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Biochimica et Biophysica Acta 1266 (1995) 31–44

BBA Biochimica et Biophysica Acta

Minireview

Towards an understanding of the signal transduction pathways for interleukin 1

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Received 9 August 1994; accepted 11 November 1994

Keywords: Interleukin 1; Signal transduction; Second messenger; Protein kinase; MAP kinase; Transcription factor

Contents

Abbreviations: cPLA2, cytosolic phospholipase A2; EGF, epidermal growth factor; hsp27, heat shock protein 27; IL, interleukin; IL1RA, interleukin 1 receptor antagonist; IL1RI/II, Type I and II IL1 receptors; MAP kinase, mitogen activated protein kinase; MAPKAP kinase 2, MAP kinase activated protein kinase 2; PMA, phorbol myristate acetate; TNF, tumour necrosis factor.

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SSDI 0167-4889(94)00217-7

1. Introduction

The interleukin 1 (IL1) family of proteins currently comprises 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]. Much interest has surrounded the many responses induced by IL1 as its effects both in vitro and in vivo have led to it being implicated in a number of pathological conditions, most notably chronic inflammatory diseases such as rheumatoid arthritis. Whether IL1 has a role to play in normal physiology is still a matter of debate. Originally described as a comitogen for T lymphocytes [2], it is now clear that IL1 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 and IL1 may be thought of as an extracellular agent which allows cells to exhibit their activated phenotype. For example, fibroblasts when activated produce a range of proteins, including tissue degrading enzymes and cytokines, and will also increase the output of prostaglandins. IL1 will enhance all of these activities through an alteration in the expression of discrete genes. The molecular basis for the changes in gene expression is unclear, despite being an area of close scrutiny for the last ten years. Efforts have focused on attempts to implicate known second messengers and protein kinases in IL1 signalling and to link such changes with transcription factor activation. Such attempts have often proved controversial with no clear consensus emerging [3,4]. More recently, novel pathways and protein kinases have been described. This review will attempt to distil the key features of IL1 signal transduction published to date and speculate on the importance of observations made.

2. IL1 receptors

Two distinct plasma membrane receptors have been described for IL1. The first to be described was an 80 kDa glycoprotein [5]. Early reports characterised this receptor by performing receptor cross-linking analysis and ligand binding studies on a variety of cell types. Features of the receptor included its high affinity for IL1 (dissociation constants in the picomolar range), the fact that both IL1α and IL1β bound to the same receptor and the relatively low number of receptors per cell (on average 1–200) [6]. Although the 80 kDa form was the main species seen on cross-linking of T cell or fibroblast membranes with [^{125}I]IL1, a 60 kDa species was seen on B cells [7]. These were eventually shown to be two distinct proteins and were named Type I and Type II IL1 receptors (IL1RI and IL1RII, respectively) [8,9].

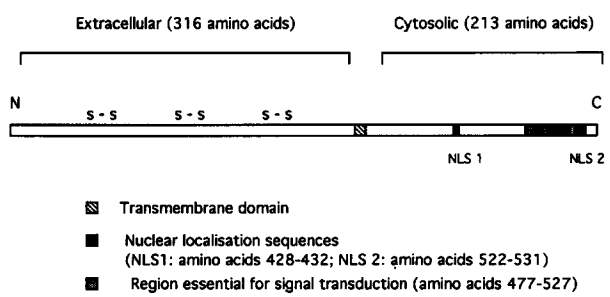

Fig. 1. Characteristics of the Type I IL1 receptor. Amino acid sequence analysis and site-directed mutagenesis studies have revealed the main features of the Type I IL1R, as shown above. Deletion of the region from amino acids 477 to 527 abrogates signal transduction [17]. Six amino acids (arginine 431, phenylalanine 513, tryptophan 514, lysine 515, arginine 518 and tyrosine 519) are conserved in human, mouse and chicken IL1RI and the *Drosophila melanogaster* protein Toll [17,18]. Mutation of these residues produces a receptor incapable of signalling.

2.1. IL1RI

IL1RI was cloned from mouse and human cells and was shown to occur on T cells, fibroblasts and endothelial cells [10–12]. When transfected into Chinese hamster ovary cells IL1RI conferred responsiveness to IL1 in these cells [13]. Disappointingly, its structure gave no clues as to a possible signal transduction pathway. The key structural features of the receptor are shown in Fig. 1. IL1RI does not possess any intrinsic protein kinase activity. The murine form of the receptor has been shown to become phosphorylated on threonine 537 in response to phorbol esters although this did not affect receptor function [14]. The human form has no sequence homology in this region and is not phosphorylated [14]. IL1 does not increase phosphorylation of murine IL1RI. Phosphorylation of IL1RI therefore does not appear to be important for receptor signalling.

Nuclear translocation motifs have been found in both human and murine IL1RI [11]. These sequences occur near the C-terminus in the vicinity of amino acids 522–531 and 428–432. There have been demonstrations of a translocation of IL1/IL1 RI complexes to the nucleus suggesting that such translocation is important for IL1-induced gene expression [15,16]. Elegant receptor mutation studies carried out by Heguy et al. and Kuno et al. [17,18], however, have ruled out this possibility. They have demonstrated that mutating the nuclear translocation motif in the murine IL1RI prevents receptor trafficking without affecting changes in gene expression [18]. They have further shown that deletion of most of the cytoplasmic portion of IL1RI had no effect on IL1-induced PGE2 and GMCSF in fibroblasts or IL2 production in Jurkat T cells [17]. A crucial 50 amino acid segment spanning residues 477 to 527 was identified, however, and shown to be highly homologous to the avian and human IL1RI [17].

Another sequence similarity has been described by Kuno et al. [18] in the murine form of IL1RI. Amino acids 435–484 are homologous to gp130 of the IL6 receptor. Truncation mutants and point mutations within this segment were found to abolish the ability of the receptor to induce IL8 expression. As IL6 is unable to induce IL8 it

was suggested that the gp130 homologous region may contribute to the maintenence of the basic structure of the receptor rather than to the interaction with a putative second messenger molecule(s).

