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Cell Type-specific Role for Reactive Oxygen Species in Nuclear Factor-kappaB Activation by Interleukin-1

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ABSTRACT. The role of reactive oxygen intermediates (ROIs) in nuclear factor-kappaB (NF-κB) activation remains a matter of controversy. We have studied whether ROIs played any role in NF-κB induction by interleukin-1β (IL-1β) in different cell types. Our studies indicated three different pathways. IL-1β stimulation of lymphoid cells generates ROIs, which are required for IκB-α degradation and NF-κB activation. The source of these ROIs is the 5-lipoxygenase (5-LOX) enzyme. In monocytic cells, ROIs are also produced in response to IL-1β and necessary for NF-κB induction, but their source appears to be the NADPH oxidase complex. Finally, epithelial cells do not generate ROIs after IL-1β stimulation, but do rapidly activate NF-κB. Interestingly, transfection of epithelial cells with the 5-LOX and 5-LOX activating protein expression vectors restored ROI production and ROI-dependent NF-κB activation in response to IL-1β. Our data thus indicate that ROIs are cell type-specific second messengers for NF-κB induction by IL-1β. BIOCHEM PHARMACOL **59**;1:7–11, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. NF-κB; reactive oxygen species; cytokines; interleukin-1

Transcription factors of the NF-κB§/Rel family regulate the expression of genes involved in inflammation, cell proliferation, and apoptosis [1, 2]. The prototype member of the family, NF-κB, is composed of a dimer of p50 and p65/RelA. NF-κB is present in the cytoplasm of resting cells and enters the nucleus in response to multiple stimuli, including viral infection, ultraviolet irradiation, and exposure to proinflammatory cytokines such as TNF-α and IL-1 [1]. NF-κB is also activated by various chemical stimuli such as phorbol esters, chemotherapeutic agents, oxidizing agents, and phosphatase inhibitors. NF-κB is sequestered in the cytoplasm in an inactive form by virtue of its association with inhibitory proteins, named IκB [1]. The IκB family members, such as IκBα, IκBβ and IκBε, have ankyrin-like repeat domains and regulate the DNA binding and subcellular localization of NF-κB/Rel proteins by masking their nuclear localization signal. NF-κB activation by TNF-α or IL-1 is achieved through the signal-induced proteolytic degradation of IκB in the cytoplasm (Fig. 1). These cytokines initiate a signaling cascade leading to the activation of two IκB kinases, IKK1 (IKKα) and IKK2 (IKKβ), which phosphorylate IκB at specific N-terminal serine residues (S32 and S36 for IκBα, and S19 and S23 for IκBβ) [3–9]. These two IKK kinases are rapidly activated after TNF-α or IL-1 interaction with their receptor, and the candidate IKK kinases are NIK and MEKK1 [10–12]. Following its phosphorylation by the IKKs, IκB is selectively ubiquitinated and degraded by the 26S proteasome [13, 14]. This process exposes the NF-κB nuclear localization signal, thereby authorizing NF-κB to interact with the nuclear import machinery and to translocate to the nucleus, where it binds its target sequences and activates target gene transcription.

In addition to this direct signaling pathway, a number of other intermediates that could play a role in NF-κB activation by TNF-α or IL-1 have been described, at least in some cell systems. Among intermediates are Raf-1, MAP kinases, protein kinase C ζ and λ/ι, Rho and Rac proteins, ceramides, and ROIs [5–25].

ROIs have been shown to activate NF-κB [21–23]. Indeed, treatment of cells with micromolar amounts of hydrogen peroxide results in NF-κB activation. Conversely, antioxidants such as PDTC and NAC block NF-κB activation by a wide range of stimuli (IL-1, TNF, phorbol 12-myristate 13-acetate etc.), suggesting that ROIs may act as signaling molecules in NF-κB activation in response to a variety of agents. Moreover, NF-κB activation by ROIs is considered as a central process in inflammation and HIV replication [2, 26, 27]. Several biochemical reactions lead to the production of ROIs, including the biosynthesis of prostaglandins and leukotrienes, the stimulation of NADPH oxidases in the plasma membrane, or the detoxification of xenobiotics by the cytochrome P450 enzymes

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§ *Abbreviations:* NF-κB, nuclear factor-kappaB; TNF, tumor necrosis factor; IL-1, interleukin-1; ROIs, reactive oxygen intermediates; 5-LOX, 5-lipoxygenase; FLAP, 5-LOX activating protein; IKK, IκB kinase; NIK, NF-κB-inducing kinase; MEKK1, mitogen-activated protein (MAP) kinase kinase kinase-1; NAC, *N*-acetyl-cysteine; and PDTC, pyrrolidine-9-dithiocarbamate.

