REVIEW

Interleukin-1, immune activation pathways, and different mechanisms in osteoarthritis and rheumatoid arthritis

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The properties of interleukin-1 suggest that it has a central role in the immunopathology of many arthritides (figure). It is now more than 10 years since it was realised that numerous biological activities were due to a single agent called interleukin-1 (IL-1)¹ (now known to be two related proteins, IL-1 α and IL-1 β ²). The functions of IL-1, such as T cell activation,³ stimulation of cellular production of prostaglandin E₂ and I₂,⁴ interleukin-1,⁵ and interleukin-6,⁶ help for B cell growth,⁷ induction of endothelial cell adhesion molecule expression,⁸ stimulation of chondrocyte stromelysin,⁹ synovial cell collagenase, and plasminogen activator production,^{10–12} induction of bone resorption,¹³ and hepatic acute phase protein synthesis, show its enormous potential for mediating pathological processes. The 10th birthday of this 'first interleukin' was recently celebrated in a comprehensive review of our current knowledge by di Giovine and Duff.¹⁴ In addition to extending the role of IL-1 as an activator of immune function, recent data have provided a more complete picture of a physiological system, with negative feedback pathways and inhibitors of IL-1 activity. This review of IL-1 covers recent *in vitro* findings together with data from *in vivo* studies and discusses the implications for rheumatoid arthritis and osteoarthritis.

Interleukin-1: a complex biological system

CONTROL OF PRODUCTION

Interleukin-1 α and IL-1 β protein production is controlled at several levels, including gene transcription, messenger RNA (mRNA) stability, translation to form the 31 kD precursor protein, and release of the processed mature 17.5 kD protein.¹⁵ The complexity of IL-1 production is illustrated by the high concentrations of IL-1 mRNA that can be stimulated by adherence of monocytes to glass.¹⁶ This mRNA is not translated into protein, however, unless a second signal (usually endotoxin) is supplied. Human monocytes are very sensitive to endotoxin and can produce IL-1 in response to the low concentrations commonly found in tissue culture media.¹⁶ Both endotoxin and IL-1 stimulate transcription and translation of IL-1.⁵ Glucocorticoids can reduce IL-1 transcription and translation,^{17 18} in contrast with prostaglandins, which reduce translation without changing gene transcription.¹⁹

Unlike most secreted proteins, IL-1 lacks a

distinct cleavage site for the N-terminus, resulting in much IL-1 remaining cell associated.²⁰ Interleukin-1 β is secreted more efficiently than IL-1 α and makes up most extracellular IL-1 (in cell supernatants and body fluids).²¹ Interleukin-1 α is found associated with cells and may form a 'membrane bound' biologically active IL-1.²² This finding is still in contention, however.²³ Despite having only 26% homology, IL-1 α and IL-1 β bind to the same receptors and seem to have similar activity.^{15 24} Interleukin-1 β is vulnerable to oxidation, in contrast with IL-1 α , which is a stable molecule.²⁵

INTERLEUKIN-1 INHIBITORS

Inhibitors of the biological activities of IL-1 were noted when it was first detected in body fluids, including synovial fluid.^{26–28} The recent characterisation of the 22 kD inhibitor is a major advance in IL-1 biology.^{29 30} This inhibitor is related to the interleukin-1 family with 26% sequence homology to IL-1 β and 19% homology to IL-1 α and competes for the IL-1 receptor. The natural inhibitor has shown functional inhibition of IL-1 induced neutrophil adherence to endothelial cells and cartilage degradation,³¹ and the recombinant protein inhibits IL-1 stimulated prostaglandin synthesis *in vitro*.³⁰ It is produced by monocyte lineage cells with different kinetics and in response to differing stimuli than IL-1. Apparently, immature monocytes predominantly secrete IL-1, in contrast with mature macrophage-like cells, which produce the inhibitor.³² It is unclear if the same cell can produce both.

Natural inhibitors of cytokines make measurement of *in vivo* cytokine concentrations difficult, and this difficulty is increased by the presence of binding factors in plasma, including α_2 macroglobulin.³³ Additionally, specific cytokine binding immunoglobulin autoantibodies have been shown for IL-1 α and tumour necrosis factor α .³⁴ These antibodies may function as physiological carriers for cytokines as their binding affinity for IL-1 is less than the high affinity receptor found on T lymphocytes but greater than the affinity of receptors found on cells such as endothelial cells. Recent findings suggest that these two forms of the IL-1 receptor with low and high affinities for IL-1 are expressed on different cell types.³⁵ High affinity IL-1 receptors have been identified on synovial cells from normal subjects and those with osteoarthritis and rheumatoid arthritis.³⁶

Biological activities of interleukin-1 (IL-1) (established by *in vivo* studies). PGE=prostaglandin E; CNS=central nervous system; ACTH=adrenocorticotrophic hormone; GM-CSF=granulocyte-macrophage colony stimulating factor; TNF=tumour necrosis factor.

