[Frontiers in Bioscience 9, 575-580, January 1, 2004]

CYTOKINES IN OSTEOARTHRITIS-CURRENT STATUS ON THE PHARMACOLOGICAL INTERVENTION

Juergen Steinmeyer

Orthopaedic Research Laboratories, Department of Orthopaedic Surgery, Justus-Liebig-University of Giessen, Paul-Meimberg-Str. 3, D-35385 Giessen, Germany

TABLE OF CONTENTS

1. ABSTRACT

Cytokines and their broad spectrum of effects have been investigated since the 1980s. The already existing preliminary scientific results have been highly suggestive of the idea that cytokines play an essential role in the pathogenesis of OA. Nevertheless, the extent to which cytokines participate in the origin of OA, or are taken as a consequence of the OA process, remain unanswered questions. Unlike the case with rheumatoid arthritis, studies on the application of anti-cytokine medications with OA remain in their infancy. At the present time no clinical studies relating to OA have confirmed that anti-cytokine medications are antiphlogistically effective and/or prevent the origin of morphologically recognizable cartilage defects or at least decelerate the increasing destruction of joint cartilage. As with every other therapeutic approach the risk-benefit scenario is decisive for deciding on the current application of anti cytokine medications for rheumatoid arthritis as well as their potential future use against OA. Considering the long-term consequences of an anti-cytokine based therapy, our poor state of knowledge should be seen in a very critical light, since the discussed approach represents an immunesuppressive therapy that entails consequences with regard to defence against infections and tumour suppression. Also, little is currently known about the interplay between pro- and anti-inflammatory cytokines and growth factors in OA; the resulting specific and fundamental therapeutic possibilities for performing a structure-modifying basic therapy in OA are worthy of further study on the part of academic and industrial institutions.

2. INTRODUCTION

The term arthrosis (osteoarthrosis, degenerative joint disease, osteoarthritis) ultimately refers to an aetiologically unexplained, slowly progressing degenerative disease of one or more joints which is associated with a loss of joint cartilage, restructuring processes in the bone and reactive changes in the joint capsule tissue. The term osteoarthritis (OA) which is usually employed in the Anglo-American world is phenomenologically defined; this reflects the fact that an arthrotic joint leading a patient to consult a physician also generally indicates the appearance of a usually sporadically appearing, painful-inflammatory symptomatology. More recent investigations have indicated that there is a chronic and less marked synovitis with an increased perfusion, a raised inflammatory cell infiltration and an elevated production of cytokines also in the early stages of OA (1). Cytokines play an essential role in the pathogenesis of OA (2, 3). Despite this, however, research on cytokines and the use of anti-cytokine medications against OA (unlike rheumatoid arthritis) is still in its infancy.

3. GENERAL DEFINITION AND CLASSIFICATION OF CYTOKINES

Cytokines are tissue hormones, as a rule small proteins and/or polypeptides, that are usually responsible for acting as messengers for communication between cells. They unleash their effects mostly in the immediate surroundings of their site of secretion. Cytokines are usually not constitutively expressed, but instead are secreted or expressed on the cell surface after activation of the producing cells. However, with pronounced inflammatory processes (e.g. severe infections, severe autoimmune diseases such as rheumatoid arthritis) considerable systemic effects of cytokines can be expressed since they are then capable of entering the bloodstream and distributing themselves to numerous tissues of the body.

Cytokines are subdivided into a number of classes that include the interleukins (IL) and the

chemokines; however, growth factors should also be assigned to cytokines in their broadest definition since they often adopt important regulatory functions in the cytokine network, as is especially the case with transforming growth factor-β (TGF-β). Since cytokines can also potentially mediate dangerous functions through their signalling effects on destructive or modelling cellular systems, their production and effector functions are usually finely tuned.

In a crude schematic manner cytokines are subdivided into groups of pro- and anti-inflammatory cytokines, whereby the distinctions between the two can be rather fluid. Normally, the effects of these two systems are held in equilibrium. With OA, current thinking assumes that a faulty homoeostasis exists between the pro-inflammatory cytokines [e.g. IL-1, IL-6, IL-8, IL-17, tumor necrosis factor (TNF)-α, leukemia inhibitory factor (LIF)] and the anti-inflammatory cytokines [e.g. IL-4, IL-10, IL-11, IL-13 and IL-1 receptor antagonists (IL-1RA)].

