Oncostatin M in the anti-inflammatory response

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Abstract

Oncostatin M (OM) is a pleiotropic cytokine of the interleukin 6 family, whose in vivo properties and physiological function remain in dispute and poorly defined. These in vivo studies strongly suggest that OM is anabolic, promoting wound healing and bone formation, and anti-inflammatory. In models of inflammation OM is produced late in the cytokine response and protects from lipopolysaccharide (LPS)-induced toxicities, promoting the re-establishment of homoeostasis by cooperating with proinflammatory cytokines and acute phase molecules to alter and attenuate the inflammatory response. Administration of OM inhibited bacterial LPS-induced production of tumour necrosis factor α and septic lethality in a dose dependent manner. Consistent with these findings, in animal models of chronic inflammatory disease OM potently suppressed inflammation and tissue destruction in murine disease OM potently suppressed inflammation and tissue destruction in murine models of rheumatoid arthritis and multiple sclerosis. T cell function and antibody production were not impaired by OM treatment. Taken together, these data indicate that the activities of this cytokine in vivo are anti-inflammatory without concordant immunosuppression.

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Initiators of the inflammatory process, including interleukin 1α/β (IL1α/β) and tumour necrosis factor α (TNFα), are rapidly produced in response to infection or injury. They give rise to effector components, including chemokines and growth factors, matrix proteases, and superoxide, that produce inflammation and tissue destruction. In the normal progression of inflammation (fig 1), factors are then produced to attenuate this destructive and inflammatory response and facilitate resolution and wound healing and a return to homoeostasis. These latter factors include acute phase proteins. Generally considered problematic indicators of active inflammation, these feed back to inhibit the action of destructive proteases and, as we will discuss here, modify the actions of initiator cytokines. Thus a normal response to infection or other insult is a self limiting process which by a temporal expression of both regulators, and effector molecules, causes a resolution of the initiating event. A failure to resolve the causative insult or to redress the balance of pro- and anti-inflammatory agents results in the tissue injury and destruction which characterise various chronic inflammatory diseases. 1 2

The exact participation of individual cytokines in the inflammatory disease process is poorly understood, in part, owing to their complex interplay. Importantly, these molecules do not function in isolation but rather in a constellation of other mediators in settings such as that described above. Their pleiotropy is both a blessing and a curse to their development as therapeutic agents. None the less, the demonstrated ability of numerous cytokine and cytokine agonists to alter the severity or course of inflammatory diseases is an impressive testament to their clinical value as drugs for therapeutic intervention. 2 3 Such data have been accrued using animal models of disease, and in the now numerous clinical trials of cytokine inhibitors. 4 5 Inhibitors of proinflammatory cytokines, most notably TNFα inhibitors, have been successful in moderating unward inflammatory responses. 2 Antibodies to TNFα and soluble receptors are currently in clinical trials against a variety of diseases, including rheumatoid arthritis (RA), multiple sclerosis, and Crohn’s disease. 2 6 Their efficacy has helped to establish a set of common effectors in these apparently disparate diseases. Alternatively, cytokines whose normal physiological role is to cause a response from the inflammatory effector phase back to homoeostasis are also being evaluated for their clinical potential as drugs. The cytokines IL10 and

Figure 1 The cycle of inflammation.
IL11 both seem to accelerate this process and their administration has proved effective in resolving several animal models of chronic inflammatory disease.10

Oncostatin M (OM) is a pleiotropic cytokine produced by activated T cells and macrophages and has shown in vitro properties that would be expected to influence the course of inflammatory responses.11,12 The protein is structurally and functionally related to IL6, leukaemia inhibitory factor (LIF), and IL11, proteins that also influence immune and inflammatory function.13 Despite each member signalling via a family of related receptors, and sharing various common properties, each is endowed with a unique array of biological functions.13 Numerous activities have been ascribed to OM in vitro, including the differentiation of megakaryocytes, inhibition of tumour cell growth, induction of neurotrophic peptides, regulation of cholesterol metabolism, and effects on bone derived cells.7-14 Recently, a collective picture of OM has emerged that strongly suggests a natural role for the cytokine in wound healing and attenuation of the inflammatory response. We have previously found that OM can modulate the expression of IL6, an important regulator of various aspects of the host defence system.14 OM has been shown to regulate the expression of human acute phase proteins and protease inhibitors that have been implicated in modulating cytokine function and limiting tissue damage at sites of inflammation. Many of these in vitro effects have also been found to occur after OM administration in rodents and non-human primates.15 Here, we discuss recent work extending these in vivo findings and furthering our understanding of how OM regulates cytokine networks after inflammatory stimuli. We present data on the effects of OM treatment in a murine model of RA and discuss our previous findings of its activity in other diseases, in which common proinflammatory cytokines have been previously shown to have key roles.18

