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COMMENTARY

SIMILAR REGULATION OF CHONDROCYTE FUNCTIONS BY CELLULAR STIMULANTS OF UNKNOWN MECHANISM

RETINOIDS, CYTOKINES, AND BACTERIAL LIPOPOLYSACCHARIDE

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Cartilage is a protective tissue overlying the surfaces of weight-bearing joints in order to prevent damage to the underlying bone. Articular chondrocytes are resident cells which maintain the integrity of cartilage by regulating the synthesis and turnover of a highly specialised extracellular matrix. The major components of this matrix are collagens (types II, VI, IX and XI) and proteoglycans which, together, maintain the compressive properties of the tissue.

Experiments utilising explants and isolated primary cell culture of cartilage have demonstrated that several classes of molecules activate chrondrocytes to initiate digestion of the surrounding matrix. The behaviour of these culture systems may have predictive value in elucidating the *in vivo* mechanisms underlying the progressive cartilage destruction which occurs in acute inflammatory disorders, such as rheumatoid arthritis, and underlying the sustained changes in matrix composition which take place during osteoarthritis and ageing. While it is often premature to draw meaningful parallels with such complex clinical conditions, it is clear that *in vitro* experiments are essential to analyse the cellular basis of cartilage maintenance, loss and reconstruction and also to analyse the molecular mechanisms of action of endogenous and exogenous regulators of these processes. The retinoids, cytokines and bacterial lipopolysaccharide have, in each case, diverse biological activities, but, strikingly, each class of molecules contains mediators which interact with chondrocytes in a similar manner, altering the cartilage matrix integrity. The purpose of this commentary is to draw attention to their shared actions on chondrocytes and to discuss both the early and late events which occur after interaction of these molecules with the cell. This will provide a basis for speculation concerning possible similarities and dissimilarities in the mode of action of each class of mediators and will allow an assessment of their relative pathophysiological importance.

Retinoids

Early experiments on embryonic limb bones [1] demonstrated that Vitamin A (retinol) deficiency had a direct catabolic action on skeletal tissues in explant culture. The enzyme papain had similar actions, causing a rapid loss of chondroitin sulfate from all cartilage of the body. This was first demonstrated by the reversible collapse of rabbit ears after intravenous papain injection [2]. These experiments led to a comparison of the actions of papain and Vitamin A on cartilage [3] and resulted in the hypothesis that Vitamin A induced loss of cartilage matrix may be the result of activation of a proteolytic enzyme(s).

Both retinol and retinoic acid have been demonstrated to regulate growth and differentiation in a wide range of cellular systems. Retinoid activation of chondrocytes is very like that described later for responses to the cytokines, interleukin-1 (IL-1) and tumour necrosis factor (TNFα). The retinoid-enhanced degradation of cartilage, originally observed as a loss of metachromatic material from the matrix [1], is accompanied by an inhibition in synthesis of new matrix molecules, including proteoglycan [4] and the collagens, type II and type IX [5]. Chondrocyte collagenase production is also stimulated by retinol [6]. Experiments utilising chondrocytes embedded in an agarose culture system [7] demonstrated that the partially degraded proteoglycans released during retinol treatment did not differ from those released in control cultures. Thus, similarly to IL-1 (see later), it appears that retinol merely increases the rate of the normal turnover processes. For many decades the mechanisms mediating the cellular responses to retinoids have remained a mystery. However, a major step towards elucidating the nature of these responses has occurred recently as a result of cloning of receptors which bind retinoic acid [8–10]. These receptors were isolated by constructing chimaeric genes which linked the conserved DNA-binding domains of previously cloned nuclear steroid hormone receptors with the regulatory regions of unknown related genes. These chimaeric genes were then evaluated for novel receptor-binding ligands. These procedures [8–10] identified two distinct retinoid binding receptors that have high affinity for retinoic acid within concentration ranges compatible with those found to elicit physiological responses to the retinoids. Both receptors have reduced affinity for retinol compared to that for retinoic acid. By structural analogy

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between these newly cloned retinoic acid receptors and the steroid hormone receptors, these experiments identify retinoic acid as a member of the class of ligands which operate via interaction with nuclear receptors.

The main difference between proposed mechanisms of action for retinoids from established mechanisms for steroid hormone receptors is the presence of retinoid-specific cellular binding proteins (CRABP for retinoic acid and CRBP for retinol). Although both binding proteins have been cloned, their precise roles in mediating cellular responses to the retinoids are still not understood. One attractive possibility is that they mediate the transfer of the retinoids from the plasma membrane, through the cytoplasmic domain, to their nuclear receptors [8].