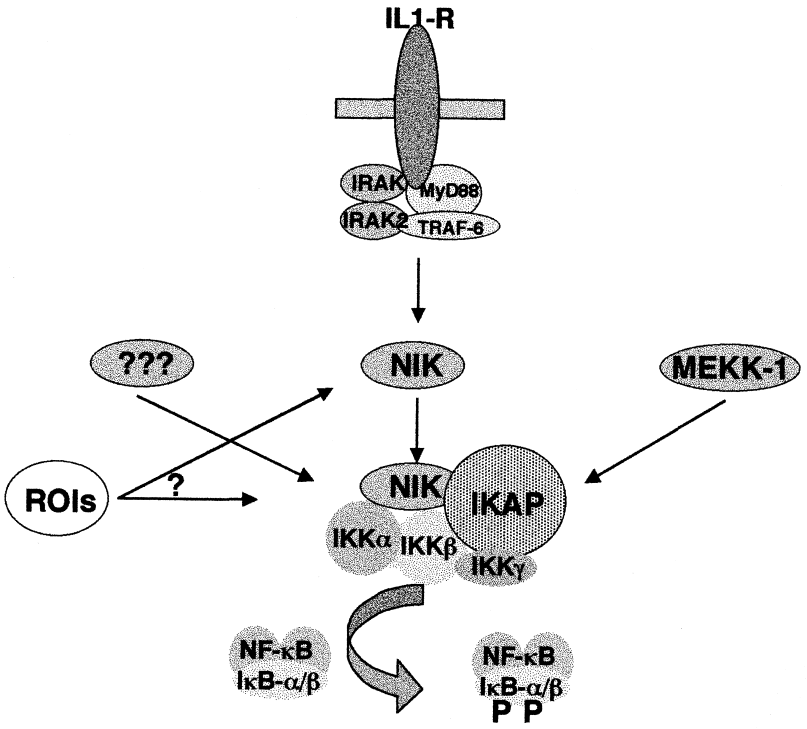

FIG. 1. IL-I-dependent NF-κB activation pathway. Following IL-1β interaction with its type 1 receptor, this receptor recruits the kinases IRAK (IL-1 receptor-associated kinase) and IRAK2 and the MYD88 and TRAF6 (TNF receptor-associated factor 6) adaptor proteins. The signal is then transmitter to the NIK or MEKK-1 activating kinases, but other kinases could also be involved at this step. NIK then associates with the signalsome and the IKAP (IKK-complex-associated) scaffold protein facilitates the activation of IKKs (the IKKγ associates with IKKβ and enhances its activation). The IKK kinase can then phosphorylate (P) the IκB inhibitor, leading to its degradation and to NF-κB nuclear translocation.

[28]. A variety of cellular enzymes, including cyclooxygenases (COX-1 and COX-2), lipoxygenases (5-lipoxygenase, 8(S)-lipoxygenase, 1-lipoxygenase, and 15-lipoxygenase), and NADPH oxidase are important ligand-activated ROI-generating systems. The lipoxygenase enzymes are present in the nuclear membrane and in the nucleus and catalyze the stereospecific insertion of molecular oxygen into unsaturated fatty acids [29, 30]. 5-LOX is the first enzyme of the leukotriene biosynthesis pathway; it catalyzes the insertion of molecular oxygen on C-5 of arachidonic acid. 5-LOX catalytic activity requires its association with the FLAP protein. Another enzyme implicated in ROI production is the NADPH oxidase, which is expressed in neutrophils, monocytes/macrophages, and lymphocytes and generates toxic metabolites in order to fight microorganisms.

In summary, ROIs had long been considered as essential second messengers for NF-κB activation, but more recent data have focused on the role of NIK, MEKK-1, and IKK kinases in NF-κB induction by TNF-α or IL-1. We therefore explored the role of ROIs for NF-κB activation by IL-1β in different cell types and demonstrated that at least three distinct cell type-specific signaling pathways lead to the induction of NF-κB following cellular stimulation with IL-1β (Fig. 2) [15, 31–33].

LYMPHOID CELLS

Stimulation of lymphoid cells (70Z/3, Raji, or EL-4 cell lines) with IL-1β generates ROIs and rapidly induces nuclear NF-κB DNA-binding activity. We could demonstrate that ROI production was required for IL-1β-induced NF-κB DNA binding, IκB-α degradation, and NF-κB-dependent transactivation, as these activities were blocked by the antioxidants NAC and PDTC. We then identified 5-LOX as the main source of ROIs in these cells after IL-1β treatment. Indeed, the above-mentioned lymphoid cells express both the 5-LOX enzyme and its activating protein, and cell treatment with the FLAP inhibitors MK886 and 5,8,11,14-eicosatetraynoic acid (ETYA) prior to IL-1β stimulation completely abolished ROI production and NF-κB activation. In conclusion, NF-κB activation by IL-1β in lymphoid cells involves a signaling pathway which implies ROI production by 5-LOX, probably upstream of IKK activation.