Suppression of inflammation and joint destruction in a mouse model of RA
To investigate the antiarthritic properties of OM its effects were studied in an antibody-induced model of RA.16 In this model inflammation occurs in the absence of a primary immune response, allowing one to distinguish between two immunoregulatory pathways, immune response and inflammation, which are often interdependent and therefore difficult to separate experimentally. To induce joint inflammation animals received a cocktail of four monoclonal antibodies to collagen type II (1 mg each) followed 72 hours later by lipopolysaccharide (LPS; 25 µg).17 This protocol induces a severe arthritis about 24 hours after the LPS injection. OM treatment was started on day 4, after joint inflammation was clearly established, and continued for seven days thereafter (5 µg, twice a day). As shown in Fig 2 the severity of joint inflammation was significantly reduced in OM treated mice compared with control animals when the incidence and severity of arthritis was assessed.18 Nine of 10 control animals were afflicted with arthritic injury by day 6 and had a median (SD) score of 3.8 (1.35). In contrast, only one OM treated animal had macroscopic evidence of disease with a score >1 and the median arthritic score was 0.4 (1). At day 11 after induction of disease the animals were killed, and disease in the rear limbs examined microscopically. Histological examination showed that treatment with OM completely inhibited the influx of inflammatory cells seen in control animals and prevented the tissue damage associated with a severe inflammatory reaction.18

The inflammation and tissue injury were quantified. Nine of ten control animals had severe inflammation and tissue injury, including pannus formation, connective tissue destruction, and erosion of cartilage and bone, with an average score of 26.9 (16.2). The OM treated mice had histological measures of inflammation and injury consistent with macroscopic evidence, and this group had significantly better score of 2.4 (7.6) (p<0.001). In two additional independent studies similar efficacy was seen and stopping OM treatment at day 7 was not followed by a delayed onset of inflammation in animals monitored for an additional 14 days (data not shown).

A basis for using OM in inflammatory diseases
TEMPORAL EXPRESSION OF OM AFTER ACTIVATION
The above findings are consistent with previous data suggesting that OM is a late phase cytokine which alters the activities of initiators of the inflammatory response. The temporal expression of OM by activated T cells and macrophages follows by days the production of cytokines associated with the initiation phase of host defence, IL2 and TNFα, respectively.18 The peripheral blood mononuclear cell secretion of TNFα is maximal two hours after LPS...
stimulation and declines thereafter. Production of OM by these cells is not increased until 24 hours and continues to rise at 48 hours. Similar findings are seen upon activation of T cells, where IL2 secretion is maximal at 24 hours and declines thereafter, whereas increased OM secretion by these T cells is not seen until 44 hours and continues to increase at 96 hours. The part IL2 may play in inducing OM from T cells has not been elucidated, yet the kinetics of cytokine expression are consistent with the findings that the murine OM gene is inducible by IL2,21 and the idea that delayed OM production may represent a regulatory function involved in a feedback mechanism after an initial response.

SUPPRESSION OF ENDOTOXIN-INDUCED TNFα

Patients with septic shock have raised levels of OM,22 and the inhibition of endotoxin-induced TNFα production by OM potentially supports a role for OM in an attenuation phase after an inflammatory stimulus rather than in the initiation or effector phases.18 The significance of this attenuation was demonstrated by increased survival of animals treated with OM that were exposed to endotoxin (table 1). Animals were injected with increasing doses of LPS (25–200 µg) either alone or in combination with OM (10 µg) given intraperitoneally four hours, two hours, and one hour before and at the time of the LPS injection. As shown in table 1, OM treatment increased twofold the maximum tolerated dose of LPS in mice, when compared with animals treated with control diluent. Thus the altered cascade of cytokines produced by co-administration of OM is functionally reflected in increased animal protection from endotoxic shock.14

The efficacy of OM treatment in murine disease models of RA shown above, was also demonstrated in the experimental autoimmune encephalomyelitis model of multiple sclerosis.19 Studies of both RA and multiple sclerosis have clearly shown the importance of TNFα in disease progression, and in models of each disease a variety of TNFα antagonists have proved efficacious.23 It is therefore reasonable that inhibition of TNFα by OM would provide protection from inflammatory stimuli driven by autoimmune conditions in addition to that caused by endotoxin.

The mechanisms by which OM inhibits TNFα remain to be elucidated. No direct effects of OM on TNFα production could be demonstrated on macrophages either treated ex vivo or isolated from OM treated animals. IL6 has been found to inhibit TNFα production in vivo,22 and IL6 is induced by OM.17 However, both the inhibition of TNFα by OM in IL6 deficient mice (Wallace P, unpublished results), and the kinetics of IL6 induction in our study, make it unlikely that IL6 is necessary for TNFα inhibition when LPS and OM are given together. IL6 may, however, participate in the sustained effects of OM when LPS administration is delayed.