It will be relevant to the evaluation of the role of retinoids in the physiological activation of chondrocytes to establish whether one or both the retinoic acid receptors and retinoid-binding proteins are present in these cells.

Cytokines

Factors which powerfully stimulated joint synovial cells [11, 12] to increase production of prostaglandins and collagenases, and activated cartilage explant cultures [13] to degrade their surrounding matrix, were later purified [14–16], and shared properties with a macrophage-derived polypeptide originally described by its ability to activate lymphocytes [17]. All these substances, purified from different animal tissues, are now known to be interleukin 1 (IL-1), a cytokine with a wide range of biological targets. IL-1 activates chondrocytes to stimulate release of existing matrix molecules from the surrounding cartilage matrix [15, 18] and also inhibits the synthesis of new matrix [19, 20]. Chondrocytic production of the enzymes collagenase and stromelysin is also stimulated by IL-1 [21, 22]. All of these actions are shared with those reported earlier for the retinoids.

The majority of cytokines, including other members of the interleukin family such as interleukin-2 and interleukin-6, the interferons and the colony stimulating factors, do not share the ability of IL-1 to regulate chondrocyte activity with the exception of TNFα. This cytokine, originally identified by its ability to promote hemorrhagic necrosis in transplanted tumours [23] is the same molecule as cachectin, so named because of its ability to induce cachexia and shock. TNF has now been cloned [24, 25] and is routinely examined as a recombinant product. TNFα causes dose-dependent degradation of proteoglycan in cartilage [26] in a comparable manner to IL-1. Both TNF [27] and IL-1 [28] stimulate bone resorption and production of prostaglandins and collagenase [11, 12, 29] by synovial fibroblasts. IL-1 may be a central element in the pathology of inflammatory joint diseases because proteoglycans are degraded in cartilage after direct injection of IL-1 into rabbit synovial joints [30], and the presence of IL-1 has been demonstrated in human synovial fluids [31, 32]. *In vivo* experiments of this type have not yet been reported using TNFα.

IL-1 occurs in two structurally dissimilar forms, termed α and β. Both have now been cloned and sequenced [33]. The two forms of IL-1 show only 26% homology but, surprisingly, compete for the same surface receptors on target cells [34–37]. TNF has surface receptors distinct from those for IL-1 but, like IL-1, two forms of TNF share the same receptor site [38], despite only 30% homology. An IL-1 receptor has been cloned recently [39] and shows characteristics of a single-transmembrane-segment receptor with homology to the immunoglobulin gene superfamily.

The signal transduction mechanisms of IL-1 remain obscure, and little is known of the early events following receptor binding. The later events following interaction of IL-1 with the chondrocyte cell surface have been studied more extensively. In explant cultures, the IL-1-induced depletion of large aggregating cartilage proteoglycan [15, 18] appears to be the result of an increased rate of the normal proteoglycan turnover [40], consistent with the mechanism of degradation observed after retinol treatment [7]. The majority of degraded proteoglycan is released into the culture medium as large fragments, lacking the hyaluronic acid binding region, but some smaller fragments are present [18, 40]. However, the enzymes responsible for cleaving the intact proteoglycans have not been identified. The IL-1-stimulated proteoglycan release is also seen in explants cultured from late foetal and neonatal animals [41]. The broad range of IL-1 activities on mesenchymal cells and actions shared with other developmental regulatory agents, retinoids, suggests that this cytokine may play a role in tissue remodelling. Indeed, recent data support a role for both IL-1 and TNF in tissue remodelling processes [42].

It is likely that the increased production of stromelysin in chondrocytes in response to IL-1 [21, 22] proceeds via direct induction of transcription from the stromelysin promoter. Transcriptional regulation of this enzyme by IL-1 has been demonstrated in synovial fibroblasts [43], and recent work also shows that TNFα directly stimulates collagenase gene transcription [44].

The mechanisms by which matrix synthesis is reduced in response to cytokines have also been experimentally investigated. IL-1-induced inhibition of proteoglycan synthesis results from direct inhibition of proteoglycan core protein synthesis without alteration in the rate of glycosylation and secretion of the newly-synthesised proteoglycan [41]. Similarly, purified IL-1 does not alter the rate of intracellular degradation of newly synthesised type II collagen but inhibits specifically production of collagen mRNA [22].