2.2. IL1RI and Toll

The only other protein so far identified that exhibits significant sequence similarity to IL1RI is 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 [19,20]. The similarity is only apparent in the cytoplasmic domain of both proteins, extending for 135 amino acids throughout most of the domain. The significance of the highest scoring matches is extremely high, with identical or conservatively substituted amino acids being found at 45% of positions. This unexpected sequence similarity suggests that the signal transduction pathway triggered by IL1RI and Toll are likely to by shared. In particular, the events stimulated by IL1 which lead to activation of the transcription factor NFκB are likely to be identical to those stimulated by Toll which 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* [21,22]. Both *tube* and *pelle* have been cloned [21,22] and *pelle* codes for a serine/threonine protein kinase related to raf-1 [22]. Raf-1 has been shown to directly activate NFκB in Jurkat T cells [23,24]. Whether a similar system is operating in the activation of NFκB by IL1 has yet to be demonstrated (see below for further discussion on NFκB). Because of the similarities in the Toll/dorsal and IL1RI/NFκB pathway it is likely that the protein(s) which interact with the cytoplasmic domains of both Toll and IL1RI will be closely related. Possible points of contact have been suggested from the work of Heguy et al. [17] who have identified six key amino acids in IL1RI from chicken, human and mouse and in Toll [21] (Fig. 1). In human IL1RI these correspond to Arg431, Phe513, Trp514, Lys515, Arg518 and Tyr519. All of these have been shown to be critical for the activation of the IL2 promoter in T cells by IL1, suggesting that these key amino acids for IL1RI function will be similarly crucial for Toll function. Interestingly, Kuno et al. [18] have found that the three basic residues in murine IL1RI which correspond to those described by Heguy et al. [17] in human IL1RI are not essential for the induction of IL8 by IL1. This suggests that different cytoplasmic elements are required for IL2 and IL8 gene activation, indicating that the signals generated by IL1 may depend on the particular final response under investigation.

2.3. IL1RII

IL1RII was first identified on cells or cell lines representative of B cells, monocytes, neutrophils, bone marrow cells and hepatoma cells [7,25–29]. Initially a second receptor type for IL1 was suggested because of differences in size and/or antigenicity from the receptors found on T cells and fibroblasts. The receptor was subsequently shown to be a product of a different gene and was cloned from murine and human B cells [9]. Structurally, the extracellular domain of IL1RII is 28% similar in amino acid sequence to IL1RI, with three immunoglobulin-like domains. Most interestingly, the intracellular 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 predominantly express IL1RII [30,31]. Secondly, in murine cells the human IL1RA will only inhibit binding to IL1RI and is inhibitory for IL1 signals [30]. A role for IL1RII in IL1 signalling involving dimerisation between IL1RI and II has also been ruled out [32]. The most likely function of IL1RII is as a regulator of IL1 levels extracellularly as it has been shown to be shed from cells [33,34], and an interesting sequence similarity has been found with a protein from Vaccinia virus which is used to decrease host immune responses to the virus [35,36]. IL1RII may therefore function as an extracellular IL1 inhibitor, binding to IL1 and preventing it from interacting with IL1RI [34].

3. G proteins and second messengers

Early attempts to determine the number of IL1 receptors per cell revealed that most cell types expressed < 500 [6]. Further investigations involving receptor occupancy studies, transfection of receptor-negative cells with the IL1RI gene [11] and studies with radiolabelled human IL1RA on 70Z/3 murine B cells [30] demonstrated that less than 10 receptors per cell were sufficient to cause cellular activation. These data suggest that a major amplification in signal must occur following IL1 binding. Classical hormonal signal amplification occurs through the coupling of receptors to guanine nucleotide binding proteins (G proteins) with the subsequent generation of second messengers such as cyclic AMP (cAMP), diacylglycerol and inositol trisphosphate and calcium. These systems have been investigated for IL1 and have been the subject of much controversy with data being presented both for and against their involvement in the IL1 signalling pathway.

3.1. Is a pertussis toxin-sensitive G protein important for IL1 signalling?

Several groups have published evidence for G protein involvement in IL1 action. Much of the evidence rests upon the inhibitory effect of pertussis toxin on post-receptor events triggered by IL1 [37–43]. The toxin has been

found to inhibit several such responses as listed in Table 1. Pertussis toxin inactivates G_i and G_o-class G proteins [44] and as a consequence G_i-like G proteins have been invoked as transducers for the IL1 signal. In conjunction with these studies IL1 has been shown to increase the binding of a non-hydrolysable analogue of GTP, GTPγS, to membranes prepared from EL4.NOB-1 thymoma cells [39]. Increased hydrolysis of GTP has also been reported in IL1-stimulated membranes from EL4.NOB-1 and 70Z/3 pre-B cells [37,39]. There have been reports of IL1 inhibiting adenylate cyclase activity in pituitary cells [45] and of another non-hydrolysable analogue of GTP, p[NH]ppG, causing a higher affinity state for IL1 binding in EL4 membranes [46]. Taken together these data suggest that a pertussis toxin-sensitive G protein (most probably G_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 NFκB, phosphorylation of the EGF receptor, induction of collagenase, IL6 and IL1RII have been shown to be insensitive [43,47]. Furthermore, for responses which are sensitive to pertussis toxin (for example, induction of IL2 and PGE2 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 [47]. The basis for the inhibitory effect of pertussis toxin therefore awaits elucidation.

