Cytokines and eicosanoids in rheumatic diseases

It is almost 20 years since non-steroidal anti-inflammatory drugs (NSAIDs) were shown to inhibit the synthesis of prostaglandins. Although our knowledge of the molecular nature and pharmacology of prostaglandins and other eicosanoids has increased considerably during this period, their role in tissue physiology and inflammation is still incompletely understood.\(^1\)\(^2\) It seems generally agreed, however, that inhibiting their synthesis has not helped in modifying the progression of inflammatory rheumatic diseases.

The eicosanoids, like other low molecular weight regulatory factors, such as platelet activating factor, the kinins, histamine, and serotonin, seem to act as local hormones and mediate quite rapid tissue responses. More recently, interest has focused on a group of protein based cell regulatory factors, or cytokines, which usually act in a similarly localised fashion but mediate longer term effects.\(^3\)\(^4\) Although the definition of cytokines is somewhat loose at present, regulatory factors that fall within this category include the interleukins, tumour necrosis factor(s), the interferons, bone marrow colony stimulating factors, and a variety of other ‘growth factors’ (fibroblast, epidermal, transforming, and platelet derived growth factors). This nomenclature is not accurately descriptive and is to some extent misleading. For example, most of the interleukins act as growth and differentiation factors not only for leucocytes but for a variety of other cells, including bone marrow, fibroblasts, and neural cells. Interferons can regulate the growth of haemopoietic and other cells, and cytokines in each category can regulate lymphocyte and inflammatory cell activity. These activities, and the ability of many of them to regulate growth and function of connective tissue cells, indicate that cytokines are likely to be directly or indirectly involved in the abnormal regulation of various tissues affected by rheumatic diseases. A few, such as interleukin 1, interleukin 6, tumour necrosis factor \(\alpha\), and interferon gamma, have received special attention. Interestingly, and with one notable exception (interleukin 6), many cytokines have been found to influence eicosanoid metabolism. The question therefore arises as to the importance of this activity and how it is regulated. The problem in answering this is that although there are considerable data relating to the molecular nature of these mediators and the phenomena that are regulated by them, there is little indication of how any of them act at the cellular level, let alone how they relate to each other.\(^1\)\(^3\)\(^4\)

**Regulation of eicosanoids by cytokines**

The pathways by which arachidonate and related fatty acids are metabolised, through the cylooxygenase pathway to the various prostanoids (prostaglandins, prostacyclins, and thromboxanes), or through the lipoxygenase pathway to the hydroxy eicosanetranotes and leukotrienes, have been quite well described. The way in which this metabolism is regulated is unclear.\(^1\)\(^2\) Different cells may regulate eicosanoid metabolism differently, depending on variations in phospholipid composition of the cell membrane and differential regulation of enzyme pathways. Fibroblasts have most commonly been used to investigate cytokine regulation of eicosanoid metabolism and a recent paper noted that the NSAID indomethacin modified the proliferative activity of interleukin 1 and tumour necrosis factor on synovial but not skin fibroblasts.\(^5\) The reason for such differences is unclear but emphasises the difficulty in interpreting experimental results too generally. Omega-3 (n-3) fatty acids will take the place of arachidonic acid (an omega-6 fatty acid) in cell membrane phospholipids. Their inclusion in the diet has been used to decrease prostaglandin \(E_2\) and leukotriene \(B_4\) production in favour of their less active counterparts, prostaglandin \(E_3\) and leukotriene \(B_5\). In a study in which n-3 fatty acids were included in the diet of healthy volunteers the production of prostaglandin \(E_2\) by their isolated monocytes fell, together with the ability to produce interleukin 1 and tumour necrosis factor.\(^6\)