A comparison of the effects of recombinant TNF-α and IL-1 on glycosaminoglycan and DNA synthesis in rat costal chondrocytes showed different sensitivities to each cytokine [45]. TNF-α was the most potent inhibitor of glycosaminoglycan synthesis [half-maximal dose (ED_{50}) 2 ng/ml]. IL-1α (ED_{50} 5 ng/ml) was substantially more active than IL-1β (ED_{50} 30 ng/ml). In both pig articular chondrocytes [41] and rat costal chondrocytes [45] the cytokines failed to suppress completely glycosaminoglycan synthesis. It is not known whether this incomplete inhibition reflects a sub-population of chondrocytes which fail to bind or respond to cytokines or whether

a class of proteoglycan within each cell is uniformly unaffected by these mediators. DNA synthesis is stimulated by TNF-α but not by IL-1α or IL-1β [45]. Similarly, IL-1 fails to stimulate DNA synthesis in pig chondrocytes [41], although it has been reported as a proliferative agent in fibroblasts [46].

Many chondrocytic responses to IL-1 cannot be detected at time points before 1–4 hr. IL-1-induced collagenase and stromelysin production were not detected until at least 4 hr after treatment [23], and a similar delay was reported for IL-1-induced release of cartilage glycosaminoglycan from explant cultures [47]. A delay in IL-1 induced inhibition of new glycosaminoglycan synthesis was also reported and may not be explained entirely by the time taken to deplete intracellular stores of proteoglycan core protein [41]. Until the early events following IL-1 receptor-binding are elucidated, these delays in output response leave open the question of whether the cytokine actions on chondrocytes are direct. In a variety of cells, IL-1 stimulates synthesis of secondary mediators which may, in turn, be capable of activating chondrocytes. In rabbit chondrocytes, IL-1 treatment leads to rapid activation of cell-associated phospholipase A_2: but secretion of phospholipase A_2 and prostaglandin E_2 were not detectable until several hours later [48]. IL-1 has been shown to stimulate diacylglycerol production [49] in T-lymphocytes but does not stimulate intracellular calcium mobilisation [50, 51] and hence is not a candidate for activation of the inositol lipid signalling pathway. It should also be noted that, although the majority of joint IL-1 is probably derived from synovial cells, chondrocytes themselves are capable of secreting IL-1 [52], and numerous factors regulate secretion of the cytokine, which implies the possibility of autocrine control of chondrocyte function by IL-1.

Bacterial lipopolysaccharides

The third, independent class of agents which induce direct cartilage matrix degradation is bacterial lipopolysaccharides (LPS). LPS stimulate release of proteoglycan from cartilage matrix and inhibit new matrix synthesis [53]. Both effects are fully reversible and parallel chondrocyte-activating actions of cytokines and retinoids.

Recent reviews [54, 55] give a detailed discussion of current knowledge concerning the structure and functions of endotoxin in biological systems. A severe potential problem in the increasing use of recombinant cytokines derived from bacterial expression is cross-contamination with LPS (endotoxin). In experiments utilising recombinant IL-1, contamination of the injected IL-1 with bacterial endotoxin was measured as less than 100 pg/ml [30]. Concentrations of bacterial endotoxin used to stimulate directly cartilage degradation were several orders of magnitude higher [53, 56], making it unfeasible that endotoxin contamination accounted for chondrocyte activation by recombinant cytokines. Conversely, the demonstration that bacterial endotoxin is a potent inducer of both IL-1 [57] and TNF [58] in monocyte/macrophage systems suggests that endotoxin may be stimulating autocrine production of cytokines from chondrocytes. However, the primary role of endotoxin in mediating cellular events has not been fully elucidated, and the possibility that endotoxin may directly activate a cellular signalling pathway should not be ruled out. When these issues are resolved, it will be possible to determine whether endotoxin is a primary mediator of cartilage matrix integrity or whether its actions are the result of induction of secondary mediators.

The lipopolysaccharide molecule consists of both a lipid moiety, termed lipid A, and polysaccharide moieties. The biological activity of the molecule used in cartilage explants resides in the lipid portion of the molecule [53]. Monosaccharide precursors of lipid A are capable of activating protein kinase C [59], and evidence for a specific lipid A receptor is accumulating [55].

Possible signalling pathways mediating the actions of cartilage degrading agents

The responses of chondrocytes to the agents reviewed in this commentary are not incompatible with an intracellular pathway shared between the various agents. Indeed, data reviewed in preceding sections of the commentary suggest that the mechanisms of regulation of cartilage integrity by each agent are very similar. Therefore, retinoids, IL-1, TNF and LPS may represent pharmacological mediators stimulating at different points of a single, conserved gene regulatory pathway.