MONOCYTIC CELLS

Stimulation of monocytic cells (U937 and THP-1 cell lines) with IL-1β also generates ROIs and potently activates NF-κB. As in lymphoid cells, preincubation of these cells with the antioxidants NAC and PDTC inhibits both the production of ROIs and NF-κB nuclear activity, thus indicating that the pathway leading to IκB-α degradation requires ROI production. However, the source of ROIs was different than in lymphoid cells. Indeed, the monocytic cell lines we analyzed do not express the 5-LOX enzyme and, consequently, the FLAP inhibitors MK886 and ETYA failed to inhibit ROI production and NF-κB activation. In these cells, the source for ROIs was likely to be the NADPH oxidase complex, as this enzyme is active in monocytic cells. Indeed, diphenyleneiodonium chloride and phenylarsine oxide, two inhibitors of the NADPH oxidase, could block, in a dose-dependent manner, the ROI production and NF-κB activation in IL-1β-stimulated

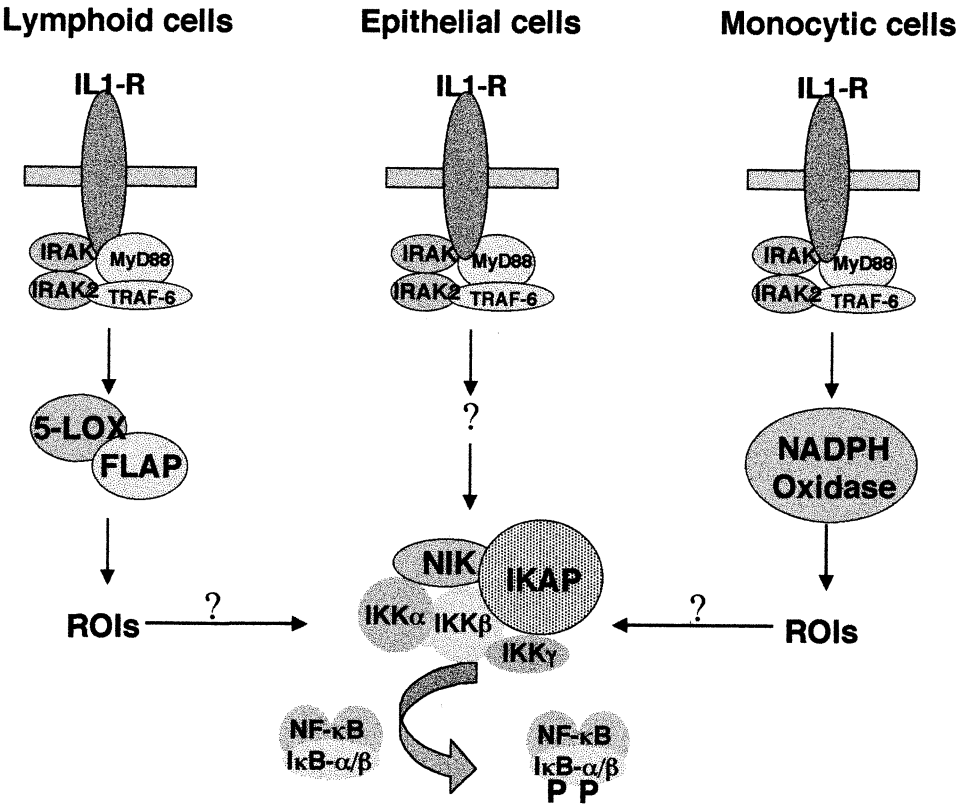

FIG. 2. Models for the role of cell type-specific ROIs in NF-κB activation by IL-1β.

U937 or THP-1 cells, while being inactive in lymphoid or epithelial cells. We also demonstrated that the small GTPases Cdc42 and Rac1 were involved in NF-κB activation by IL-1β in monocytic cells, possibly through their association with the NADPH oxidase complex [15].