In spite of this, the reported changes in GTP binding and hydrolysis and the effect of p[NH]ppG on IL1 binding would suggest that the IL1 receptor can couple to a G protein. The mechanism of this coupling is likely to be different to that exhibited by conventional G-protein linked receptors as the receptor does not possess the classical 7 transmembrane domain structure of most G protein-coupled receptors [48]. This is further suggested from the observation that p[NH]ppG actually *increases* IL1 binding rather than causing the characteristic decrease (termed negative heterotropic interaction) which is observed with classical trimeric G proteins [46]. Evidence has been provided for other agents whose receptors do not possess the seven transmembrane domain motif coupling to G proteins. These include IL2 [49], EGF [50], TGFβ [51], insulin [52], IGFII [53] and TNF [54] whose receptor has been co-purified with a G protein of unknown identity [55]. As TNF and IL1 share many biological responses and most probably induce similar post-receptor signals it is possible that IL1RI complexes to and increases the activity of a similar G protein. Such coupling may involve phosphorylation as the activity of the a subunit of G_i is modified by phosphorylation [56]. The nature of such a G protein and its importance for IL1 signalling, however, awaits determination. As may be the case with other reported signals for IL1, changes in G protein activity may be a consequence of overall cellular activation, rather than having a determining role in IL1 signal transduction.

3.2. cAMP and IL1

In 1988, Shirakawa et al. [57] reported that IL1 could increase cAMP and activate adenylyl cyclase in a variety of cell lines and that forskolin, which directly stimulates adenylyl cyclase could mimic the effects of IL1 in lymphocytes and thymocytes. The increase in cAMP was subsequently shown to be sensitive to pertussis toxin [37]. Earlier workers had attempted to implicate cAMP in IL1 signalling in chondrocytes and failed [58]. Furthermore, other workers showed that increasing cAMP in lymphocytes rather than causing activation, inhibited proliferation and IL2 production [59,60]. Subsequent studies by several other laboratories failed to find changes in cAMP in response to IL1 in several cell types, of both lymphoid and non-lymphoid origin [46,61–64]. 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 [65]. Whether IL1 can activate protein kinase A as a consequence of cAMP accumulation has also not been demonstrated directly and the phosphorylation changes characterised to date in response to IL1 have not been shown to be due to protein kinase A (see below). Finally, Munoz and co-workers [66] have dissociated changes in cAMP

Table 1
Inhibition of IL1-induced responses by pertussis toxin

Cell type	Response	Ref.
70Z/3 pre-B cells	κ Ig L chain expression	[37]
YT natural killer cells	IL2 receptor α chain expression	[37]
Rheumatoid synovial cells	PGE2 production	[37]
EL4.NOB-1 thymoma cells	Diacylglycerol accumulation	[38]
EL4.NOB-1 thymoma cells	IL2 production	[39]
MIA PaCa pancreatic cells	M-CSF production	[40]
Mesangial cells	Phosphatidate phosphohydrolase activation	[41]
Hippocampal neurons	Inhibition of calcium channel currents	[42]
Rinm5F pancreatic β cells	Induction of IL1 RI	[43]

from several IL1 responses in Th2 cells. The conclusion must therefore be that cAMP is unlikely to be a second messenger for IL1.

3.3. Phospholipid-derived second messengers

IL1 has been shown to cause the breakdown of phospholipids in a variety of cell types. An increase in arachidonic acid release from membrane phospholipids via phospholipase A2 activation has been demonstrated in synovial fibroblasts [67], chondrocytes [68] and mesangial cells [69]. Recent work carried out by Gronich et al. [70] has demonstrated that IL1 activates the cytosolic form of phospholipase A_2 ($cPLA_2$) by phosphorylating the enzyme. As $cPLA_2$ in other systems has been shown to be phosphorylated by mitogen activated protein kinase (MAP kinase) [71], the authors suggest that the effect of IL1 is similarly due to activation of MAP kinase, as IL1 has been found to activate MAP kinase [72,73].

Increases in diacylglycerol have been reported in different cell types although the phospholipid source varies: in T cells this has been shown to be phosphatidylcholine [74], in macrophages, phosphatidylinositol [75], and in mesangial cells phosphatidylserine [76]. The breakdown of phosphatidylinositol in response to IL1 does not occur in most cell types, however, and there is a consensus that IL1 does not cause an increase in intracellular calcium [77,78], which is consistent with the lack of evidence for inositol trisphosphate generation. However, a recent report has shown activation of nuclear phospholipase Cβ with subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate in SaoS osteosarcoma cells [79].

There have also been reports of phosphatidic acid generation in EL4 thymoma cells with subsequent generation of diacylglycerol via the activation of phosphatidate phosphohydrolase [41]. IL1 has also been demonstrated to activate phosphatidylinositol-3-kinase activity in fibroblasts [80]. The activation of this enzyme is a common event for many different growth factors [81]. The significance of its activation by IL1 may relate to the observation that the product of phosphatidylinositol-3-kinase, phosphatidylinositol-3,4,5-trisphosphate has been shown to activate protein kinase Cζ [82]. If IL1 is able to activate protein kinase Cζ by such a mechanism, this may explain the differences in phosphorylation patterns observed in cells stimulated with IL1 and phorbol myristate acetate (PMA) (see below), as protein kinase Cζ is insensitive to PMA [83].

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 cyclooxygenase by IL1 would appear to have a more important role in the triggering of this response [84,85]. Recently, evidence has been presented for arachidonic acid released in response to IL1, activating the transcription factor AP1 (see below) and from there inducing granulocyte-macrophage colony stimulating factor in a bone marrow stromal cell line [86]. 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. Furthermore, in a cell type where diacylglycerol generation has been shown to occur (Detroit 532 fibroblasts) no protein kinase C activation has been observed [87].

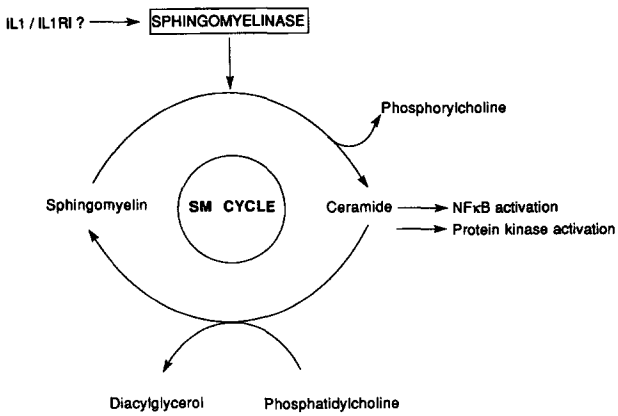

Fig. 2. The sphingomyelinase (SM) cycle. Sphingomyelin hydrolysis in response to IL1 has been reported [88,89]. This scheme illustrates a putative cycle which may operate in stimulated cells. The product of sphingomyelinase, ceramide, may act as a second messenger as indicated and can be re-esterified to sphingomyelin through a reaction with phosphatidylcholine [90].