Due to their particularly great efficacy and pluripotence, IL-1 and TNF-α are considered as dominating mediators of cartilage destruction. In animal models it has already been shown quite impressively that joint diseases occur particularly when IL-1 or TNF-α are produced excessively. Although IL-1 and TNF feature differing primary amino acid structures, their effects on mesenchymal cells are very similar. Both induce the production of prostaglandin-E_2 (PGE_2) and matrix degrading enzymes in synovial fibroblasts and chondrocytes, and also inhibit the production of proteoglycans in cartilage cells; an inhibition of the activity of these cytokines may therefore appear to be a reasonable therapeutic approach. Animal studies have shown that the therapeutic effectiveness of anti-TNF treatment primarily prevents synovitis, whereas IL-1 blockade preferentially blocks cartilage destruction. However, it should be stressed that our knowledge regarding their interactions with, as well as the role and importance of other cytokines is still rather incomplete.

4. CYTOKINE EXPRESSION DURING OA

Both *in vitro* and *ex vivo,* OA altered joint cartilages reveal an increased expression of cytokines. Attur et al. (4) reported that a range of inflammatory mediators including cytokines are expressed to a higher degree in OA joint cartilage than in normal cartilage, e.g. IL-1β, IL-6, IL-8, IL-18, TNF-α, TNF-α converting enzyme (TACE), PGE_2, COX-2, nuclear factor kappa B (NF-κB), NO, monocyte chemoattractant protein-1 (MCP-1), TGF-β, myeloperoxidase, heat shock protein 70 (HSP70), as well as the matrix metalloproteinases (MMP)-1, -3, -9, -10, and -13.

More recent studies have attempted to identify the cellular origin of the cytokines immunohistochemically both during the late stages of OA and in rheumatoid arthritis. These studies dealt above all with the synovial tissue of rheumatoid arthritis patients whereby corresponding tissue was compared in OA patients so that disease-specific differences and/or factors could be looked for. The results of these interesting studies are summarised in table 1 and show that most cytokines were found in the synovial tissue of patients irrespective of whether they suffered from OA or rheumatoid arthritis. Wherever differences were found, these were basically of a quantitative and less of a qualitative nature.

Using molecular biological techniques, the production of the cytokines IL-1 and TNF-α were quantified in the "lining cells" of the synovial membranes of patients with knee OA by Smith et al. (1), whereby as expected the densities of IL-1- and TNF-α positive cells were correlated with the severity of OA. Remarkably, these cytokines could be demonstrated even in the early stages of OA and also in arthroscopically normal joints of the same individuals with basal expression the same cytokines.

The extent to which these varyingly high IL-I levels (that depended on the severity of OA) in the synovia exerted significant effects on the metabolism of chondrocytes could only be roughly estimated from concentration thresholds established in *in vitro* studies by Dingle et al. (5) and Neidel and Zeidler (6). Thus, either an inhibition of synthesis of proteoglycans or an increased expression of catabolic enzymes could be shown in response to IL-I in human chondrocytes. Here, a significant dependence on the concentration of IL-1 was found. While with human cartilage the synthesis of proteoglycans is inhibited at relatively low IL-1 concentrations, a hundredfold higher concentration was necessary to bring about an enzymatic degradation of proteoglycans. The principle of differing thresholds for cytokine effects on the anabolic or catabolic functions of the cells was also illustrated in studies on bovine cartilage, although at considerably higher concentrations (6). If one considers the absolute concentrations of IL-1 in the synovial fluid of OA patients (in the order of pg/ml), with those of the above-mentioned *in vitro* experiments (in the order of ng/ml), this suggests that although different absolute thresholds play a role in determining the effects of cytokines, considerably more complex processes are important *in vivo*. For instance, a normal, non-activated chondrocyte has approx. 2000 specific IL-1-receptors while twice as many IL-1 receptors have been found on chondrocyte membranes of OA cartilage (7).