MODULATION OF OTHER INFLAMMATORY MEDIATORS

The anti-inflammatory properties of OM are not limited to inhibition of TNFα. Cell based studies have shown that OM can block and modify the response to IL1, the agent provocateur most closely associated with TNFα in mediating tissue damage at sites of inflammation.24 In vivo, inflammatory stimuli resulting in production of IL1 initiate a cascade of effectors, including IL8 and granulocyte macrophage colony stimulating factor (GM-CSF), which amplify the inflammatory response by recruiting, expanding, and activating inflammatory cells.25 Although OM has no apparent effect on IL1 production, it does affect the ability of IL1 to induce downstream mediators. Richards et al have shown that gene expression of GM-CSF and IL8 induced by treatment of synovial fibroblasts with IL1 is suppressed by co-treatment with OM in a dose dependent manner.26 IL8 is not only the primary chemottractant of neutrophils but also stimulates their oxidative burst and degranulation at sites of inflammation.26 Surprisingly, coincident with suppression of GM-CSF and IL8, OM acted synergistically with IL1 to induce expression of IL6 and tissue inhibitor of metalloproteinase-1 (TIMP-1) in these same cells. The ability of OM to synergise with IL1 to suppress inflammatory cytokine expression and induce expression of IL6 was not paralleled by other members of this cytokine family.25

In vivo, OM may also act indirectly on the function of IL1 and TNFα by inducing a constellation of protein antagonists. The acute phase proteins, serum amyloid A (SAA) and α1-glycoprotein, are produced locally after tissue injury to minimise damage proximal to the site of injury. In addition, systemic release of cytokines results in acute phase response by the liver to down regulate the inflammatory response and re-establish homeostasis. These proteins are normally produced by adult mammals in response to tissue injury or infection, or both; and in a normal, self limiting process are induced by IL1 and TNFα themselves. Administration of α1-glycoprotein protects animals from TNFα-induced lethality.28 SAA and α1-glycoprotein produced during the acute phase are thought to decrease inflammation by sequestering circulating IL1 and decreasing TNFα expression, respectively.25 30 31 We have previously shown that administration of OM can up regulate the expression of SAA and α1-glycoprotein in vivo in both mice and in non-human primates.7 Corticosteroids are also potent inhibitors of proinflammatory cytokines, including IL1, IL8 and TNFα. OM, in combination with IL1, stimulates the

Table 1 Effect of oncostatin M (OM) on the survival of mice after lipopolysaccharide (LPS) treatment

<table>
<thead>
<tr>
<th>Treatment (µg LPS)</th>
<th>Control diluent (No alive/group total)</th>
<th>OM treatment (No alive/group total)</th>
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<tr>
<td>200</td>
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Balb/c mice were injected with OM (10 µg intraperitoneally, 4, 2, 1, and 0 (co-injected) hours) before LPS challenge. The number of mice surviving to day 7 after LPS treatment is shown.
hypothalamus-pituitary-adrenal axis to secrete corticosterone, providing an additional mechanism whereby it can feed back to attenuate inflammation.

The secretion of reactive oxygen intermediates and destructive proteases, including neutrophil elastase, cathepsins, and matrix metalloproteinases (MMPs), by activated infiltrating cells results in degradation of connective tissue and cartilage in RA and the neural sheath in multiple sclerosis. In addition to attenuating the cytokines that stimulate secretion of these proteases, in vitro studies have shown that OM can induce a spectrum of protease inhibitors. The acute phase proteins induced by OM include two major serine proteinase inhibitors: α₁-proteinase inhibitor (α₁-Pi), and antichymotrypsin. α₁-Pi, the primary inhibitor of neutrophil elastase, is secreted from lung epithelial cells and synovial fibroblasts stimulated by OM (Richards C, personal communication). In comparison, other family members have little or no effect on expression of α₁-Pi. Interestingly, the stimulation of α₁-Pi by OM is greatly enhanced in the presence of IL1, again suggesting that OM works in consort with inflammatory cytokines to alter their effect. Antichymotrypsin, an inhibitor of cathepsin G and other chymotrypsin-like enzymes secreted during inflammation, also inhibits superoxide generation by activated neutrophils. Antichymotrypsin, is produced after OM treatment of hepatic and numerous non-hepatic human cells. Its expression by epithelial cells in response to treatment with OM is synergistically increased by co-treatment with OM and corticosteroid.

