Molecular Mechanisms Underlying the Actions of the Pro-inflammatory Cytokine Interleukin 1

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Introduction
The inflammatory response is the body’s way of handling tissue injury. Following tissue damage or as a consequence of infection, a complex series of events occurs that eventually leads to repair of the damaged tissue and elimination of the infectious agent. During inflammation a cascade of mediators is released that leads to well-characterized vascular changes, leucocyte migration, hyperalgesia and systemic changes such as fever and the acute-phase response. Important mediators include kinins, complement components and eicosanoids. Recently, a great deal of attention has focussed on cytokines, a large and still-expanding family of proteins synthesized during the immune and inflammatory responses. These include the interleukins, interferons, colony-stimulating factors and chemokines. Of these, interleukin (IL)1 and tumour necrosis factor (TNF) are considered key mediators that orchestrate the inflammatory response and the cytokine network. Both have been implicated in such conditions as rheumatoid arthritis and septic shock, where the uncontrolled release of these cytokines leads to the key clinical features of these pathologies.

IL1 was one of the first cytokines to be studied in detail. There are currently three members of the IL1 family, comprising IL1α, IL1β and the IL1 receptor antagonist (IL1RA). The biological activities of IL1 are shared by IL1α and IL1β, with IL1RA acting as a true receptor antagonist [1]. IL1 was originally described as a co-mitogen for T-lymphocytes [2]. It is now clear that it can activate a wide range of cell types with roles in both immunity and inflammation. The end result of IL1 action on target cells is a change in gene expression. Most of the genes induced by IL1 play a role in inflammation, and include those for other cytokines, nitric oxide synthase, cyclo-oxygenase, collagenase, stromelysin, acute-phase proteins and leucocyte–endothelial-cell-adhesion molecules. A list of these genes and the products that result from their expression is shown in Figure 1. The molecular basis for the changes in gene expression is unclear, despite being an area
IL1 increases the expression of genes that code for proteins involved in immunity and inflammation. IL1 is central to inflammation because of its ability to up-regulate the genes listed here. Target cells include T-cells, B-cells, macrophages, fibroblasts, hepatocytes, chondrocytes, glial cells and endothelial cells. The products that result are all potent mediators of inflammation.

Abbreviations used: PLA2, phospholipase A2; PG, prostaglandin; CSF, colony-stimulating factor; SAA, serum amyloid A; CRP, C-reactive protein; CAM, cell-adhesion molecule.

of close scrutiny for the last ten years [3–5]. Recently, novel pathways and protein kinases have been described. This review describes the key features of IL1 signal transduction published to date and speculates on the importance of observations made.

**IL1 receptors**

In 1988, the first report on the cloning of an IL1 receptor appeared [6]. This was subsequently named the type I IL1 receptor (IL1RI). It has recently become clear, however, that there is an IL1 receptor family, which share sequence motifs either extracellularly or intracellularly [7]. These range from IL1RI, which appears to mediate most of the actions of IL1, to cytosolic proteins such as MyD88 and proteins involved in *Drosophila* development, such as Toll. Table 1 lists these proteins.

**IL1RI**

Cross-linking studies had originally identified a protein of 80 kDa on T-cells and fibroblasts [8]. This protein was cloned and sequenced, and at the time of cloning no similarities were found with other proteins in the database. When transfected into Chinese hamster ovary cells, IL1RI conferred responsiveness to IL1 in these cells [9]. Disappointingly, its structure gave no clues as to a possible signal-transduction pathway. IL1RI does not possess any intrinsic protein kinase activity. Nuclear translocation motifs have been found in both human and murine IL1RI [6], and there have been demonstrations of a translocation of IL1–IL1RI complexes to the nucleus, suggesting that such translocation is important for IL1–induced gene expression [10]. Receptor-mutation studies carried out by Heguy et al. and Kuno et al. [11,12] have ruled out this possibility, however. They have demonstrated that mutating the nuclear translocation motif in the murine IL1RI prevents receptor trafficking without affecting changes in gene expression.