THE CYTOKINE NETWORK

Interleukin-1 is part of a network of cytokines which can interact in additive, synergistic, or inhibitory ways through direct cytokine effects or the effects of cytokines on cellular receptors.³⁷⁻³⁹ Recent examples of the complexity of *in vitro* interactions are the additive or inhibitory effects of IL-1 and platelet derived growth factor on fibroblast proliferation depending on the presence of indomethacin,⁴⁰ and the change in endotoxin stimulated U937 cell IL-1 production induced by interferon γ .⁴¹ Pretreatment of this cell line with interferon γ results in prolonged IL-1 α and IL-1 β gene expression for 16 hours, in contrast with the normal transient response. Many cytokines, including IL-1 α and IL-1 β , tumour necrosis factor α , IL-6, epidermal growth factor, transforming growth factor β , and granulocyte-macrophage colony stimulating factor, have been detected in rheumatoid synovial fluids, indicating a large number of potential *in vivo* interactions.^{31 42 43} For example, transforming growth factor β can antagonise many IL-1 mediated activities *in vitro* and is present at high concentrations in rheumatoid synovial fluids.³¹ Cytokines also have important interactions with prostaglandins and leukotrienes, which were recently reviewed in detail in this journal.⁴⁴ Interleukin-1 is a potent stimulus for prostaglandin E₂ production. Prostaglandin E₂, however, reduces IL-1 production, demonstrating another potential natural feedback loop. In contrast, leukotrienes stimulate IL-1 production. Changes in leukotriene B₄ production are thought to explain the reduction of *in vitro* IL-1 production that accompanies a high polyunsaturated omega-3 fatty acid (fish oil) diet.⁴⁵ The *in vivo* importance of interrupting prostaglandin negative feedback

on IL-1 production by non-steroidal anti-inflammatory drug treatment has not yet been resolved.

Interleukin-1 *in vivo*

IN VIVO DISTRIBUTION

Cytokines (and their inhibitors) have been detected in synovial fluids by bioassay and immunoassay and more recently in synovial tissue by mRNA analysis (northern blot, slot blot, or *in situ* hybridisation) or immunohistological techniques.^{31 42 43 46-48} Interleukin-1 α and IL-1 β have been detected in the peripheral blood of patients with rheumatoid arthritis but not osteoarthritis.⁴⁹ Concentrations of IL-1 β correlated with the Ritchie index and erythrocyte sedimentation rate in cross-sectional analysis, and synovial fluid concentrations of IL-1 β correlated with clinical signs in paired knee joint samples from patients with rheumatoid arthritis.⁴⁹ The source of circulating IL-1 β is unclear as although concentrations are increased in synovial fluids, recent studies have shown that peripheral blood cells from subjects with rheumatoid arthritis spontaneously secrete IL-1.^{50 51} These findings are difficult to interpret as most experiments were performed in conditions that did not exclude laboratory stimulation by adherence of monocytes or low concentrations of endotoxin. Both of these reports, however, did compare the rheumatoid samples with control samples from normal or osteoarthritic patients which did not spontaneously produce IL-1, though they had undergone similar laboratory procedures. The above reservations are even more relevant to many experiments using cultured synovial membrane cells where access to normal control tissue can be difficult.

By in situ hybridisation analysis of rheumatoid synovial membrane, Duff and colleagues showed that CD14 positive macrophages contain IL-1 β mRNA.⁴⁹ We detected IL-1 β , by immunohistological techniques, in cells scattered throughout perivascular aggregates and in some lining layer cells in rheumatoid synovial membrane.⁵² These techniques will be increasingly applied to assess cytokine production in disease states and models of inflammation and to assess modes of drug action in vivo. For example, we showed a significant decrease in IL-1 β staining after 12 weeks of sodium aurothiomalate treatment in sequential synovial biopsy specimens from six patients with rheumatoid arthritis. This decrease occurred without significant changes in the synovial mononuclear cell composition.⁵³ These methods will provide a picture of the cytokine network present in different disease states, and for IL-1 at least, an assessment of the balance between cytokine and inhibitor production may soon be possible.