The rate limiting step for synthesis of eicosanoids is generally considered to be the release of arachidonate from membrane phospholipids. The classical enzyme responsible for this is phospholipase \(A_2\), which is able to cleave fatty acids from the \(\beta\) position (figure). Arachidonate is a common fatty acid in this position, particularly in the inositol phosphoglycerides. Inositol phosphoglycerides are important substrates of the biochemical pathway, activated by many cell membrane receptors, that leads to the release of intracellular calcium (mediated by cleavage of inositol triphosphate from the biphosphorylated form of inositol phosphoglyceride) and activation of protein kinase C (mediated by the other product of this cleavage, diacylglycerol). At least two mechanisms of arachidonate release are then potentially possible (figure). Diacylglyceride lipase may cleave arachidonate from the diacylglycerol or the increased calcium may activate phospholipase \(A_2\), or both. Interleukin 1 increases the synthesis of both cylooxygenase and phospholipase \(A_2\).\(^7\)\(^8\) To date, however, there is little or
no evidence of inositol phosphoglyceride activation or a rise in intracellular calcium following binding of interleukin 1 to its receptor(s). Consistent with this, calcium ionophores and agonists, such as bradykinin, bombesin, or thrombin, which are able to increase intracellular calcium concentrations by activation of the phosphatidylinositol pathway, potentiate the eicosanoid inducing activity of interleukin 1 considerably.9 10 The ability of interleukin 1 to increase prostaglandin synthesis in the absence of a second stimulus may be explained by its ability to activate or induce the synthesis of other, Ca** independent, phospholipase activities.11

Most reported experimental data indicate that prostaglandin release occurs slowly (increasing over hours) after stimulation with cytokines such as interleukin 1 or tumour necrosis factor. This contrasts with the release induced by mechanisms involving phosphoinositide turnover, which occurs within minutes. This is consistent with cytokine induced synthesis of enzymes in the eicosanoid synthetic pathway. After systemic administration of cytokines such as interleukin 1 and tumour necrosis factor, however, a very rapid synthesis of prostaglandin is apparent, and there are also reports of rapid induction in vitro.12 As a result of some elegant experiments investigating phospholipid turnover in lymphoid cells induced by interleukin 1,13 Dinarello et al suggested that the source of arachidonate might be diacylglycerol released from phosphatidylcholine. The mechanism by which this occurs is not entirely clear, though some phospholipase C-like activity is presumably activated. Lymphoid cells are not noted for their ability to produce large amounts of eicosanoids but if this pathway operates in endothelial cells it might explain the rapid production of interleukin 1 induced prostaglandin. Platelet derived growth factor also releases prostaglandins in a rapid and slow phase.14 The slow phase (peaking at six hours) is consistent with induction of cyclooxygenase synthesis, while the rapid phase (peaking at 10 minutes) indicates activation of some other protein synthesis-independent process. Platelet derived growth factor occurs as dimers of A chain proteins, B chain proteins, or as an AB heterodimer. Identification of two distinct platelet derived growth factor receptors, with differential affinities for the various dimers, has indicated that each receptor activates different pathways.15 16 Possibly this may explain the dual effect on eicosanoid metabolism.

Another potential mechanism for regulation of eicosanoid activity is to influence eicosanoid receptors. This area is largely unexplored, though it has recently been observed that leukotriene B4 receptors on neutrophils are reduced in number and affinity after preincubation with tumour necrosis factor.17

Cytokine activity mediated and regulated by eicosanoids
Many of the effects of systemically injected interleukin 1 and tumour necrosis factor, such as fever and hypotension, can be inhibited by pretreatment with NSAIDs. Interleukin 1 induced release of plasminogen activator by synovial cells seems to be mediated by eicosanoid synthesis, and bone resorption stimulated by interleukin 1, tumour necrosis factor, epidermal growth factor, and transforming growth factors α and β can be shown to be at least partially mediated by prostaglandins in some systems. A study by Dewhirst et al, however, noted that although bone resorption induced by interleukin 1 could be inhibited by indomethacin, the synergistic resorbing activity of interleukin 1 and parathyroid hormone could not.18

Tumour necrosis factor and interleukin 1 can increase the rate of proliferation by fibroblasts. This effect can be inhibited if the concentration of prostaglandins is allowed to rise and act as a negative regulator. An interactive role in regulation of fibroblast function is further suggested by the ability of prostaglandins to increase the number of interleukin 1 receptors.19 Elias found that interleukin 1 and tumour necrosis factor synergistically inhibited fibroblast proliferation and that this inhibition was blocked by indomethacin.20 In contrast, interferon gamma, which has been shown to increase the numbers of receptors for tumour necrosis factor and also to inhibit fibroblast proliferation, together with tumour necrosis factor synergistically inhibited fibroblast proliferation in an apparently prostaglandin independent manner. The effect of interferon (especially interferon gamma) on eicosanoid synthesis is somewhat contentious in itself. Interferon stimulates an increase in eicosanoid metabolism in many cells and there is even some evidence that this may be important for its antiviral and antiproliferative activities. In contrast, a number of studies have shown that interferon gamma inhibits prostaglandin
production by monocytes/macrophages, and it has been suggested that this activity may, at least partially, account for its demonstrated efficacy in rheumatoid arthritis.21