IL1RI also has a motif that predicts G-protein coupling. This was first identified in the IGFII receptor, which was shown to couple directly to G2, and comprises B-B-X-B (where B represents basic or aromatic residues and X represents non-basic residues) [13]. This motif occurs in both mouse and human IL1RI, at positions 429–432 and 428–431 respectively [6]. Interestingly, mutation of Arg-431 in the mouse IL1RI results in a receptor that is unable to signal, suggesting that G-protein coupling might be essential for IL1 signalling [11]. Evidence for the involvement of G-proteins in IL1 action has been reported (see below). Five other amino acids were also identified that when mutated produced a receptor incapable of signalling. In mouse IL1RI these are Arg-431, Phe-513, Trp-514, Lys-515, Arg-518 and Tyr-519 [11].

As mentioned previously, several other proteins have been identified that exhibit significant sequence similarity to the cytosolic portion of IL1RI [7]. The first to be identified was Toll
protein, a membrane protein in *Drosophila melanogaster*, which has been shown to play a key role in the generation of dorsoventral polarity in the early *Drosophila* embryo [14]. The similarity is only apparent in the cytoplasmic domain of both proteins, extending for 135 amino acids throughout most of the domain. This suggests that the signal-transduction pathways triggered by IL1RI and Toll are likely to be shared. In particular, the events stimulated by IL1 that lead to activation of the transcription factor nuclear factor κB (NFκB) are likely to be identical to those stimulated by Toll that lead to activation of dorsal, the *Drosophila* NFκB homologue. Genetic analysis has revealed that two other genes, *tube* and *pelle*, are expressed during the generation of dorsoventral polarity, and that these act sequentially in the pathway leading from Toll to dorsal [15,16]. Both *tube* and *pelle* have been cloned; *pelle* codes for a serine/threonine protein kinase related to Raf-1 [16]. Raf-1 has been shown to activate NFκB directly in Jurkat T-cells. Whether a similar system is operating in the activation of NFκB by IL1 has yet to be demonstrated (see below for further discussion of NFκB).

In addition to Toll, five other proteins have been identified that have similar sequences to the cytosolic portion of IL1RI (reviewed in [7]). The first of these was called T1 by one of the groups that reported it and ST2 by the other [17,18]. It was initially characterized as a soluble molecule that has growth-promoting activity. A membrane-associated form was then cloned and shown to be similar both in its extracellular and intracellular domains to IL1RI. The signalling domain of T1/ST2 has recently been shown to be functional [19]. A chimaeric protein involving the extracellular portion of IL1RI and the signalling domain of T1/ST2 was capable of signalling, indicating that the sequence similarity between IL1RI and T1/ST2 is also a functional similarity. Interestingly, in spite of the similarities between T1/ST2 and IL1RI extracellularly, T1/ST2 does not bind IL1. A ligand has recently been cloned [20] but does not appear to trigger any signal upon binding. Further information on this system is therefore required.

Apart from Toll, another *Drosophila* protein has been described, 18-wheeler, which is similar to IL1RI [21]. 18-wheeler also plays a role in development. Two mammalian proteins have also been described that are similar to the cytosolic domain of IL1RI. These are MyD88 and rsc786. The MyD88 gene was described as a gene induced by IL6 in leukaemic cells [22]. Interestingly, it is not membrane associated but occurs in the cytosol. Its role is unclear. rsc786 is an anonymous clone present in Genbank. Recent chimaeric studies with extracellular IL1RI and MyD88 or rsc786 have indicated that they are unable to signal [19], indicating further complexity.

Finally, a protein in the tobacco plant *Nicotiana* has been described whose N-terminus is similar to the cytosolic portion of IL1RI [23]. This protein (termed N protein) provides resistance to tobacco mosaic virus infection.

As to whether IL1RI interacts with any other proteins in the membrane, a recent report described the cloning and characterization of a second subunit of the IL1 receptor complex [24]. This protein also shows similarities to IL1RI and has been termed IL1 receptor accessory protein. It appears to be required for high-affinity binding of IL1, although whether it also has a role in signalling awaits determination.