Depending on their effect on cartilage metabolism, cytokines may also be subdivided into the following three categories: destructive cytokines, modulatory cytokines/factors, and growth factors (Figure 1). The metabolic response of the chondrocytes to these diverse biochemical stimuli, i.e. the net effect, depends on

- the balance between cytokines amongst themselves
- the timepoint of the expression of individual cytokines
- the extent of expression
- the hierarchy of the cytokines amongst themselves
- the stage of disease

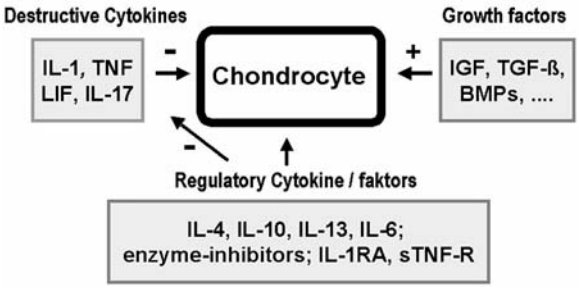

Figure 1. Balance between cytokines and growth factors.

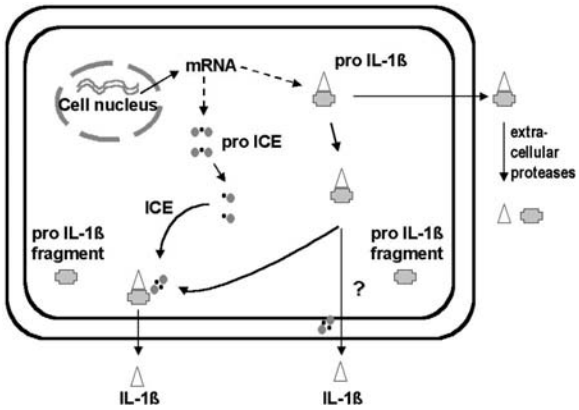

Figure 2. Synthesis and secretion of IL-1. The inactive pro-IL-1β is not usually able to traverse the cell membrane. Cleavage by the specific IL-1β converting enzyme (ICE, caspase 1) is necessary. The largest proportion of ICE exists as a precursor protein (pro-ICE, molecular weight 45 kDa), and the two subunits arising from proteolytic cleavage (molecular weight of 10 kDa and 20 kDa) are required in concert (after cleavage) in order to unleash the catalytic activity. In addition to intracellular cleavage into the mature IL-1β form, a cleavage into ICE-associated membrane channels is also assumed to occur. There is no membrane-bound form of pro-IL-1β, and only IL-1β can pass the cell membrane. If cytolysis occurs, pro-IL-1β can also be cleaved by extracellular proteases.

- other factors (e.g. mechanical stimuli and hormonal influences)

Our knowledge regarding this complex interplay is rather incomplete and currently the subject of intense academic and industrial research.

5. EXPERIMENTAL APPROACHES FOR THE BASIC THERAPY OF OA USING ANTI-CYTOKINE MEDICATIONS

New information is being gathered at great pace regarding the molecular and cellular "pathways" involved in rheumatic diseases. The number of potential target molecules for a pharmacological intervention is not just continuously increasing, but findings are being resolutely pursued by the pharmaceutical industry and numerous biotechnological firms for application as therapeutic strategies. The development of anti-cytokine medications a.k.a biologicals or immunobiologicals is still in its infancy with respect to OA (unlike rheumatoid arthritis). Table 2 shows that a great number of new substances and therapeutic principles based on an anti-cytokine principle have been developed and are already undergoing clinical testing for the indication rheumatoid arthritis. The enormous activity in the field of cytokine research can be explained by the numerous potential indications for anti-cytokine medications which are listed in table 3.

