1988; Nuvert-Zwart *et al.*, 1988; Aitman *et al.*, 1989; Allen *et al.*, 1991; Keyzer *et al.*, 1995). However, it has now become clear that lower levels of IGF-1 in serum or synovial fluid and/or decreased availability of IGF-1 are not necessarily the main factors that regulate cartilage metabolism.

Recently, it was demonstrated in our laboratory that IGF-1 is essential to maintain chondrocyte proteoglycan synthesis during culture of intact cartilage (Schalkwijk *et al.*, 1989a). However, cultured, experimentally induced, arthritic cartilage obtained from inflamed joints was nonresponsive to IGF-1 (Schalkwijk *et al.*, 1989b). This phenomenon was not caused by total nonresponsiveness of the chondrocytes, since forskolin, which is an upregulator of the adenylate cyclase pathway, could stimulate chondrocyte proteoglycan synthesis. It was suggested that this nonresponsiveness to IGF contributes to cartilage destruction (Schalkwijk *et al.*, 1989b, Van den Berg *et al.*, 1989). Therefore, the exact role of IGF-1, and the mechanism by which IGF-1 regulates chondrocyte proteoglycan metabolism in normal and experimentally induced arthritic cartilage, were studied (Table 1). These studies are reviewed in the following paragraphs.

Regulation of IGF-1 nonresponsiveness by growth factors and glucocorticoids

The *in vitro* effects of IGF-1 and triamcinolone acetonide on the hydrodynamic volume of newly synthesized proteoglycans in normal and arthritic mouse cartilage have been investigated (Van der Kraan *et al.*, 1993; Verschure *et al.*, 1994a). *In vitro* IGF-1 stimulation of chondrocyte proteoglycan synthesis in normal cartilage resulted in the synthesis of hydrodynamically large proteoglycans, which is identical to the *in vivo* situation. In arthritic cartilage, IGF-1 was unable to stimulate chondrocyte proteoglycan synthesis and only small proteoglycan monomers were synthesized, demonstrating that in arthritic cartilage not only are the amounts of proteoglycan that are synthesized decreased, but also that their quality is inferior. In the presence of triamcinolone acetonide, hydrodynamically large proteoglycans were synthesized both in normal and

Table 1. Overview of the role of IGF-1 and the mechanism by which IGF-1 regulates chondrocyte proteoglycan metabolism in mouse normal and experimentally-induced arthritic and human osteoarthritic cartilage to provide insight in IGF-1-related processes underlying cartilage destruction

Normal cartilage
IGF-1 can maintain *in vitro* synthesis of proteoglycan monomers with large hydrodynamic size at similar levels as occur *in vivo*
Glucocorticoids are able to exert similar effects as IGF-1 on the regulation of *in vitro* chondrocyte proteoglycan metabolism
PDGF and bFGF have an additional effect on IGF-1 regulation of chondrocyte proteoglycan synthesis during culture. EGF and TGFβ have an inhibitory effect on chondrocyte proteoglycan synthesis
IGF-1 receptors are located at the cell membrane of chondrocytes in the middle and deeper zones of cartilage, whereas intracellular labelling is found in surface zone chondrocytes
Arthritic cartilage
IGF-1 nonresponsiveness during arthritis is highly correlated with the severity of arthritis
The failure of IGF-1 to stimulate *in vitro* chondrocyte proteoglycan synthesis in arthritic cartilage accompanies the synthesis of small proteoglycan monomers, demonstrating that not only the amount but also the quality of synthesized proteoglycan has deteriorated in arthritic cartilage
Chondrocytes from arthritic cartilage do not respond to other growth factors besides IGF-1 during culture
Glucocorticoids are able to bypass disturbed IGF-1 responsiveness and may therefore play an important role in maintaining cartilage integrity in arthritic cartilage
Metalloproteinases and cysteineproteinases, reactive oxygen species, IL-1, and TNFα are not key factors in the maintenance of IGF-1 nonresponsiveness. IL-1 does not induce IGF-1 nonresponsiveness but is indirectly involved in the generation of this phenomenon
The correlation of chondrocyte IGF-1 receptor expression with *in vitro* IGF-1 stimulation and metabolic activity is disturbed in arthritic cartilage
Chondrocytes in arthritic cartilage do not exhibit decreased amounts of IGF-1 receptor on their cell membrane. Therefore, IGF-1 nonresponsiveness cannot be explained by a decreased receptor expression
Osteoarthritic cartilage
IGF-1 has different effects on chondrocytes in the different cartilage zones in normal cartilage. This metabolic heterogeneity is lost in osteoarthritic cartilage. In such cartilage, chondrocyte proteoglycan synthesis and IGF-1 receptor distribution are dependent on the stage of osteoarthritis