**IL1RII**

The second major IL1 receptor is IL1RII. It was first identified on cells or cell lines representative of B-cells, monocytes, neutrophils, bone-marrow cells and hepatoma cells and was cloned from murine and human B-cells [25]. Structurally, the extracellular domain of IL1RII is 28% similar in amino acid sequence to IL1RI, with three immunoglobulin-like domains. The extracellular portion of IL1RII comprises only 29 amino acids, in contrast to the 215 amino acids of IL1RI. This initially suggested that both receptors would interact with different signal-transduction pathways but it has since become clear that IL1RI, and not IL1RII, is the signalling receptor for IL1. This conclusion is based on the fact that blocking antibodies to IL1RI but not IL1RII inhibit IL1 action even on cells shown to express IL1RII predominantly [26]. Secondly, in murine cells the human IL1RA will only inhibit binding to IL1RI and is inhibitory for IL1 signals [26]. The most likely function of IL1RII is as a regulator of IL1 levels extracellularly, as it has been shown to be shed from cells [27]. Furthermore, an interesting sequence similarity has been found with a protein from Vaccinia virus that is used to decrease host immune responses to the virus. IL1RII may therefore function as an extracellular IL1 inhibitor, binding to IL1 and preventing it from interacting with IL1RI [27].
G-proteins and IL1

Receptor-occupancy studies have shown that fewer than 10 IL1RI receptors per cell are sufficient to cause cellular activation. This suggests that a major amplification in signal must occur following IL1 binding. Evidence has been presented suggesting that G-proteins may be important for such amplification in the IL1 pathway. This area is somewhat controversial, however, as changes in G-protein activity have not been detected by all groups, and much of the evidence has rested on the inhibitory effect of the G-protein-modifying agent pertussis toxin. Recently, however, studies involving rapid ion changes induced by IL1 and the use of recombinant pertussis toxin preparations have indicated that for some IL1 responses G-proteins may be involved.

Pertussis toxin-sensitive G-proteins and IL1 signalling

Pertussis toxin has been reported to inhibit a range of IL1 responses. These include induction of IL2, prostaglandins, IL2 receptor, M-colony-stimulating factor and IL1RI, and the proliferation of thymocytes (reviewed in [5]). Increases in GTP binding and hydrolysis have also been reported [28], although others have failed to show such changes [29]. A non-hydrolysable GTP analogue has also been shown to cause a higher affinity state for IL1 binding [29]. Taken together these data suggest that a pertussis toxin-sensitive G-protein (most probably G\textsubscript{i}) has a role to play in IL1 signalling. Recent evidence, however, questions this conclusion. Firstly, pertussis toxin does not inhibit all responses to IL1. The activation of NF\textsuperscript{kappa}B, phosphorylation of the epidermal growth factor receptor, induction of collagenase and IL6, and the suppression of L-type calcium currents in myocytes have been shown to be insensitive [30,31]. Furthermore, for responses that are sensitive to pertussis toxin (for example, induction of IL2 and prostaglandin E\textsubscript{2} production) the B oligomer of the toxin, which is devoid of ADP-ribosylating activity and would therefore not interfere with G-protein function, has been shown to be as inhibitory as the holotoxin [30]. More recently, however, evidence has been presented that B oligomer effects may be due to the contamination of holotoxin [32]. Furthermore, a mutant pertussis toxin, defective in its ability to ADP ribosylate, was without activity [32].

A role for a pertussis toxin-sensitive G-protein in IL1 action is therefore still somewhat ambiguous, although the presence of a motif in IL1RI which predicts G-protein coupling and which if mutated abolishes signalling would favour a role for G-proteins. It is also possible that IL1 triggers multiple pathways, one of which may involve a G-protein.

cAMP

IL1 has been reported to increase cAMP and activate adenylate cyclase in a variety of cell lines [33]. However, several reports have also appeared in which such changes were not found (reviewed in [3]). The reason for these discrepancies is unclear. Most of the studies have been carried out in transformed cells, and differences in cell types or strains may be partly responsible. There is also the possibility that increases in cAMP may be indirect and may be secondary to prostaglandin production [34]. Whether IL1 can activate protein kinase A as a consequence of cAMP accumulation has also not been demonstrated directly, and the phosphorylation changes characterized to date in response to IL1 have not been shown to be due to protein kinase A (see below). Finally, Munoz and co-workers [35] have dissociated changes in cAMP from several IL1 responses in Th2 cells. The conclusion must therefore be that cAMP is unlikely to be a second messenger for IL1.