Most recently the sphingomyelin breakdown product ceramide has been shown to be generated in response to IL1 in both EL4 thymoma cells [88] and fibroblasts [89]. The 'sphingomyelin cycle' represents a novel signal transduction pathway and is shown in Fig. 2 (reviewed recently in [90]). Ceramide has been shown to activate a protein kinase and a member of the protein phosphatase 2A family [90] indicating that it may act as a second messenger. Sphingomyelin turnover was originally shown to be stimulated by TNF, interferon-γ and vitamin D-3 in the myeloid cell line HL60 [91]. A series of experiments have suggested that sphingomyelin hydrolysis may also be a key step in IL1 signalling. Evidence includes the ability of sphingomyelinase to mimic IL1-induced IL2 production in EL4 [88], the potentiating effect of sphingosine, ceramide and sphingomyelinase on IL1-induced prostaglandin production in fibroblasts [89,92], the activation of a ceramide-regulated protein kinase by IL1 [88] and finally sphingomyelin hydrolysis and ceramide generation in reponse to IL1 [88,89]. A similar pathway has been invoked in studies with TNF, again suggesting a common signal [91]. Finally, ceramide and sphingomyelinase have been shown to activate the transcription factor NFκB in HL60 [93]. As mentioned previously, activation of NFκB is an early signal triggered in response to IL1. The effect of ceramide on NFκB, however, may be cell-type or cell-strain specific as other reports have failed to show a link between sphingomyelinase activation by TNF and NFκB

in Jurkat and HL60 [94,95]. Whether the sphingomyelin pathway represents a general signal transduction pathway in all cell types and for all IL1 responses therefore awaits further investigation. The fact that sphingomyelin breakdown products on their own failed to cause prostaglandin production in fibroblasts [89] would suggest that IL1 may provide other signals necessary for this effect. Further complexity is indicated from the suggested role of sphingolipid breakdown products in tumour suppression and inhibition of proliferation [96,97]. Reports on the effects of IL1 on cell proliferation have been conflicting, with IL1 at best being a weak mitogen which probably acts indirectly [98]. This would suggest that the sphingomyelin cycle may have other roles to play in cellular activation, or alternatively may represent another general change which occurs in the activation of cells by IL1. A role for ceramide in IL1 signalling is further questioned, however, from studies demonstrating that ceramide activates protein phosphatase 2A. Other reports have indicated that IL1 inhibits this enzyme as described in more detail in section 4.3 below. Further experiments are therefore necessary to clarify how important this pathway is likely to be in IL1 signalling.

4. Protein phosphorylation changes and IL1

IL1 causes a wave of protein phosphorylation within minutes of binding to fibroblasts. Studies involving high resolution two-dimensional gel electrophoresis have identified at least 116 polypeptides whose phosphorylation state changes in response to both IL1 and TNF [73]. The majority of these changes are an increase in phosphorylation, although some represent a decrease. Most of the changes were shown to occur on serine or threonine residues with a small number occurring on tyrosine residues. A range of other agents were tested in order to determine how specific the changes were for IL1. PMA, epidermal growth factor (EGF), cAMP agonists, bradykinin and interferons failed to mimic IL1, suggesting that neither growth factors nor protein kinases A or C were capable of triggering similar changes to IL1 and thereby arguing against the involvement of these protein kinases in IL1 action here. Only TNF and the protein phosphatase inhibitor okadaic acid caused identical changes to IL1. The major phosphorylation change observed was of the small heat shock protein hsp27 [99]. Changes in phosphorylation of stathmin, eIF-4E, myosin light chain, nucleolin, EGF receptor, c-abl, retinoblastoma and p53 were also identified. Earlier studies had demonstrated that IL1 increased the phosphorylation of proteins in several cell types, including a 65 kDa protein in B cells [100] subsequently identified as 1-plastin [101]; in fibroblasts hsp27 [102,103], the EGF receptor [104,105] and the cytoskeletal protein talin [106] and in At-20 pituitary cells, stathmin [107,108]. The identity of the other substrates in fibroblasts is still ill-defined and the protein kinases which become activated in response to IL1 have been under intense investigation.

4.1. Protein kinase C

Early work attempted to implicate known protein kinases in IL1 signalling. Initially this focused on protein kinase C, as PMA, which directly activates protein kinase C, mimics many of the actions of IL1 [72,109–111]. Reports have appeared showing activation of protein kinase C in T cells [112], mesangial cells [76], mouse fibroblast NIH 3T3 cells [113] and EL4 6.1 thymoma cells [114]. However, evidence has been presented questioning the importance of protein kinase C in IL1 action. The phosphorylation pattern induced in fibroblasts by PMA differs markedly from that seen with IL1 [73]. 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 [109,115–121]. Interestingly, protein kinase C inhibitors such as staurosporine and/or H7 have been shown to potentiate in response to IL1, IL2 and IL4 production in EL4 cells [118], PGE2 production in fibroblasts [117], proliferation in Th2 lymphocytes [66] and the induction of IL6 in monocytes [121]. Furthermore, staurosporine has also been found to potentiate TNF action [122]. This would suggest that protein kinase C may have a negative effect on IL1 and TNF signal transduction. Alternatively, staurosporine may trigger responses of its own which lead to the observed potentiation. This is further suggested from studies showing a dramatic upregulation of TNF receptors by staurosporine in myeloid and epithelial cells [122]. Staurosporine may therefore also upregulate IL1 receptors and thereby increase the effect of IL1 on cells.