arthritic cartilage. It was concluded that glucocorticoids may play an essential role in maintaining cartilage integrity in both normal and arthritic cartilage.

Furthermore, *in vitro* investigations were carried out to study the effects of other growth factors besides IGF-1, such as bFGF, EGF, PDGF and TGFβ on the regulation of chondrocyte proteoglycan metabolism under normal and pathological conditions (Verschure *et al.*, 1994b). It was investigated whether these factors could compensate for IGF-1 nonresponsiveness of the chondrocyte either during repair or during early and/or later phases of experimental arthritis. In normal cartilage, none of the growth factors tested stimulated *in vitro* chondrocyte proteoglycan synthesis as IGF-1 did. EGF and TGFβ even inhibited chondrocyte proteoglycan synthesis. Combinations of bFGF or PDGF with IGF-1 exerted significant additional stimulation of chondrocyte proteoglycan synthesis. Normal cartilage that was previously exposed to IL-1 *in vivo* was responsive to IGF-1 and to other growth factors in a similar way as to normal cartilage. However, experimentally induced arthritic cartilage exhibited a state of nonresponsiveness to all individual growth factors tested, as well as to combinations of growth factors. This suggests that during the inflammatory process in the joint, chondrocytes display a general deficiency of receptor functions. Apparently, inflammatory processes during experimental arthritis are more complex than processes caused by injecting IL-1 into the knee joints alone.

Regulation of IGF-1 nonresponsiveness by inflammatory mediators

Studies were carried out to see whether the state of nonresponsiveness to IGF-1 during arthritis was caused by either inflammatory mediators such as IL-1, TNFα, or by proteolytic enzymes or reactive oxygen species (Verschure *et al.*, 1995a). Mediators from tissues surrounding patellar cartilage were able to suppress *in vitro* responses of chondrocytes to IGF-1. Priming of normal cartilage with IL-1 did not completely induce IGF-1 nonresponsiveness, but enhanced the ability of suppressive mediators to downregulate the IGF-1 response. Treatment of the cartilage with antibodies against IL-1α and IL-1β, or IL-1 receptor antagonist, or antibodies against TNFα, or selective inhibitors of proteolytic enzymes or of reactive oxygen species, did not counteract the

nonresponsiveness to IGF-1 *in vitro*. However, treatment with anti-IL-1α and anti-IL-1β antibodies before induction of arthritis abolished nonresponsiveness to IGF-1. It was concluded that neither metalloproteinases and cysteine proteinases nor reactive oxygen species are key factors in IGF-1 nonresponsiveness, and that IL-1 and TNFα alone are not essential for the maintenance of nonresponsiveness. Moreover, IL-1 does not induce IGF-1 nonresponsiveness but is indirectly involved.