EPITHELIAL CELLS

In the various epithelial cell lines we explored (MCF7 A/Z, OVCAR-3, HCT116, and SKOV-3), IL-1β induced a strong NF-κB nuclear activity, but did not activate ROI production. Therefore, NF-κB activation and IκB-α degradation were not affected by antioxidants in these cells. There are two possible explanations for the lack of ROI production in these cells. Epithelial cells, such as OVCAR-3 cells, express high levels of catalase activity. Moreover, none of the epithelial cell lines we analyzed express both the 5-LOX and FLAP proteins, indicating that an important enzymatic system for ROI generation is missing in these cells. Interestingly, reconstitution of the 5-LOX/FLAP system through transfection of expression vectors in these cells restored IL-1β-induced ROI production and ROI-dependent NF-κB-mediated transactivation of a reporter plasmid. These last experiments indicate that, once the 5-LOX/FLAP/ROI pathway is present, it predominates over alternative pathways for the degradation of IκB-α. We attempted to identify other intermediates for NF-κB activation by IL-1β in epithelial cells and obtained some indication that the production of ceramide by the acid sphingomyelinase might play an important it role in these cells. However, these experiments were based on the inhibition of NF-κB activation in the presence of nonspecific acid sphingomyelinase inhibitors (chloroquine and NH_4Cl) and need to be confirmed by genetic studies.

DISCUSSION

Although the role of ROIs in NF-κB activation by proinflammatory cytokines and other stimulating agents was reported several years ago, it has become very much a matter of controversy since the cloning of the IKKs. Several authors have recently described a direct pathway for NF-κB activation by IL-1β which links the receptor, receptor-associated adaptor proteins and kinases (MyD88, Il-1 receptor-associated kinase (IRAK), and TNF receptor-associated kinase factors 6 (TRAF6)), the NIK or MEKK-1 kinases, and the IKKs (Fig. 1). Is there a possible role for ROIs in such a scheme? We think that there is and that it is cell type specific. Three hypotheses could explain the importance of ROIs for NF-κB activation.

First, ROIs could influence the DNA-binding and transactivating activity by NF-κB family members without interfering with IκB-α phosphorylation and degradation. Indeed, most NF-κB-related proteins are modified post-translationally, and it has been shown that the redox status of p50 cysteine-62 residue is important for DNA binding [34, 35]. However, this hypothesis is not sufficient to explain our observations, as we demonstrated that IκB-α degradation in IL-1β-treated lymphoid cells is inhibited by antioxidants. We therefore believe that the cellular redox

status influences IκB-α phosphorylation and degradation. Secondly, ROIs could influence, directly or indirectly, the activity of one of the kinases of the NF-κB signaling pathway. The activity of these kinases is regulated by phosphorylation [36], and other modifications cannot be excluded. The generation of ROIs could be required for optimal and cell-specific kinase activity. The measure of kinase activity in the presence of antioxidants or in genetically modified cells will need to be performed to test this hypothesis. Finally, the redox status and the production of ROIs could influence the assembly of the signalsome. This large multiprotein complex is formed of the IKKs associated with the NIK kinase, and the whole system is held together by a scaffold protein (IKAP, IKK-complex-associated protein) (Fig. 1). The stability of such a large complex (700 to 900 kDa) is probably influenced by a number of cellular conditions, and one could easily imagine that the signal cannot be properly transduced if this stability is hampered. Whether or not the ROIs can influence, in a cell type-specific way, the assembly or stability of the signalsome is, however, still to be explored.

An intriguing aspect of our work is that, once we had restored the 5-LOX/FLAP/ROI pathway in epithelial cells, this pathway became dominant. If such a pathway is faster and more efficient than the others, it could turn off an intermediate which is also required for the other pathways (for instance, receptor-associated factors). The antioxidants or other inhibitors acting downstream of this intermediate would therefore completely block the signaling without authorizing the other pathway to transmit the signal. Alternatively, if ROIs favor the assembly of parts of the signalsome, their inhibition in a reconstituted system might not be sufficient to allow the assembling of the other complex.

These studies seem to indicate that the pathways leading to NF-κB activation in different cell types are at least partially divergent. Interestingly, IKKβ knockout animals show major defects in NF-κB activation in response to TNF-α or IL-1β, but the deficit is less striking with IL-1β at least in embryonic fibroblasts [37]. Moreover. IKKα −/− animals have a very different phenotype and are characterized by a thick skin [38–40]. This skin phenotype might be due to a defect in NF-κB activation in epidermal cells [40]. These knockout reports therefore suggest, as did our work, that the signaling pathways leading to IκB-α degradation might differ in fibroblastic or epithelial cells.

We therefore conclude that there exist distinct cell type-specific signaling pathways for NF-κB activation by IL-1β and probably by TNF-α. The role of ROIs in these pathways is specific, but remains to be precisely characterized. It will also have to be determined whether these distinct pathways result in the activation of different target genes and thus in specific biological activities. Of note, NF-κB and ROIs are implicated in important processes such as inflammatory reaction, defense against microorganism, apoptosis, cancer, and AIDS. The identification of the role of ROIs for NF-κB activation is thus of major biological importance.

M-P.M. and V.B. are research associates and J.P. is a research director at the National Fund for Scientific Research (FNRS, Belgium). G.B. is a fellow from the Biotechnology Programme, European Commission.

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