Further evidence against protein kinase C involvement has come from studies into the EGF receptor [72,104,105,123]. Similar to PMA, IL1 decreases the affinity of this receptor for EGF, a process termed transmodulation [104]. The insensitivity of the IL1 response to staurosporine, coupled with differences in phosphopeptide maps of the receptor generated by PMA and IL1 indicated that protein kinase C was not involved in this response to IL1 [105]. Subsequent work has gone on to demonstrate that IL1 causes phosphorylation of the EGF receptor on threonine 669, whereas PMA causes phosphorylation on threonine 654 [72].

Taken together, these data strongly argue against protein kinase C involvement in IL1 action. However, as most of the evidence is based on comparing IL1 with PMA or the use of protein kinase C inhibitors, it is possible that an isoform which is insensitive to PMA and the inhibitors may be activated, for example protein kinase Cζ [83], as described above. This has yet to be tested, however.

4.2. MAP kinases

The protein kinase responsible for the phosphorylation of the EGF receptor by IL1 has been purified from KB epidermoid carcinoma cells and evidence has been pre-

sented for this enzyme being a member of the MAP kinase family [72]. Other workers have shown that IL1 can activate p42/p44 MAP-kinase and MAP kinase kinase in fibroblasts [73,124,125]. In common with several growth factors and TNF [73,126], IL1 can therefore activate the MAP kinase pathway. Whether the kinases activated are identical to those triggered by other agents has yet to be demonstrated. Activation of the MAP kinase pathway by IL1 may again be a consequence of overall cellular activation, perhaps by cross-talk from other pathways, and the relevence of the changes induced for the specific signal generated by IL1 is yet to be established. It is possible that activation of the MAP kinase pathway may be a common early event for many cellular activators with specificity being achieved downstream, as suggested recently by Brunner et al. in *Drosophila* [127] and in studies comparing EGF and NGF signal transduction in PC12 cells [128]. Both studies indicated that although phenotypic responses to different stimuli varied according to the stimulus, MAP kinase activation was common to all stimuli studied. Signalling pathways must either therefore diverge after MAP kinase, or alternatively involve additional early signals, perhaps other protein kinases, specific for each stimulus.

Phosphorylation of hsp27 in response to IL1 in KB cells has been shown to occur downstream of MAP kinase [129]. The phosphorylation sites have been identified as Ser78 and Ser82. These have also been shown to be phosphorylated by the MAP kinase substrate MAP kinase activated protein kinase 2 (MAPKAP kinase 2) [130] which may therefore be the IL1-activated MAP kinase substrate which phosphorylates hsp27. Putative protein kinase cascades culminating in hsp27 phosphorylation are shown in Fig. 3. Other evidence indicates that the hsp27 kinase cascade activated by IL1 may be novel (see below).

4.3. Protein phosphatases

The phosphorylation changes induced by IL1 in fibroblasts are similarly induced by the phosphatase inhibitor okadaic acid [99]. This has led to the suggestion that IL1 inhibits the action of a phosphatase, possibly protein phosphatase-2A. The substrate for this phosphatase could be a kinase suppressor which is active in a de-phosphorylated form. Direct evidence has been provided for IL1 and TNF inactivating a phosphatase, possibly protein phosphase 2A, one of whose substrates is hsp 27 [132,133]. This scenario may therefore have a role to play in increased phosphorylation of hsp27, as described in Fig. 3. In addition,

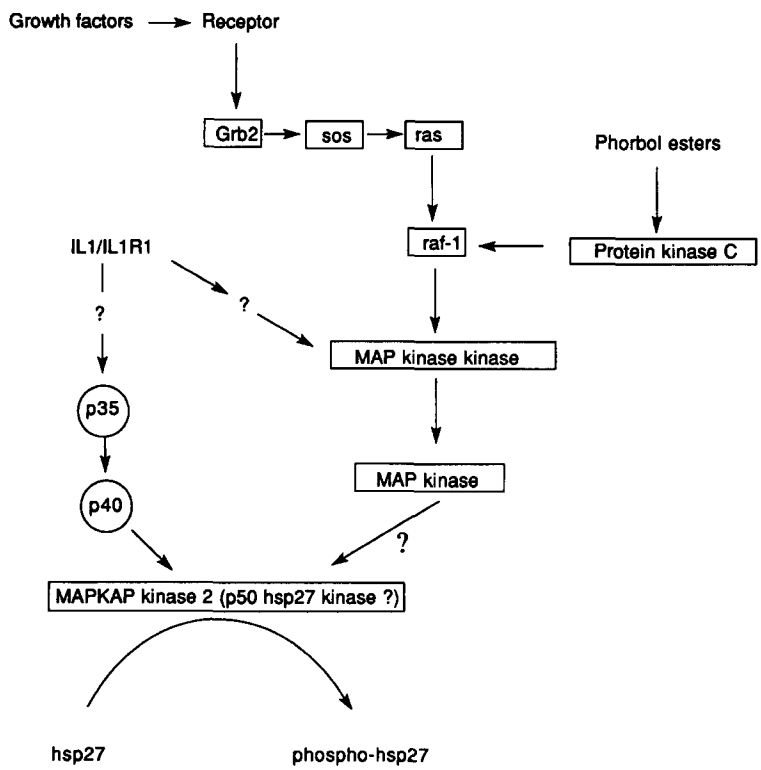

Fig. 3. Protein kinase cascades leading to phosphorylation of hsp27. As described in the text, signals leading to the phosphorylation of hsp27 may converge on the enzyme MAPKAP kinase 2. The sequence of events leading from growth factor receptors and protein kinase C to MAP kinase are well documented [131]. IL1 has been shown to trigger the MAP kinase cascade in some cell types [72,99,124,125,129]. Evidence has also been presented for the activation of a novel protein kinase cascade which may culminate in a protein kinase related to or identical to MAPKAP kinase 2 [134]. The pathways indicated are all sensitive to protein phosphatase 2A which will dephosphorylate and thereby inactivate MAP kinase kinase, MAP kinase, MAPKAP kinase 2, p35, p40 and hsp27 itself [132–134]. IL1 may therefore inhibit this enzyme which is susceptible to inactivation by phosphorylation on tyrosine [132,133].

evidence has been presented for both protein phosphatase 2A and phosphotyrosine phosphatase 1B inactivating IL1-activated protein kinases [123,134]. This suggests that protein phosphatases are likely to be critical in the regulation of IL1 action. IL1 signalling may therefore involve a co-ordinate activation of kinases and inactivation of phosphatases.