Phospholipid-derived second messengers

IL1 has been shown to activate lipid-metabolizing enzymes in a variety of cell types, including activation of phospholipase A\textsubscript{2} and phospholipase C.

The importance of these observations for subsequent events is unclear. The generation of arachidonic acid is the first step in prostaglandin production, although the induction of cyclo-oxygenase by IL1 would appear to have a more important role in the triggering of this response [36]. The generation of diacylglycerol would suggest that protein kinase C activation may be important, although, as discussed below, there is a consensus that this kinase is not activated in response to IL1 in most cell types.

Most recently the sphingomyelin breakdown product ceramide has been shown to be generated in response to IL1 [37]. Further experiments are therefore necessary to clarify how important this is likely to be in IL1 signalling.
**Protein phosphorylation changes and IL1**

In common with other cellular activators, IL1 changes protein phosphorylation in cells. Such changes have predominantly been studied in fibroblasts. High-resolution two-dimensional gel electrophoresis has identified at least 116 polypeptides whose phosphorylation state changes in response to both IL1 and TNF [38]. Most of these are increases and occur on serine or threonine residues, with a small number occurring on tyrosine residues. Only TNF and the protein phosphatase inhibitor okadaic acid cause identical changes as IL1, suggesting that some of the kinases involved are novel.

**Protein kinase C and tyrosine kinases**

In spite of evidence for activation of protein kinase C by IL1, the consensus is that this kinase is not IL1 sensitive (reviewed in [5]). Most studies have shown that inhibitors of protein kinase C such as staurosporine fail to block a wide range of IL1 responses in different cell types.

There is similar divergence over tyrosine kinases and IL1 signalling. Increases have been reported in several cell types, with a consistent change occurring in proteins of 42–44 kDa, the molecular-mass range for mitogen-activated protein (MAP) kinases, and it has been suggested that these proteins are members of the MAP kinase family. Direct evidence has been provided for increased tyrosine phosphorylation of p42 and p44 MAP kinases in response to IL1 in KB cells [39].

Most recently, studies have appeared suggesting that the extracellular matrix may play a role in IL1 signalling, with evidence being presented for activation of the tyrosine kinase FAK (focal adhesion kinase) [40].

**MAP kinase, p54 MAP kinase and p38/HOG-1**

**MAP kinase**

Most recently, several reports have appeared concerning MAP kinases. Three main MAP kinase cascades have been described in cells. These are p42/p44 MAP kinase, p54 MAP kinase and p38 MAP kinase (a homologue of the yeast kinase HOG-1). All of these are related in protein sequence, and IL1 has been shown to activate all three [41–43], as shown in Figure 2. Activation of such pathways is unlikely to involve second messengers, and the cascade of phosphorylations that occur would possibly allow for the amplification required in IL1 signalling. Much work has yet to be done on these pathways, however, as the upstream regulators have yet to be clearly identified and the consequences of their activation are still unknown. Most recently, an IL1 receptor-associated kinase (IRAK) has been cloned [44]. Whether this kinase couples directly to the receptor is not known, nor is it known whether it activates any of the kinase pathways described above. Interestingly, it is similar to the *Drosophila* protein pelle, which is activated by the IL1RI homologue Toll, as described above.