For OA, a range of pharmacotherapeutic strategies have been developed and tested with the goal of inhibiting the synthesis and/or activity of pro-inflammatory and catabolically active cytokines such as IL-1; a range of *in vitro* and *in vivo* findings have already been published. The following therapeutic approaches appear at least to be hypothetically promising:

5.1. Inhibition of cytokine synthesis

Cytokine synthesis can be blocked by inhibition of IL-1 converting enzyme (ICE; Caspase-1) or TNF-α converting enzyme (TACE), and application of IL-4 or IL-10. A range of well-known medications inhibit the synthesis of IL-1 and TNF-α, such as corticosteroids, estrogens, cyclosporin and pentoxifyllin. Corticosteroids are also IL-1 inhibitors since they suppress IL-1 gene expression and secretion *in vitro* and *in vivo* and induce the production of soluble IL-1 type II receptors. The development of ICE inhibitors is already well advanced. Generally it is assumed that more IL-1β is produced than IL-1α during inflammation. A direct inhibition of ICE or an inhibition of the cleavage of ICE precursors appear as potential therapeutic strategies (Figure 2) since ICE deficient mice produce much less IL-1β (8). However, other proteases can convert pro-IL-1β and in so doing reduce the therapeutic effect. In addition, temporal co-ordination seems important since (for example) with rheumatoid arthritis a prophylaxis rather than a therapeutic success can be achieved using an ICE inhibitor.

5.2. Inhibition of pro-inflammatory/destructive cytokine effects through application of anti-inflammatory cytokines

Anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 are also potential candidates for a basic therapy of OA, whereby these cytokines are either produced by recombinant techniques or applied gene-therapeutically. The synthesis of IL-1RA (9) in particular is induced by cytokines such as IL-4, IL-10 and IL-13, while the first two also suppress the transcription and translation of IL-1. IL-4, IL-10 and IL-13 also express their destruction inhibiting effect on joint cartilage via inhibition of MMP production and stimulation of TIMP synthesis, the endogenous inhibitors of MMPs. IL-10 is already being tested in clinical trials for the treatment of rheumatoid arthritis (table 2). Only recently Miagkov et al. (10) reported on a remarkable gene-therapeutic experiment using a rat model of rheumatoid arthritis, in which the expression of IL-10 could even be autoregulated in a manner dependent on the degree of inflammation.

Table 1. Relative occurrence of cytokines in the synovia of patients with OA and rheumatoid arthritis (modified according to 3)

	OA	Rheumatoid arthritis
IL-1	++	+++
IL-1RA	+++	++
TNF-α	+	+++
sTNF-R	++	++
LIF	++	+++
IL-4	-	+
IL-6	++	+++
IL-8	++	++
IL-10	+	++
MCP-1	++	++

IL: interleukin; RA: receptor antagonist; TNF-α: tumor necrosis factor alpha; sTNF-R: soluble tumor necrosis factor-receptor; LIF: leukemia inhibitory factor; MCP-1: monocyte chemoattractant protein-1

Table 2. Clinical studies employing anti-cytokine medications with rheumatoid arthritis

Cytokine receptor antagonists • IL-1 RA Cytokine receptors • soluble IL-1 type II receptor • soluble IL-15 receptor • soluble TNF-p55 receptor (Onercept) Anti inflammatory cytokines • IL-10 • IL-11	Monoclonal antibody (mAb) • human anti-IL-8 mAb • chimeric anti-IL-6 receptor mAb • anti-C5 mAb • anti-TNF-α mAb • anti-CD4 mAb Enzyme-inhibitors • oral ICE inhibitors (for example Pralnacasan) • oral p38-MAPK inhibitor • oral type IV phosphodiesterase inhibitor • oral MMP inhibitors

Not listed in this table are medications that are already authorized for the therapy of rheumatoid arthritis, but which act through another mechanism (e.g. COX-2 inhibitors) as well as human and/or humanized anti-TNF α antibodies. If not stated otherwise, the above-mentioned substances are applied parenterally. p38-MAPK: mitogen-activated protein kinase with a molecular weight of 38 kDa.