Regulation of IGF-1 nonresponsiveness by IGF-1 receptor

The lack of effects of IGF-1 on chondrocytes during arthritis may be due to a defect at the level of IGF-1 receptor binding or postreceptor signalling. Therefore, the amounts and distribution patterns of IGF-1 receptors on the cell membrane of chondrocytes were investigated in normal and arthritic cartilage by immunohistochemical localization and by quantification of IGF-1 receptor (Verschure *et al.*, 1994c). Immunohistochemical labelling of the IGF-1 receptor was performed on cryostat sections of mouse patellas using antibodies against the α subunit of the receptor, and was visualized with confocal laser scanning microscopy (CLSM). Optical sectioning using this technique resulted in improved spatial resolution of the localization of the IGF-1 receptor in chondrocytes, and allowed peripheral labelling to be distinguished quantitatively from intracellular labelling in mouse normal and arthritic cartilage (Verschure *et al.*, 1995b; Verschure *et al.*, 1996a), as well as in human normal and osteoarthritic cartilage (Verschure *et al.*, 1996b) (Figs 5 and 6). Discrimination of specific signals from background labelling, which is difficult when labelling is of low intensity, and background and autofluorescence levels are high, can be accomplished by the application of confocal microscopy (Wilson *et al.*, 1989; Van Oostvelt & Bauwens, 1990; Wells *et al.*, 1989). This is a problem that is inherent to articular cartilage, and therefore CLSM will become increasingly important in cartilage research. Most of the IGF-1 receptor fluorescence was found on chondrocytes of the middle and deeper zones of the cartilage, whereas surface zone chondrocytes exhibited negligible fluorescence. The heterogeneous distribution of IGF-1 receptor in chondrocytes of articular cartilage suggests that effects of IGF-1 on chondrocytes may be distinctly different in the specific cartilage zones.

Confocal laser scanning microscopy was applied to compare labelling of IGF-1 receptor by anti-IGF-1 receptor antibody with labelling of binding sites with biotinylated IGF-1 in the various cartilage zones of normal and experimental arthritic knee joints (Verschure *et al.*, 1996c). In normal cartilage, IGF-1 receptors were found at the cell membrane of chondrocytes of the middle and deep cartilage zones with anti-IGF-1 receptor antibody, whereas intracellular labelling was highest in chondrocytes of the surface zone. After incubation with biotinylated IGF-1, distinct membrane labelling was not present. In normal cartilage, fluorescence was found to be distributed homogeneously in chondrocytes of the middle and deep zones but not in the surface zone. In cartilage from inflamed knee joints, labelling with anti-IGF-1 receptor antibody was not significantly different from normal cartilage, whereas labelling with biotinylated IGF-1 was increased in chondrocytes of the middle and deeper zones of arthritic cartilage as compared with normal cartilage. The lack of intracellular labelling with biotinylated IGF-1 of surface zone chondrocytes may suggest that intracellular IGF-1 binding sites have low affinity for IGF-1, or that the binding site of the receptor is shielded. Labelling patterns obtained with biotinylated IGF-1 also display IGF-1 binding proteins (IGFBPs). It was suggested that during joint inflammation larger amounts of these binding proteins are present.

Both the distribution patterns of IGF-1 receptor, and *in vitro* IGF-1 stimulation of chondrocyte proteoglycan synthesis were examined in articular cartilage of normal and experimental arthritic mouse knee joints during various phases of arthritis (Verschure *et al.*, 1995b). The distribution patterns of IGF-1 receptor expression in the different cartilage zones of normal cartilage reflected IGF-1 stimulation and proteoglycan synthetic activity of chondrocytes in these layers. Concurrently with the development of arthritis, cartilage lost its capacity to react to IGF-1, but when the inflammatory response was waning, IGF-1 responsiveness returned. Shortly after induction of experimental arthritis, a clear decrease in IGF-1 receptor expression was observed, whereas in later phases of arthritis receptor expression returned to normal levels. It was found that IGF-1 nonresponsiveness was highly correlated with severity of arthritis. The correlation of IGF-1 receptor expression with IGF-1 stimulation and metabolic activity is disturbed in arthritic cartilage, suggesting inadequate or abolished intracellular signal transduction in chondrocytes.