Studies into Hsp27 phosphorylation have revealed the third 'MAP kinase-like' enzyme cascade activated by IL1. Originally called Hsp27 kinase, this enzyme was found to use GTP or ATP, was insensitive to a wide range of protein kinase inhibitors and was highly specific for Hsp27 [41]. These data suggested that the enzyme may be novel, and a protein kinase cascade activated in response to IL1 in KB cells that culminated in phosphorylation of Hsp27 was described [41]. Three components were isolated in the cascade, an upstream activator of molecular mass 35 kDa and two downstream components of 40 kDa and 50 kDa. *In vitro* reconstitution experiments have demonstrated that a protein kinase cascade is likely to be operating comprising p35→p40→p50→Hsp27. Biochemical evidence indicated that the enzymes participating in the cascade were novel. It has recently become clear that p40 is the human homologue of a recently reported murine protein...
kinase, p38, that becomes activated in response to endotoxin and hyperosmolarity [45]. The murine protein is related to mammalian p42 and p54 MAP kinases and to the *Saccharomyces cerevisiae* HOG1 gene, which is also activated in response to changes in osmolarity. p50 Hsp27 kinase resembles MAP kinase-activating protein kinase 2.

**β-casein kinase**

A final IL1-sensitive kinase to be described has been termed β-casein kinase [46], because of its ability to phosphorylate β-casein. This is shown in Figure 2. The enzyme has so far been shown to be activated by IL1 and TNF alone. This is unlike Hsp27 kinase, which is activatable by a wide range of agents, as described above. The biochemical characteristics of the enzyme indicate that it has not been described before. It may be a multimeric enzyme, with a 90 kDa form being the smallest active component. Interestingly, unlike the other protein kinases described above, β-casein kinase is not inactivated by protein phosphatases. The fact that the enzyme is only activated by IL1 and TNF makes it particularly interesting and suggests that it may be a key component in the specific signal triggered by these cytokines. Much work has yet to be done, however, and in particular its substrate(s) in cells have yet to be determined.

**IL1 and transcription factors**

The activation of protein kinases by IL1 is likely to lead to enhanced gene transcription through the activation of transcription factors by phosphorylation. IL1 has been shown to activate and/or induce several transcription factors, including Jun, Fos, NFkB, Myc, Egr-1, NAK-1, IRG-9, Myb, c/EBP and NFIL6 (reviewed in [5]).

**NFkB**

In virtually every cell type tested, IL1 activates NFkB. This event is likely to be particularly important, as many IL1-regulated genes contain NFkB-binding sites in their promoter regions [5]. The predominant form of NFkB exists in resting cells in the cytosol as a dimer, p50 and RelA (formerly called p65), complexed to an inhibitory protein, IkB. To activate NFkB, IkB must dissociate, allowing translocation of the NFkB dimer to the nucleus. IL1 has been shown to activate NFkB in numerous cell types.

Phosphorylation and proteolysis of IkB are required for NFkB activation. It has recently been demonstrated that IL1, but not phorbol 12-myristate 13-acetate or TNF, can induce degradation of both IkBα and IkBβ [47]. The basis for this is still unknown. The protein kinase responsible for IkB phosphorylation has also yet to be determined. The tyrosine kinase inhibitor herbimycin A has been shown to block activation by IL1, suggesting the involvement of a tyrosine kinase. However, we have provided evidence that the mechanism of action of herbimycin A involves covalent modification of the p50 subunit of NFkB rather than inhibition of tyrosine kinases [48].

Reactive oxygen intermediates have been implicated as second messengers for NFkB activation [49]. The evidence was based on the observations that H2O2 activates NFkB in certain cell types and that anti-oxidants such as N-acetylcysteine and pyrollidine dithiocarbamate inhibit NFkB activation [50]. Activation of NFkB by IL1, however, has been shown to be insensitive to the anti-oxidant N-acetylcysteine in EL4 cells and 1231 N1 astrocytoma cells, indicating that, unlike other activators of NFkB, oxygen radicals may not be important for the activation of NFkB by IL1 [50].

**Conclusions**

The area of IL1 signal transduction has been difficult and controversial, and a clear pathway has yet to emerge. Recently, however, the involvement of MAP kinase cascades and the ability of IL1 to affect transcription factors such as NFkB have improved our understanding of the processes involved. The use of such recent techniques as the yeast two-hybrid system and efforts to elucidate upstream regulators of the kinase cascades activated by IL1 are likely to yield much information on how this important cytokine works.

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