In vivo, the action of collagenase and gelatinase is regulated by the relative level of their cognate inhibitor, TIMP-1. OM increased the expression of TIMP-1 by synovial fibroblast and did so more effectively than other members of the IL6 cytokine family. Similarly, OM stimulated the production of TIMP-1 from human articular chondrocytes and cartilage explant culture more effectively than IL11, LIF, or IL6. Expression of TIMP-1 from fibroblasts in response to OM occurs with no effect on matrix MMP levels, resulting in a net decrease in MMP activity. The effects of OM on α₁-Pi, antichymotrypsin, and TIMP-1 expression are greatly enhanced in the presence of IL1, again suggesting that OM works in consort with proinflammatory molecules as part of an anti-inflammatory feedback loop. Based on the numerous cell types responsive to OM it is reasonable to expect that this synergistic feedback may occur at sites of inflammation.

The synergy of OM with proinflammatory mediators is also seen for the induction of IL6. Although the role of IL6 in inflammation remains controversial, in vivo IL6 induces IL1 receptor antagonist and the soluble TNF receptor p55 that attenuate inflammation. IL6 is a key inducer of protease and cytokine inhibitors that reduce inflammation and initiate healing. Its protective effects are also inferred from the failure of IL6 deficient mice to repair tissue and recover from inflammation, to attenuate TNFα, or produce proteins that limit damage at sites of injury. In this light, the ability of OM to enhance IL6 production is consistent with a role for the protein in tissue repair. The synergistic interaction of OM and TNFα on increased IL6 expression supports the concept that OM activity is enhanced in the presence of this proinflammatory cytokine at sites of injury and inflammation. This synergy between pro- and anti-inflammatory cytokines is not unique to OM and TNFα, as OM combined with IL1 also yields a similar enhancement of IL6. As described above, the production of acute phase proteins is also maximised by the combined presence of OM and inflammatory mediators.

We have shown that expression of OM from activated T cells and macrophages is temporarily delayed and increases coincident with a decline in expression of TNFα and IL2. We, and others, have demonstrated synergy between OM and inflammatory cytokines in suppression of inflammatory mediators, and we have shown that when given systemically, the molecule is efficacious in three different models of acute disease with common proinflammatory mediators.

**Discussion**

Others have reported that commercially available OM, expressed in bacteria, is proinflammatory and induces the expression of adhesion molecules on endothelial cells in vitro, and an inflammatory infiltrate in vivo. Using highly purified mammalian protein or yeast derived protein, Brown et al previously studied the effects of OM on endothelial cells in vitro and injected protein in vivo in five species (Wallace PM, unpublished results), with no evidence of these findings. In vitro OM combined with IL1 has been shown to stimulate the release of proteoglycan and collagen from bovine nasal cartilage and human articular cartilage in explant culture, while in the hands of others OM activates osteoblasts and bone formation and inhibits bone resorption. In contrast with the information presented here, recombinant adenovirus, producing murine OM, injected into mouse joints was shown to promote joint inflammation.

The recent cloning of the murine OM-specific receptor has also called into question the interaction of human OM in murine studies. These studies suggest that human OM can interact with the murine TNF receptor and indirectly suggest that the effects of the human protein observed in mice might be attributed to LIF.

Collectively, these findings are in contrast with our experience with OM and may reflect differences in the sources and purity of the proteins, in addition to experimental design differences that remain to be resolved. Clearly, cytokine production and the generation of acute phase proteins, described and referenced herein, occur from human cells treated with human OM and, in our hands, numerous comparative studies with LIF and OM on human cells suggest no strong parallels between LIF and the anti-inflammatory activities of OM.
Additionally, we have established that OM functions in non-human primates as in mice to inhibit LPS-induced TNFα production and up regulate IL-6 and acute phase proteins (Wallace PM, Wahl AF, unpublished results).17

Conclusions

Taken together, these in vivo findings suggest that OM participates in attenuating the inflammatory response and restoring normal homoeostasis after tissue injury or infection, or both. In an inflammatory cycle, initiators such as TNFα and IL-1β, which promote inflammatory cell activation and secretion of chemoattractants and proteases, would remain maximal at the peak of the inflammatory response. Local expression of OM in concert with these activators then potentiates a return to homoeostasis as the proinflammatory mediators are suppressed. The ability of OM to enhance the negative feedback of proinflammatory cytokine production, in addition to inhibiting their biological effects, distinguishes the therapeutic potential of this molecule from those of individual cytokine antagonists such as IL-1 receptor antagonist or anti-TNFα soluble receptor. Moreover, some key anti-inflammatory activities of OM, such as its direct effect on fibroblasts and epithelial cells and its induction of protease inhibitors, are not seen with other anti-inflammatory cytokines, such as IL-6, IL10, or IL11. Such OM-specific responses may reflect the tissue distribution of the recently characterised OM-specific receptor complex which is coupled to a specific set of OM inducible genes. These data indicate that OM may act therapeutically in the treatment of inflammatory diseases by regulating the spectrum of events that comprise a natural feedback loop to return active inflammation to homoeostasis. The key to its development as a therapeutic agent lies in further elucidating these interactions.