It was also studied whether chondrocyte proteoglycan synthesis correlates with chondrocyte IGF-1 receptor expression in human normal cartilage, as well as in cartilage known to display a shift in proteoglycan synthesis, such as cultured cartilage and osteoarthritic cartilage (Verschure *et al.*, 1996b). Osteoarthritic cartilage shows markedly enhanced proteoglycan synthesis. Cartilage specimens were obtained *post mortem* from human knees within 18 hours after the death of donors with no known clinical history of osteoarthritis. The samples were dissected from macroscopically normal regions as

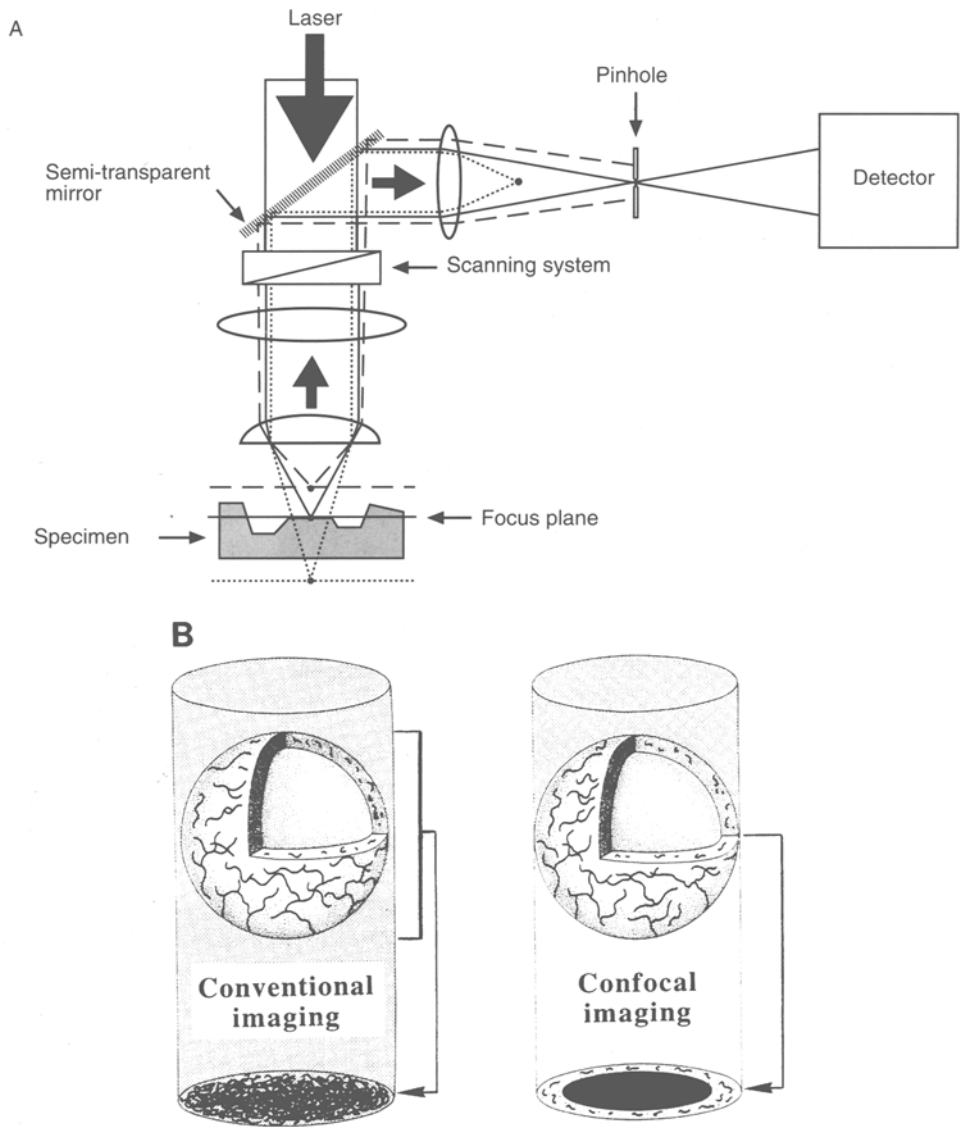

Fig. 5. (A) Schematic drawing of a confocal laser scanning microscope (CLSM). It collects images that are almost free of out-of-focus signals. This type of analysis results in improved spatial resolution as compared with conventional light microscopy and allows optical sectioning of the specimen (White *et al.*, 1988; Wilson, 1989). (B) The possibility of making optical sections through the entire chondrocyte has been applied to the analysis of IGF-1 receptor labelling in the chondrocyte in the middle optical section.

well as from damaged regions. This procedure yielded a range of grades from mild to moderate and severe osteoarthritis. In normal cartilage, both chondrocyte proteoglycan synthesis and the amount of chondrocyte IGF-1 receptor were low in the surface zones, and high in the middle and deep zone of the cartilage. After culture, an increase in chondrocyte proteoglycan synthesis in the surface zone coincided with upregulation of IGF-1 receptor localisation. In mild osteoarthritis, particularly high levels of chondrocyte synthetic activity were found in the upper cartilage layer, whereas IGF-1 receptor expression was low in this layer, suggesting that factors other than IGF-1 are involved in enhancing chondrocyte proteoglycan synthesis. High proteoglycan synthesis activity and chondrocyte IGF-1 receptor labelling were found in moderate osteoarthritis, whereas low levels of proteoglycan synthesis and IGF-1 receptor labelling were observed in severe osteoarthritis.

Final considerations

A critical aspect of the structural integrity of articular cartilage is the ability of chondrocytes to synthesize and degrade proteoglycans. IGF-1 is the most important growth factor that regulates chondrocyte proteoglycan metabolism, by binding to the type 1 receptor. In the present review it is shown that IGF-1 receptor distribution patterns in surface, middle and