4.4. Tyrosine kinases

As mentioned previously, IL1 has been shown to increase tyrosine phosphorylation in fibroblasts [99]. Increases in tyrosine phosphorylation have also been reported in K562 cells [135], A375-C6 human melanoma cells [136], Th2 cells [137] and EL4 thymoma cells [138]. A consistent change occurs in proteins of 42–44 kDa [72,99,136,137], the molecular mass range for 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 [72]. This is likely to be due to MAP kinase kinase activity since, as mentioned previously, IL1 activates this enzyme [125] and MAP kinase kinase activates MAP kinases by phosphorylating tyrosine and threonine residues [139,140]. The tyrosine kinases responsible for the increased tyrosine phosphorylation of the other proteins reported in A376-C6 and Th2 cells [136,137] have yet to be identified.

Studies with tyrosine kinase inhibitors have suggested that tyrosine kinase activation may be important for IL1-induced changes in gene expression. Induction of *gro* genes in melanoma cells, IL5 in T cells and PGE2 production in mesangial cells are inhibited by the tyrosine kinase inhibitor genistein [136,137,141]. Activation of the transcription factor NFκB can also be blocked by genistein and another tyrosine kinase inhibitor herbimycin A [136,138,142] (see below) which has also been shown to inhibit IL2 production in EL4 cells [142]. The precise target of these inhibitors has yet to be determined.

4.5. Novel protein kinases

The possibility that IL1 is activating novel protein kinase(s) which initiate a specific signalling cascade has also been explored. These studies have focused on three substrates: a peptide derived from the EGF receptor, hsp27 and β-casein [102–105,123,124,134,143,144].

p54 MAP kinase

As mentioned previously, a report has appeared characterising the protein kinase responsible for phosphorylation of a peptide derived from the EGF receptor in KB cells and suggesting that the enzyme responsible is a member of the MAP kinase family [72]. Recently, more extensive analysis has been carried out which has suggested that the enzyme is unlikely to be p42/p44 MAP kinase, based on substrate specificity (myelin basic protein and microtubule-associated protein-2 are weak substrates), lack of reactivity with antisera to p42/p44 MAP kinases and finally failure to reactivate the dephosphorylated form of the enzyme with MAP kinase kinase [123]. Most recently a study carried out in rabbit has indicated that this kinase is likely to be p54 MAP kinase α [145], a member of JNK1/SAP family of kinases which phosphorylate c-jun [146]. This study involved treating rabbits with IL1 and then purifying the kinase from liver. The isolated kinase is highly likely to be the rabbit form of the enzyme described in KB cells [123]. The p54 family appear to have their own upstream regulators which have yet to be determined [146]. Finally, TNF has recently been shown to activate JNK1 in NIH3T3 fibroblasts [146], further emphasising a role for this kinase in IL1 and TNF signalling.

hsp27 kinase cascade: HOG1 homologue / MAPKAP kinase 2

hsp27 phosphorylation can be induced by a range of stimuli, including TNF, platelet derived growth factor, bradykinin, acid fibroblast growth factor, ATP and PMA [116]. It is therefore possible that hsp27 may be a substrate for several serine kinases, and recent evidence has suggested that the hsp27 kinase cascade activated by IL1 may differ from that activated by PMA [124,134]. The hsp27 kinase can use GTP or ATP, is insensitive to a wide range of protein kinase inhibitors and is highly specific for hsp27 [124]. These data suggest that the enzyme may be novel. Indeed most recently, evidence has been presented for a novel protein kinase cascade activated in response to IL1 in KB cells which culminates in phosphorylation of hsp27 [134] (described in Fig. 3). Three components were isolated in the cascade, an upstream activator of molecular mass 35 kDa and two downstream components, of molecular mass 40 kDa and 50 kDa, respectively. In vitro reconstitution experiments have demonstrated that a protein kinase cascade is likely to be operating comprising p35 → p40 → p50 → hsp27, as shown in Fig. 3. Similar to the EGF peptide kinase described above [123], all three components are sensitive to inactivation by protein phosphatase 2A. Biochemical evidence indicates that the enzymes participating in the cascade are novel. Very recent evidence suggests that p40 is the human homologue of a recently reported murine protein kinase, p38, that becomes activated in response to endotoxin and hyperosmolarity [147]. The murine protein is related to mammaliam p42 and p54 MAP kinases and to the *Saccharomyces cerevisiae HOG1* gene product, which is also activated in response to changes in osmolarity. p50 hsp27 kinase resembles MAPKAP kinase 2 as previously described by Bird et al. [129] and may represent the human homologue of MAPKAP kinase 2, which was first described in rabbit [148]. Based on data presented by Bird et al. [129], and as suggested by Freshney et al. [134], MAPKAP kinase 2

may therefore be a point at which the MAP kinase and hsp27 cascades converge. However, recent evidence presented by Rouse et al. [149] has suggested that MAPKAP kinase 2 is not a substrate for p42/p44 MAP kinase, indicating that both pathways are unlikely to converge. This work described a novel protein kinase cascade triggered by stress and heat shock which culminated in hsp27 phosphorylation. This cascade appears to be identical to that triggered by IL1. Whether both this cascade or the 'classical' p42/p44 MAP kinase cascade are triggered in response to IL1 may be cell-type specific. In KB cells, IL1 only weakly activates MAP kinase [123] whereas in MRC-5 fibroblasts, there is a stronger activation [124]. Further complexity is suggested from the observation that PMA, which strongly activates MAP kinase in KB, does not activate the IL1-sensitive hsp27 kinase [124], distinguishing the enzyme from the MAPKAP kinase 2 which lies on the MAP kinase pathway. The precise relationship between the p40/p38/HOG1 pathway and the p42/p44 MAP kinase pathway therefore remains ambiguous.