Table 3. Clinical studies employing anti-cytokine medications for different diseases

Anti-TNF-α based therapy (e.g. Ethanercept, Infliximab)	**IL-1 receptor-Antagonist (e.g. Anakinra)**
• Rheumatoid arthritis • Psoriasis • Psoriatic arthritis • Ulcerative colitis • Still's disease • Rheumatoid spondylitis (Bechterev's disease) • Behçet's disease • Crohn's disease • Uveitis • Advanced cardiac defect • Wegener's granulomatosis	• Rheumatoid arthritis • Diabetes mellitus • Leukemia • Arteriosclerosis • Multiple sclerosis • Osteoarthritis

5.3. Regulation of cytokine receptor expression

Cytokine receptor expression can be modified for example by TGF-β, whereby this can either be produced recombinantly or applied gene-therapeutically. TGF-β, however, numbers amongst the dualistically effective growth factors with anabolic and catabolic effects, depending on target cell, tissue localization and concentration. From animal-experimental studies it is also known that TGF-β can induce the formation of osteophytes, i.e. a "classical" feature of OA (15). Here we should be reminded of the increased sensitivity of OA chondrocytes towards IL-1 which is based on the doubled numbers of IL-1 receptors (7). This raised sensitivity means that only 1 % instead of 4 % of the receptors need to be occupied by IL-1 in order for a half-maximal increase in the synthesis of MMPs to be brought about. Since it suffices to occupy only a small number of receptors with IL-1 to induce a metabolic response, an amplification mechanism is assumed to be involved in signal transduction. In a similar way increased numbers of TNF-α receptors were found on the membranes of OA altered chondrocytes (11). This enrichment was verified particularly in areas of OA knee

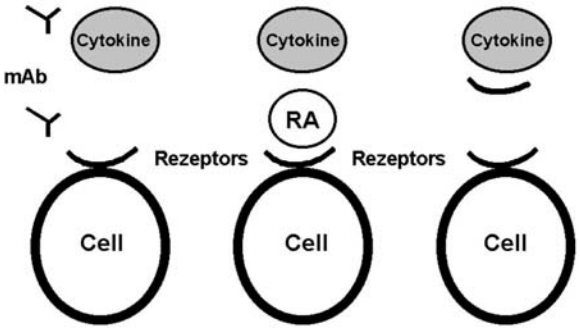

Figure 3. Selective inhibition of cytokine effects. Monoclonal antibody (mAb) binds either to the cytokine or its receptor (left); receptor antagonists (RA) bind competitively to the receptors and block them, without the signal cascade being activated (middle); soluble receptors that represent a truncated version of the cell membrane receptors due to their lacking transmembrane and intracytoplasmic domains have an approx. 1000-fold higher affinity towards cytokines compared to the mAb (right).

joint cartilage that were mechanically stressed and damaged, a fact which offers a potential explanation for the initially focally appearing cartilage destruction that occurs with OA.

5.4. Inhibition of cytokine effects through application of monoclonal antibodies

Monoclonal antibodies against for example IL-1, TNF-α or their receptors can suppress the effect of cytokines (Figure 3). Already now, a gene-technologically produced monoclonal humanised anti-TNF-α antibody (Infliximab, Remicade®) is being used with positive effect for rheumatoid arthritis as well as for Crohn's disease. Infliximab is a chimeric drug: its unchangeable region consists of a human immunoglobulin G1 (IgG1) part, and its variable regions are derived from a murine antibody. The antibody binds highly selectively to human TNF-α. As a result the release of pro-inflammatory cytokines such as IL-1 and IL-8, the expression of endothelial adhesion molecules and thereby the infiltration of leukocytes is reduced.

5.5. Inhibition of cytokine effects through application of soluble cytokine receptors

Figure 3 explains the principle. Using Etanercept (Enbrel®), a fusion protein synthesized by gene expression from Chinese hamster ovarian cell lines consisting of the extracellular binding portion of human TNF-α receptors and the Fc portion of human IgG1, adults with active rheumatoid arthritis have been treated in Germany already since June 1st 2000; such patients could no longer be adequately treated with basic medications including methotrexate. The abovementioned fusion proteins have the benefit that they reveal a higher affinity towards cytokines and an extended half-life in the bloodstream. However, such benefits are balanced by the potentially higher risk of immune reactions. TNF-α exerts its pro-inflammatory effect via two receptor subtypes that occur both in soluble and membrane-bound forms. TNF circulates in the form of trimeric molecules. Etanercept binds at two points on these trimers and in this way prevents cytokine interaction with the membrane-bound receptors. With therapeutic application of this TNF-R_{p75}-Fc fusion proteins (Etanercept), not only was an effective neutralization of TNF-α shown in a recently published study with rheumatoid arthritic patients, but also a clear improvement of the disease symptoms was achieved (12).