IN VIVO FUNCTION

Most of the activities ascribed to IL-1 have been determined in vitro and must be regarded as potential functions. The in vivo function of cytokines can be assessed by the use of recombinant cytokines and cytokine inhibitors, by the use of specific anticytokine monoclonal antibodies, or by the use of transgenic animal models. Henderson and Pettipher showed that intra-articular administration of IL-1 α or IL-1 β in rabbits causes rapid loss of cartilage proteoglycan and a rapid increase of neutrophils and monocytes in the synovial compartment.⁵⁴ Interestingly, synovial fluid prostaglandin concentrations did not rise and no clinical signs of inflammation such as swelling or warmth were seen. Tumour necrosis factor α had less potent but similar effects and, together, IL-1 β and tumour necrosis factor α showed synergistic effects, confirming in vitro predictions.⁵⁵ Repeated intra-articular injections of IL-1 into the ankles of normal rats produces a synovial cellular infiltrate and fibrosis in the absence of cartilage or bone destruction.⁵⁶ Interleukin-1 given intra-articularly or systemically can exacerbate low grade antigen induced arthritis in animal models.⁵⁶⁻⁵⁷ The in vivo picture is complicated, however, as the exacerbation seen in collagen induced arthritis varied depending on the relative timing of disease stimulus and cytokine.⁵⁸ Jacobs *et al* reported that intra-articular IL-1 β actually decreased antigen induced arthritis when given both before or during the arthritis.⁵⁹ The timing and dose of cytokine in relation to a stimulus is crucial. Although high dose IL-1 given intravenously causes toxic effects, such as hypotension, fever, and neutrophilia,⁶⁰ low dose IL-1 given before noxious stimuli, such as endotoxin, falciparum malaria, or radiation, can improve survival in animal models.⁶¹ The mechanisms for these effects are not known but may involve induction of cytokine inhibitors, reduction of cytokine receptor expression, or stimulation of cortisol production, and may demonstrate some of the

physiological roles of IL-1. Many of these protective effects can be abrogated by pretreatment with non-steroidal anti-inflammatory drugs.

These models suggest that IL-1 activity in vivo might contribute to cartilage damage and promote inflammatory changes. This activity, however, will be suppressed by natural inhibitors. Inhibition of interleukin-1 activity increases over time in antigen induced arthritis in rabbits.⁶² Two studies of patients with rheumatoid arthritis attempted to correlate synovial fluid IL-1 concentrations with measures of local joint damage. Saxne and colleagues showed that synovial fluid concentrations of IL-1 do not correlate with synovial fluid proteoglycan fragment concentrations.⁶³ Miyasaka and colleagues, however, reported that concentrations of IL-1 secreted by cell cultures from synovial biopsy specimens did correlate with radiographic damage.⁶⁴ A single study reported modest short term improvements in patients with rheumatoid arthritis treated with an IL-1 inhibitor.⁶⁵

Interleukin-1 in osteoarthritis

INTERLEUKIN-1 PROPERTIES AND OSTEOARTHRITIS

As outlined above, much attention has been paid to the role of IL-1 in inflammatory arthropathies such as rheumatoid arthritis. Interleukin-1 has also been consistently detected in synovial fluid samples from patients with osteoarthritis.^{26 42 66} The processes initiating osteoarthritis are unknown and it is unclear if the end stage of cartilage failure is due mainly to proteoglycan, collagen, subchondral bone, or vascular defects.^{67 68} It is known that raised levels of degradative enzymes, such as collagenase and stromelysin, are present in osteoarthritic cartilage as is plasminogen activator, an important activator of these enzymes.^{69 70} Interleukin-1 has many properties, such as stimulation of stromelysin, collagenase and plasminogen activator secretion, and direct bone resorbing activities, that might account for many of these changes. Tumour necrosis factor α produces similar changes and has also been found in some osteoarthritic synovial fluids.⁷¹ It is generally found in a lower proportion of osteoarthritic synovial fluids than is IL-1. This suggests that synergistic effects at very low concentrations may occur in some patients, contributing to the variable rates of disease progression seen between patients. In addition to its catabolic effects, IL-1 has anabolic effects, such as increasing fibroblast collagen synthesis,⁷² which might account for other changes, such as osteophyte formation.