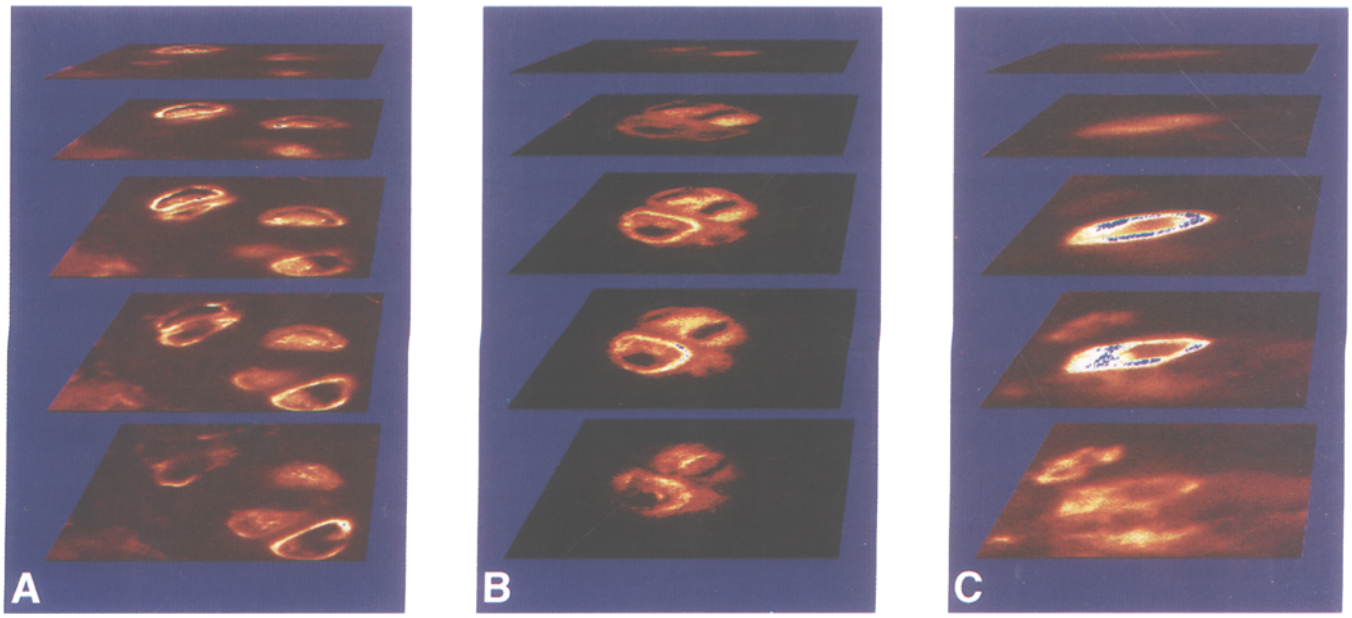

Fig. 6. Pseudo 3-D visualization of a CLSM data stack; consecutive optical sections of a chondrocyte located in the middle zone of mouse articular cartilage after being immunohistochemically labelled for the IGF-1 receptor. (A) Chondrocytes located in the middle zone of normal mouse cartilage after labelling with a polyclonal antibody against the α subunit of the IGF-1 receptor. The chondrocyte membrane contains large amounts of IGF-1 receptor fluorescence but intracellular labelling is weak. (B, C) Chondrocytes located in the middle zone of mouse normal (B) or arthritic articular cartilage (C) after being labelled with biotinylated IGF-1. Intracellular labelling is higher when using biotinylated IGF-1 than when using anti-IGF-1 receptor antibody. Chondrocytes in arthritic cartilage display strikingly higher fluorescence intensity than that observed in normal cartilage. High amounts of fluorescence are found directly below the cell membrane of the cells.

deeper zones of mouse articular cartilage appear to reflect a heterogeneity in IGF-1 stimulation and proteoglycan synthesis of chondrocytes in these layers. Intracellular IGF-1 receptors which are mainly found in chondrocytes of the surface zone, represent internalized, recycling or newly synthesized receptors. This intracellular pool of receptors may become important under conditions of stress. The cell surface receptors on chondrocytes of the middle and deeper zones may reflect receptors actively involved in triggering IGF-1 responses. After culture of human cartilage explants in the presence of serum, chondrocytes show markedly enhanced proteoglycan synthesis, mainly in the surface zone. This shift in metabolic activity coincides with an upregulation of IGF-1 receptor expression on the membrane of these cells (Korver *et al.*, 1990; Lafeber *et al.*, 1990, 1992; Maroudas *et al.*, 1990; Verschure *et al.*, 1996b). The quantities of receptors at the cell membrane or intracellularly in various cartilage zones may provide further insight regarding the regulation of chondrocyte metabolic activity. The application of confocal laser scanning microscopy provides a valuable tool to obtain more detailed information on IGF-1 receptor expression in articular cartilage chondrocytes. However, CLSM analysis of cartilage from diseased joints indicates that conclusions have to be drawn with caution from growth factor or receptor expression patterns without evaluating biological responses triggered by receptor binding of the growth factor involved.

Chronic interference with IGF-1-dependent regulation of chondrocyte proteoglycan synthesis during induced arthritis in the mouse knee joint contributes to severe depletion of articular cartilage. Intra-articular administration of IGF-1 in the treatment of rheumatoid arthritis patients results in pain relief and improvement of joint function (Silveri *et al.*, 1994). However, the present investigation did not provide evidence for the supposed beneficial effects of IGF-1 treatment during induced arthritis in the mouse knee joint with respect to protection against articular cartilage damage. Treatment with glucocorticoids or blocking of IL-1 effects seem to provide a more promising therapeutic approach.