The significance of hsp27 phosphorylation for IL1 action is unclear, as the physiological role(s) of hsp27, and hence phosphorylation of hsp27, is uncertain. It is present in almost all mammalian cells and is induced in response to heat shock and other stresses. Like other heat shock proteins it is reported to have chaperonin-like properties [150], and its overexpression has been shown to increase thermotolerance and to inhibit cell proliferation [151,152]. hsp27 has also been shown to participate in actin polymerisation [153]. Phosphorylation of hsp27 may be essential for cytoskeletal changes in response to growth factor action, as recent work in fibroblasts has shown that the expression of a dominant negative mutant of hsp27 which could not be phosphorylated prevented growth factor-induced polymerisation of submembrane actin filaments and F-actin accumulation [154]. Whether IL1 causes similar cytoskeletal changes through the phosphorylation of hsp27 has yet to be determined.

β-Casein kinase

The third novel kinase to be described has been termed β-casein kinase [124,144] because of its ability to phosphorylate β-casein. It has been described in MRC-5 gingival fibroblasts, chondrocytes and endothelial cells [124,144]. The enzyme is not activated by PMA and a wide range of other agents, including serum, basic fibroblast growth factor and transforming growth factor β1 [124,144]. 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 [144]. 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.

In summary, the novel kinases described above are likely to be part of phosphorylation cascades triggered by IL1. Apart from β-casein kinase whose amino acid sequence has yet to be determined, they are all related to p42/p44 MAP kinase. Cascades involving p38/p40/HOG1 (which leads to hsp27 phosphorylation), p42/p44 MAP kinase and p54 MAP kinase/JNK1 are therefore likely to be triggered in cells treated with IL1. The extent to which each cascade is activated may be cell-specific and so far only p54 kinase has been demonstrated to be activated in vivo [124]. All three cascades also become activated in response to endotoxins and a range of physiological stresses. The early protein kinases in the cascades and a kinase associated with the liganded IL1 receptor remain to be determined. Similarly, whether they are involved in the gene expression changes induced by IL1, possibly through phosphorylation of transcription factors, has yet to be discovered.

5. IL1 and transcription factors

Transcription factors provide the link between post-receptor signalling events and changes in gene expression. Phosphorylation plays a key role in regulating their activity and there are many examples of phosphorylation controlling their nuclear translocation, DNA binding and transactivation [155]. IL1 has been shown to activate and/or induce several transcription factors, including jun [156–158], fos [159,160], NFκB [110,161], Myc [66], Egr-1, NAK-1, IRG-9 [162], Myb [66], c/EBP [163] and NFIL6 [164]. Most studies have focused on the fos-jun dimer AP1 and NFκB as a number of IL1-responsive genes have enhancers which contain AP1 and NFκB binding sites. Some of these are shown in Table 2.

5.1. AP1

The predominant form of AP1 comprises a dimer of two proteins, fos and jun [181]. IL1 has been shown to induce expression of fos and jun in several cell types including the pituitary cell line AtT-20 [182] and HepG2 cells [160], and jun in LBRM T cells [158]. The latter study indicated that induction of jun by IL1 may be the basis of its co-stimulatory effect on T cells, whereby antigenic stimulation and IL1 are both needed to trigger IL2 production. The antigenic signal (provided by phytohemaglutinin) was found to induce fos, thus to generate AP1, IL1 induces jun and phytohemaglutinin induces fos. AP1 activation by IL1 has been shown to be critical for the induction of β-endorphin in AtT-20 pituitary cells [182]. The mechanism by which IL1 increases transcription via

AP1 may involve IL1-activated MAP kinase and p54/JNK1, as phosphorylation of jun by both of these kinases has been demonstrated and in the case of MAP kinase has been shown to be required for transcriptional activation by AP1 [183]. As mentioned previously, the generation of arachidonic acid in response to IL1 may also be important for the activation of AP1 [86].

5.2. NFκB

The predominant form of NFκB exists in resting cells in the cytosol as a dimer, p50 and RelA (formerly called p65), complexed to an inhibitory protein IκB [184]. As shown in Fig. 4, to activate NFκB, IκB must dissociate, allowing translocation of the NFκB dimer to the nucleus. IL1 has been shown to activate NFκB in numerous cell types [30,110,161,185,186]. Direct dissociation of IκB and translocation has been demonstrated [186]. NFκB can be activated by a range of other agents, including viruses, double-stranded DNA, lipopolysaccharide, UV irradiation, oxidative stress and TNF [184]. All of these agents may use a common signalling pathway. Several models have been proposed to describe how NFκB is activated. These are summarised in Fig. 4.

IκB can be dissociated chemically from NFκB in vitro with detergents such as deoxycholate [187]. In vitro phosphorylation of IκB by protein kinase C, protein kinase A and casein kinase II also lead to dissociation [188], although whether these kinases also cause phosphorylation in vivo has yet to be directly demonstrated. Whether IL1 can cause the phosphorylation of IκB is somewhat controversial, with evidence being presented both for and against this occurring [185,189]. Proteolysis of IκB has also been suggested as a key event in the activation process [189].