Figure 1 shows a proposal whereby such a shared pathway could be envisaged. In this model, the final output response would be, in all cases, direct transcriptional control of specific connective-tissue genes. The model is based on recent experimental demonstrations of retinoic acid-binding receptors with homology to nuclear steroid receptors [8–10]. Hence, retinoic acid is proposed to cross the plasma and nuclear membranes and bind directly to nuclear receptors. Cellular retinoid binding proteins may be intermediaries in this pathway. The nuclear retinoid-receptor interaction may be the rate-limiting step in a signalling mechanism which directly influences the transcription of specific genes. In turn, interaction of IL-1 and TNF with their respective plasma membrane receptors may trigger a cascade of cytoplasmic events, resulting in induction or suppression of the same set of genes. This is consistent with the demonstration of direct cytokine regulation of specific genes including the stromelysin [43] and collagenase genes [44] in connective tissue cells and also nerve growth factor [60] and colony stimulating factor [61]. The model is also supported by data showing that IL-1 is internalised and accumulates in the nuclei of human diploid fibroblasts [62]. The model allows for the possibility of interaction of cytokines at independent nuclear binding sites or of direct interaction with retinoid receptors.

LPS may represent a pharmacological modulator, potentially arising during bacterial infection, of the same pathway, influencing the same cytoplasmic cascade of events. This would be analogous to mimicry of diacylglycerol action by exogenous phorbol diesters [63], resulting in activation of one branch of the inositol lipid signalling pathway [64].

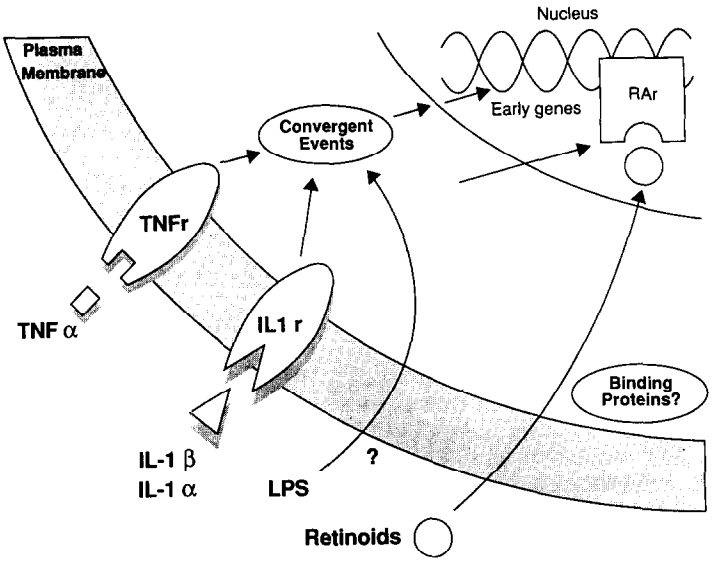

Fig. 1. Model proposing an interactive cellular pathway for cartilage-degrading agents. The schematic diagram shows retinoids binding to nuclear retinoic acid receptors (RAr). The cytokines, interleukins (IL-1α and IL-1β) and tumour necrosis factor (TNFα) bind to their respective cell surface receptors (IL-1r and TNFr) to initiate a cascade of events resulting in the regulation of nuclear activity in a manner similar to that of the retinoids. Lipopolysaccharides (LPS) may regulate this cascade of events.

Concluding comments

This commentary discusses how three seemingly unrelated classes of molecules interact with chondrocytes to produce closely comparable biological output responses. One of these agents, IL-1, has received much attention as a potential mediator in inflammatory joint diseases. The experimental data reviewed here lead to the conclusion that other classes of agents, the retinoids and bacterial lipopolysaccharides are worthy of further investigation of their pathophysiological role in the maintenance of cartilage integrity. Indeed, while cytokines are strong candidates for mediating the rapid cartilage degradation occurring in inflammatory disorders, retinoids such as Vitamin A, required as an essential chronic dietary factor, may be important in long-term changes in functional cartilage matrix which occur with ageing.

The identification of specific retinoid nuclear receptors offers the basis for the testable hypothesis proposed in the preceding model, whereby all these cartilage degrading agents may interact in a common gene regulatory pathway analogous to the pathway for steroid hormones via nuclear receptors.

While the agents discussed here represent the known single mediators of cartilage destruction, it is most likely that multiple mediators interact to contribute to the tissue damage. In this respect, it is important to assess how the cellular responses to these primary mediators of cartilage breakdown are altered by other chondrocyte activators to produce a combined output response. Chondrocytes have been shown to respond to adenylate cyclase-linked histamine receptors [65] and cyclic AMP-regulating agents have been shown to inhibit endotoxin-mediated cartilage degradation [55]. Therefore, it will be important to assess whether a physiological receptor-mediated regulator of cyclic-AMP has similar actions. The calcium-mobilising hormone, bradykinin [50], has also been shown to activate chondrocytes. Whether these substances are inhibitory, additive or synergistic with cartilage-degrading agents or whether they alter threshold responses or rates of secretion will be important in elucidating the relative roles of chondrocyte activators in the pathophysiology of joint diseases.

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