The exact mechanisms underlying IGF-1 nonresponsiveness during experimental arthritis in the mouse knee joint remain unclear. Many possible candidates, such as metalloproteinases, cysteine proteinases, reactive oxygen species, TNFα or IL-1 do not override IGF-1 signalling, although IL-1 seems to play an indirect role in the generation of IGF-1 nonresponsiveness (Joosten *et al.*, 1991; Lin *et al.*, 1992; Blount & Crawford, 1994; Verschure *et al.*, 1995a). The lack of IGF-1 response cannot be explained by receptor downregulation either. This

may indicate that, although IGF-1 receptors are present on the chondrocyte membrane, the membrane lipid bilayer composition may have changed, causing altered configuration of the transmembrane receptor, which may hamper the biological function of the receptor. Furthermore, receptors may be masked or damaged by agents produced by the inflamed synovium (Jansen *et al.*, 1989; Kwok *et al.*, 1989; Bergstedt & Wieloch, 1993; Kato *et al.*, 1993). For instance, IGFBPs may play a role in this process (Tesch *et al.*, 1992; Chevalier & Tyler, 1994). In osteoarthritic human cartilage, elevated IGFBP levels seem to be involved in capturing IGF-1 and therefore in reduced bioavailability of IGF (Dore *et al.*, 1994; Olney *et al.*, 1996; Chevalier & Tyler, 1996; Tardif *et al.*, 1996). Increased labelling in chondrocytes of arthritic cartilage after incubation with biotinylated IGF-1 may indicate increased levels of IGFBPs. However, even at unphysiologically high concentrations of IGF-1 chondrocyte proteoglycan synthesis is not stimulated *in vitro* and chondrocytes maintain their nonresponsiveness to IGF-1. Therefore, if IGFBPs are produced in high concentrations they may be involved in the IGF-1 nonresponsive state during arthritis.

Alternatively, IGF-1 receptors may be defective and unable to exhibit normal biological functions (Soos & Siddle, 1989; Raz *et al.*, 1991; Zhang & Roth, 1991; Attisano *et al.*, 1992; Miki *et al.*, 1992; Werner *et al.*, 1992). Yee *et al.* (1989) have found an alternative IGF-1 receptor transcript with a 3-base deletion in many tissues and cell lines. It would be interesting to investigate whether this alternatively spliced mRNA of IGF-1 receptor is also present in arthritic chondrocytes but not in normal chondrocytes. At present, this hypothesis is investigated by detecting the two different spliced IGF-1 receptor mRNAs with the use of the polymerase chain reaction. In addition, the composition of chondrocyte membranes may be changed and thus also the function of transmembrane proteins, such as the β subunits of the IGF-1 receptor, that are involved in signal transduction pathways. Therefore, possible defects in the phosphorylation pathways leading to receptor signalling will be another interesting item for further research. However, these experiments may introduce artefacts: isolation and subsequent culture of chondrocytes from arthritic knee joints may change the 'arthritic' phenotype of the cells. Moreover, analysis of whole chondrocyte populations leads to loss of information about individual chondrocytes located in the different cartilage layers.

Although arthritis seemed to cause a nonresponsive state to a whole range of potential anabolic growth factors, the glucocorticoid triamcinolone acetonide can maintain *in vitro* chondrocyte proteoglycan metabolism. Glucocorticoids may have an indirect effect on chondrocytes in arthritic cartilage; they can modulate the production of inflammatory mediators (Sheppard *et al.*, 1982; Steinberg & Sledge, 1983), and seem to have a priming effect on the biological action of IGF-1 in cartilage metabolism (Itagane *et al.*, 1991; Price *et al.*, 1992). In a previous study, it was shown that a single intra-articular injection in arthritic joints of a depot preparation of the steroid triamcinolone hexacetonide or rimexolone counteracts the severe suppression of chondrocyte proteoglycan synthesis in arthritic cartilage (Joosten *et al.*, 1990). These steroid-treated arthritic joints show normal responsiveness to IGF-1 (Joosten *et al.* unpublished results). In this respect, it will be interesting to analyse whether, at later stages of arthritis or after renewed exacerbation, chondrocytes still display their responsiveness induced by glucocorticoids.

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