5.6. Blockade of cytokine-receptors through application of cytokine receptor antagonists

The application of IL-1RA (Figure 3) is currently a subject of great interest. Using IL-1 as an example the effect of cytokine receptor antagonists can be explained: IL-1 binds to the membrane-bound type I IL-1 receptor. Both then form a heterodimer complex with the accessory protein IL-1RAcP that then activates a signal cascade in the cell. In the healthy joint an endogenous IL-1RA competitively inhibits IL-1 binding to the receptor without bringing about any effect. As a result, IL-1RacP is no longer able to bind. In rheumatoid arthritis as well as with OA, IL-1 and the endogenous receptor antagonist are not in equilibrium, and the IL-1 mediated effect predominates. By applying IL-1RA an opportunity becomes available to directly inhibit the destructive effects of IL-1.

An important aspect here is that although IL-1RA binds to its receptor with the same affinity as IL-1, a 50% inhibition of the effects induced by IL-1 demands at least a hundredfold surplus of IL-1RA compared to IL-1. Only such a surplus leads to the displacement of IL-1 from its receptor molecule. Since a biological response is already induced by 1-2% IL-1 occupancy, the IL-1RA must be present at a 100-2000-fold higher concentration in order to suppress the cellular stimulation *in vivo* through binding (2, 3) to "all" receptors.

IL-1RA is currently either produced by recombinant techniques (Anakinra, Kineret®), applied in animal-experimental as well as clinical studies gene-therapeutically, or is induced *ex vivo* in autologous monocytes (Orthokin®) before it is applied intra-articularly in patients with rheumatoid arthritis or OA.

The application of various gene-therapeutic approaches is designed to bypass problems associated with applying proteins, experiencing potential side effects and maintaining sufficiently high levels of active substances in the joint; in this respect gene therapy also represents a new, in future available form of medicinal application. The transfection of IL-1RA in chondrocytes or fibroblasts produces a longer-term increased secretion of IL-1RA (12). In associated animal-experimental studies on rheumatoid arthritis as well as OA the local IL-1RA secretion acts chondroprotectively (13, 14, 15). Preliminary clinical studies on patients with rheumatoid arthritis are currently being undertaken (14).

Anakinra (Kineret®) was developed by the US-American biotech enterprise Amgen and is produced by recombinant DNA technology with the aid of an

Escherichia coli expression system. It resembles the native, human IL-1-RA except for the additional amino acid methionine at the N-terminus and the absence of glycosylation. Since April 2002 Anakinra has been authorized for the therapy of rheumatoid arthritis in Germany; until now the data has been supportive of the idea that Anakinra provides a new effective weapon against rheumatoid arthritis. Animal studies have confirmed that the intra-articular injection of IL-1RA can prevent the appearance of OA lesions in the knee joints of dogs (16). At this time, the first clinical studies are being initiated with the goal of also treating OA patients with Anakinra in the future.

Unlike the case with rheumatoid arthritis, studies on the application of anti-cytokine medications with OA remain in their infancy. At the present time no clinical studies relating to OA have confirmed that anti-cytokine medications are antiphlogistically effective and/or prevent the origin of morphologically recognizable cartilage defects or at least decelerate the increasing destruction of joint cartilage. Also, little is currently known about the interplay between pro- and anti-inflammatory cytokines and growth factors in OA; the resulting specific and fundamental therapeutic possibilities for performing a structure-modifying basic therapy in OA are worthy of further study on the part of academic and industrial institutions.

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Key Words: Osteoarthritis, Bone, Inflammation, Cytokine, Drug, Pharmacotherapy, Cartilage, Review

Send correspondence to: Priv.-Doz. Dr. Jürgen Steinmeyer, Orthopaedic Research Laboratories, Department of Orthopaedic Surgery, Justus-Liebig-University of Giessen, Paul-Meimberg-Str. 3, D-35385 Giessen, Germany, Tel.: ++49-(0)641-99-42920, Fax: ++49-(0)641-99-42939, E-mail: juergen.steinmeyer@ ortho.med.uni-giessen.de