Others, however, have found that interferon gamma fails to decrease the synthesis of prostaglandins induced by interleukin 1 and tumour necrosis factor α.22

Morley and his colleagues showed that prostaglandins, produced by macrophages during an inflammatory response, may down regulate immune activation by suppressing lymphokine production.23 More recently it has been shown that prostaglandins also inhibit monocyte interleukin 1 production at the level of secretion and inhibit tumour necrosis factor α gene expression.24 25 Together with the evidence that some NSAIDs may have a negative effect on cartilage integrity, a process likely to be regulated by cytokine function, the data indicating that prostaglandins have a regulatory role seem again to raise the question as to whether it is a good idea, from the point of view of disease modification, to prevent prostaglandin production. The ability of leukotriene B4 to enhance interleukin 1 production by monocytes and stimulate lymphocyte activity,26 27 suggests that inhibition of the lipoxigenase pathway might be more effective in reducing tissue damage. Demonstration that interleukin 1, tumour necrosis factor, and prostaglandin production were each decreased by inclusion of n-3 fatty acids in the diet6 indicates that decreased production of leukotriene B4 may be responsible for reduced cytokine production or that n-3 products may be inhibitory.

So what role might NSAIDs already be playing in modifying cytokine action? As indicated above we know too little about the cellular interactions of cytokines and eicosanoids to answer this adequately at present. The receptors for bioactive eicosanoids are largely uncharacterised and the intracellular events activated by these or the cytokine receptors are essentially unknown or unclear. Prostaglandins are known to increase intracellular levels of cyclic AMP via their receptor interaction with stimulatory G proteins (receptor associated GTP-binding proteins) and adenylylase. It is also suggested that under some circumstances prostaglandins and other eicosanoids may interact with alternate receptors and activate inhibitory G proteins to prevent cyclic AMP production.2 Interleukin 1 and tumour necrosis factor have themselves recently been proposed to act by induction of cyclic AMP.28 29 It is unclear whether the activity reported is mediated directly or by induction of eicosanoids. Dayer and colleagues showed that after stimulation of synovial fibroblasts cyclic AMP levels rise as a result of secondary release of prostaglandins.30 They also showed that continued stimulation with cytokines or prostaglandins resulted in the cells becoming refractory to this effect and that NSAIDs could prevent development of this refractory state. Could it be that NSAIDs prevent the development of a refractory state during inflammation so that other cyclic AMP stimulating factors, or even relatively transient rises in eicosanoids themselves, can retain their regulatory role?

Interleukin 6 as an exception?

An exception to the general observation that cytokines of interest in inflammation regulate eicosanoid metabolism (as reported to date at least) is interleukin 6. Although biologically active concentrations of interleukin 1 and tumour necrosis factor may be produced in inflammatory tissues they seem to be found in the circulation only in extreme circumstances, such as severe sepsis and other situations where circulating activators of cytokine synthesis are present. As is evident in sepsis, the resulting systemic activation of eicosanoids and platelet activating factor can be catastrophic. Interleukin 6, however, is normally found in the circulation at low concentrations, rises rapidly after even minor trauma and inflammation, and is induced in the tissues by cytokines such as interleukin 1, tumour necrosis factor, and platelet derived growth factor. As a (if not the) major systemic regulator of hepatic acute phase protein synthesis, and therefore responsible for activating a potentially anti-inflammatory mechanism, it seems to make physiological sense for interleukin 6 not to activate inflammatory mediators.

Eicosanoids as second messengers

In addition to the ability of leukotrienes to potentiate interleukin 1 release from monocytes, arachidonate and leucotrienes stimulate interferon gamma production from lymphocytes and thus it has been proposed that they may have a second messenger role. More recently it has been suggested that after receptor mediated activation of the βy subunits of regulatory G proteins by a variety of stimuli, including hormones, the βy subunits activate phospholipase A2.32 Subsequent production of leukotrienes may then mediate a variety of intracellular events, including activation of ion channels. Intracellular receptors for prostaglandins have also been detected, although the physiological significance of this is unknown. It remains to be seen whether eicosanoids may function as intracellular signals following cell activation by cytokines and provide a further reason for the interaction between these two categories of mediators.