IN VIVO DISTRIBUTION IN OSTEOARTHRITIS

It is now clear that infiltrates of mononuclear cells are commonly found in osteoarthritic synovial membranes, even in patients with early disease.^{73 74} We and other groups investigated the distribution of IL-1 β in the osteoarthritic synovium, using immunohistological techniques. Pelletier *et al* reported IL-1 β in osteoarthritic synovial lining layer cells, with some

staining in the deeper tissues.⁷⁵ Shinmei *et al* demonstrated IL-1 β staining in osteoarthritic chondrocytes.⁷⁶ We detected IL-1 β staining consistently in synovial lining layer cells, with almost no IL-1 β being detected in the perivascular lymphoid aggregates that were commonly present.⁵² This distribution of IL-1 β staining was different from that found in rheumatoid arthritis, where it was mainly in the perivascular aggregates. This suggests that although IL-1 is present in both the rheumatoid and osteoarthritic synovial compartment, it is stimulated by different processes. The presence of IL-1 staining in the perivascular cellular infiltrates in rheumatoid arthritis suggests that these infiltrates may be the site of an active cell mediated immune process which interacts with other components of the systemic immune system. This is supported by the systemic nature of rheumatoid arthritis, with circulating IL-1⁴⁹ and IL-1 producing cells,^{50 51} raised erythrocyte sedimentation rate, and soluble interleukin-2 receptor concentrations.⁷⁷ In contrast, the localised lining cell activation in osteoarthritis would produce localised increases of IL-1 in the synovial fluid in the absence of systemic immune activation. Many cells in the osteoarthritic synovial membrane infiltrate may be inactive bystander cells, as suggested for the rheumatoid synovial membrane by Ziff in 1974.⁷⁸ The suggestion of differing mechanisms of IL-1 production in these diseases is supported by the results of Brennan and colleagues.⁷⁹ They found that in vitro osteoarthritic synovial cells produced low concentrations of IL-1 compared with the high concentrations produced by rheumatoid synovial cells. Both groups produced high concentrations of tumour necrosis factor α . When monoclonal antibodies to tumour necrosis factor α were added to the system, however, IL-1 production was reduced only in the group with rheumatoid arthritis, with no change occurring in IL-1 production by osteoarthritic synovial cells.

A POTENTIAL ROLE IN OSTEOARTHRITIS

The detection of IL-1 in osteoarthritic synovial membranes has been in patients with established disease, highlighting the difficulties of studying early disease in humans. It is therefore unclear if the lining cell layer production of IL-1 is an early or late development. Goto and colleagues reported that monocytes can be stimulated to produce IL-1 by fragments of human cartilage, in particular by collagen type II.⁸⁰ For osteoarthritis a possible mechanism relating cytokines to disease might be an initial insult to cartilage with release of cartilage fragments which then stimulate synovial lining cell production of IL-1. The properties of IL-1 may then contribute to the progress of the lesion. In this situation the balance of cytokine and inhibitor would play a crucial part.

The importance of IL-1 (and other cytokine) secretion in the early stages of disease will be best studied in animal models.⁸¹ Interleukin-1 has been detected in the synovial fluid of equine osteoarthritis.⁸² Two facts suggest this is an important point to resolve. Glucocorticoids

have been shown to prevent progression of disease in osteoarthritis models, in contrast with non-steroidal anti-inflammatory drugs, which have no effect. Glucocorticoids have many effects, including reduction of collagenase production, which may mediate this improvement. It must be remembered that another powerful activity is the reduction of cytokine expression. The second fact relates to reports that non-steroidal anti-inflammatory drugs, which reduce prostaglandin concentrations, may cause a more rapid deterioration of osteoarthritis.⁸³ The mechanisms by which this might occur are unclear but, possibly, suppression of the prostaglandin negative feedback on IL-1 production may contribute to this deterioration.

Conclusion

The increasing in vivo definition of cytokine activities and distribution promises to provide the clinician with explanations of disease pathology. Important lessons about the ease of non-physiological stimulation of cells (recently illustrated by the in vitro production of tumour necrosis factor α in patient samples⁸⁴) mean that clinical studies must be meticulously planned and executed if meaningful data are to be gained. With the availability of ultrasensitive techniques using the polymerase chain reaction to detect very low concentrations of cytokine mRNA⁸⁵ the complex control of production of many cytokines must be remembered, and cytokine production should preferably be reported at both the mRNA and peptide level. The recombinant materials, specific monoclonal antibodies, and techniques are now available to ensure a continuation of the recent encouraging increase of studies to define in vivo cytokine function.

The findings for IL-1 described in this review give an indication of the potential available information but have little meaning in isolation. A more complete picture of the cytokine network that operates in many diseases will emerge when the range of cytokines is assessed at the site of disease activity. It may become apparent that there is a hierarchy of cytokines in different diseases which might be regulated by inhibiting the actions of a limited number of cytokines. At present the great overlap of cytokine activities suggests to many in this field that this will not be the case. An interesting hypothesis would suggest that so called 'non-inflammatory' diseases such as osteoarthritis, which may have a limited number of participating cytokines, may be more appropriate targets for these immunotherapies of the future.

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