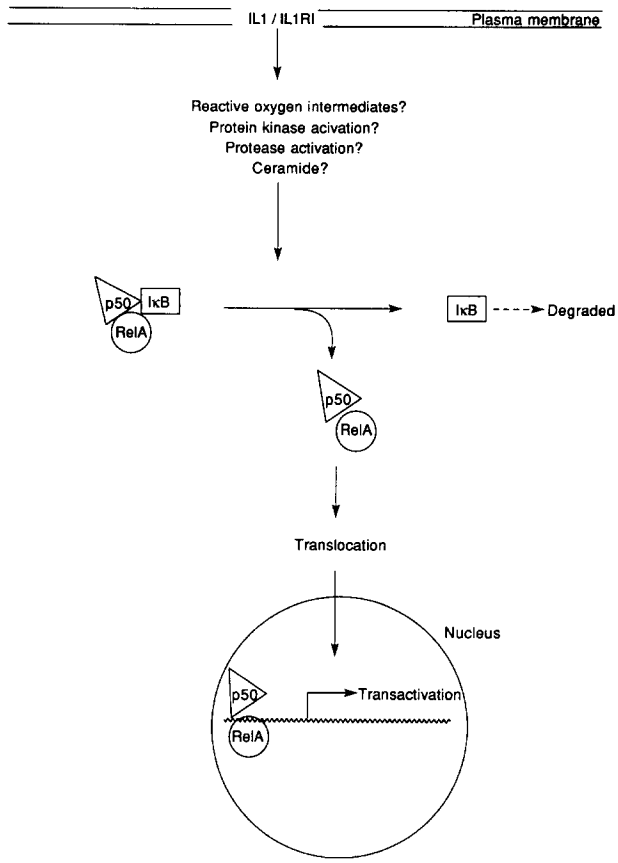

Fig. 4. Possible routes to NFκB activation. The primary event which occurs in NFκB activation is dissociation of the inhibitory component IκB, allowing the NFκB complex to translocate to the nucleus. As indicated above, several mechanisms have been proposed for this process, including generation of reactive oxygen intermediates [192,193], protein kinase activation [185,188], proteinase activation [189] and ceramide generation [93]. The importance of any of these for IL1 action has yet to be fully defined.

Table 2
IL1-sensitive gene enhancers which contain NFκB and/or AP1 consensus sequences

Transcription factor	IL1-Regulated target gene	Ref.
NFκB	Igκ light chain	[161,165]
	IL2 receptor α chain	[166]
	GM-CSF	[167]
	G-CSF	[168]
	IL2	[169,170]
	IL6	[171]
	TNFα	[172]
	Serum amyloid A	[173]
	myc	[174]
	E-selectin	[175]
	VCAM-1	[176]
	ICAM-1	[177]
AP1	Collagenase	[178]
	Metallothionein	[179]
	IL2	[158]
	IL8	[180]
	E-selectin	[175]
	VCAM-1	[176]
	ICAM-1	[177]

Proteinase inhibitors have been shown to block the activation of NFκB by IL1, indicating that IL1 may be activating a proteinase important for the process of activation [189]. As mentioned previously, the tyrosine kinase inhibitors genistein and herbimycin A have also been shown to block activation [138,140], suggesting the involvement of a tyrosine kinase. There is also a possibility that the MAP kinase pathway is involved, as recent studies involving the overexpression of MAP kinase in T cells have demonstrated constitutive activation of NFκB and AP1 in these cells [190]. This may relate to the likely similarity between NFκB activation by IL1 and dorsal activation by Toll in *Drosophila* as mentioned above. The importance of the raf-1-like kinase pelle for dorsal activation suggests that a raf-1-like kinase may be important for the activation of NFκB by IL1. raf-1 activates the MAP kinase pathway in some cell types [191] and so by analogy this pathway may be important for NFκB activation. Furthermore, dominant negative mutant studies involving raf-1 have also shown raf-1 to be critical for NFκB activation in response

to a range of stimuli, including TNF [24]. Whether raf-1 becomes activated in response to IL1 has yet to be determined. As mentioned previously, ceramide may act as a second messenger for NFκB activation, although its effect may be cell-type specific [94,95].

A final model for activation of NFκB has come from work with antioxidants which have been shown to inhibit a range of stimuli including IL1 [192,193]. This suggested that reactive oxygen intermediates may be important second messengers for NFκB activation. Hydrogen peroxide was also shown to activate NFκB [192]. Recent studies which compared the susceptibility of transformed cell lines and primary cells to hydrogen peroxide suggest that the ability of reactive oxygen intermediates to activate NFκB may be cell-specific and may depend on the intracellular oxidative state of individual cell types [194]. Activation of NFκB by IL1 has been shown to be insensitive to the antioxidant *N*-acetylcysteine in EL4 cells and 1231 N1 astrocytoma cells [30,195,196]. Another antioxidant, pyrollidine dithiocarbamate also failed to inhibit NFκB activation in 1231 N1 astrocytoma cells [195]. Interestingly, in a human endothelial cell line ECV304, the compound again failed to inhibit NFκB activation by IL1 but was able to block activation by TNF and PMA [197]. This further questions a role for reactive oxygen intermediates in the activation of NFκB by IL1. It is possible that there may be several mechanisms by which NFκB can be activated which may be dependent on the species of NFκB and perhaps more importantly, the species of IκB, as four distinct forms have so far been described [198].

Pyrollidine dithiocarbamate and *N*-acetylcysteine have also been used in studies on the induction NFκB-driven genes by IL1. Both agents were found to block IL1-induced IL2 in EL4 cells [196]. However, in 1231 N1 astrocytoma cells, where IL1 has been shown to induce the cell adhesion molecules ICAM-1 and VCAM-1, only VCAM-1 proved susceptible [195]. An identical phenomenon was observed in endothelial cells [199]. These results suggest that the signal leading from the IL1RI to changes in gene expression must diverge for these two genes.

6. Conclusions

Despite intense investigation, a clear signal transduction pathway for IL1 has yet to emerge. The dramatic activation of cells induced by IL1 may turn on a number of signal transduction pathways which may diverge, adding complexity. Furthermore, different pathways may be activated in different cell types. Recent evidence of a protein kinase cascade involving MAP kinases and/or novel protein kinases would allow for the amplification needed to generate a signal from the small number of receptors triggered by IL1. These kinases are also likely to activate transcription factors such as NFκB, thereby providing a link between the receptor and gene expression. Determining the nature of these kinases and precisely how they alter gene expression represents a major challenge and will hopefully lead to the determination of the specific signal transduction pathway for IL1.

Acknowledgements

I would like to thank Drs. Paul Moynagh and Tim Mantle for stimulating discussion and helpful comments on the manuscript. This work was supported by grants from Forbairt, Health Research Board, Cancer Research Advancement Board, Irish Heart Foundation and Bioresearch Ireland.

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