In summary, it is apparent that a number of cytokines, produced during local inflammatory or immune responses, are able to regulate eicosanoid metabolism. The mechanisms by which this is achieved are as yet unclear but may be importantly modulated by synergy with other cytokines or low molecular weight mediators. The resulting eicosanoids may in turn mediate some of the effector functions of cytokines or regulate cytokine function through classical cell surface receptor interactions, or both. Possibly, also, induced eicosanoids may have some regulatory role within the cell.

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Pigmented villonodular synovitis

In 1941 Jaffe, Lichtenstein, and Sutro introduced the term pigmented villonodular synovitis (PVNS) to describe a 'yellow-brown tumour-like tenosynovial lesion'. Before their paper this condition had gone under a variety of names, many of which implied a neoplastic disorder. Indeed the earlier descriptions assumed it was a malignant disease, and amputation was often the treatment of choice. The first report was by Chassaignac in 1852, who described a nodular lesion occurring in the flexor tendons of the hand. In 1909 Moser reported a diffuse type of lesion in the ankle. It was not until the paper of Jaffe et al., however, that a clear overall description of the condition was given.

Reporting on 20 of their own cases affecting joints, tendon sheaths, and bursae, Jaffe et al defined them as either circumscribed or diffuse and showed that the histological appearances were similar in both types of articular synovitis and also in pigmented villonodular tenosynovitis and bursitis. They described the salient histological features: deposition of haemosiderin and infiltration of histiocytes and giant cells in a fibrous stroma within the synovium of tendon sheaths and large joints. They suggested that the condition represented an inflammatory response to an unknown agent. In their experience the treatment of choice was complete excision, and recurrence was due to inadequate removal of diseased tissue. Recurrences were never malignant and could be adequately treated by radiotherapy. Much has been written about this condition since 1941, but we know little more now than that written in this classic paper.

Research into PVNS is difficult because of the rarity of the disorder and most series have had very few patients or have grouped cases from different anatomical sites. In a recent epidemiological study of this condition Myers et al found the incidence to be 1-8 per million population. This is probably an underestimate as they looked only at patients who had undergone surgery. They found a higher incidence in men, though other investigators have not found this sex difference. The age range was from 11 to 84, but as with other series young men were most often affected and the knee was the most commonly involved joint. They were unable to find any clear aetiological associations.

The cause and pathogenesis of PVNS remain obscure.

There have been many attempts to incriminate trauma as the cause, but no study clearly validates this claim. Many varied substances have been injected into the joints of experimental animals, but none has ever reproduced the typical clinical or histological features of PVNS. Myers et al. felt that chronic repetitive trauma and haemarthrosis were important factors. The cause is not seen in haemophiliacs, however, nor in patients with Charcot's joints. No organisms have ever been consistently isolated from a lesion of PVNS, though there is one isolated report of the finding of a myxovirus-like structure from the knee in an unusual bilateral case. The likelihood of an infective cause seems remote.

The most likely cause seems to be inflammatory, but the noxious agent remains unidentified. Lipids have been suggested as they are seen in increased concentration intracellularly in PVNS. There is no evidence for any systemic lipid abnormality, but Hirohata suggested a localised metabolic disturbance as the source of the lipids. Intra-articular injection of lipids does not induce PVNS, however, and probably, as Jaffe pointed out, the presence of lipids in the lesion is a secondary phenomenon as seen in other inflammatory lesions around bone.

Most authors since Jaffe have thought that PVNS is a non-neoplastic process, but there are still some dissenters. Certainly the condition is non-malignant clinically and there are no recorded deaths from PVNS nor any proved incidents of metastasis.

Clinical features

The classical clinical picture of PVNS is that of a young man with monarticular involvement of the knee with the diffuse form of the disease. Other leg joints may be involved but virtually always in isolation. The nodular form tends to affect the fingers but can occur in the knee, where it may mimic a meniscal lesion. The course of the disease is insidious with slow progression of symptoms being the rule. Discomfort rather than pain is a common complaint and swelling is invariably present. In patients with longstanding disease stiffness is a feature.

Routine haematological, biochemical, and immunological