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Interleukin-1β Production in Human Monocytes/ Macrophages is Differentially Regulated by Mek1 upon Sterile and Infectious Inflammatory Conditions

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Abstract

Deregulation of the production of IL-1β and its natural inhibitor, the secreted form of IL-1 receptor antagonist (sIL-1Ra), plays an important role in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis. Relevant to the latter conditions direct cellular contact with stimulated T cells potently triggers cytokine production in human monocytes. Identification of signal transduction pathways specific to pathogenic induction of cytokines may lead to new therapeutic approaches. Two different stimuli were compared to investigate the implication of MEK1 and MEK2 in the control of IL-1β and sIL-1Ra production by human monocytes: (i) soluble extracts of plasma membranes from stimulated T cells (CE_{sHUT}), mimicking cellular contact with T cells, i.e., chronic/sterile inflammatory conditions; and (ii) LPS that is relevant to infectious inflammation. The ATP-noncompetitive MEK1/2 (U0126) and MEK1 (PD98059) specific inhibitors diminished the expression (protein and mRNA) of IL-1β in CE_{sHUT}-activated monocytes. In contrast, only the concomitant inhibition of MEK1 and MEK2 inhibited IL-1β production in LPS-activated monocytes, whereas the inhibition of MEK1 only did not affect IL-1β production. In CE_{sHUT}- and LPS-activated monocytes, MEK1 inhibition slightly affected sIL-1Ra production that was significantly inhibited by U0126. These results suggest that MEK1 and MEK2 are differentially involved in the regulation of the IL-1 system upon chronic/sterile and infectious inflammatory conditions. MEK1 which is dispensable to IL-1β production in LPS-activated monocytes represents a potential therapeutic target whose inhibition could participate in the restoration of IL-1β/sIL-1Ra balance in chronic/sterile inflammation without affecting regular responses to pathogens.

Keywords: Chronic/sterile inflammation; IL-1β; sIL-1Ra; Human monocytes; MAP kinases; Transduction signals; Cytokines; LPS

Abbreviations

IL-1β: Interleukin-1β; sIL-1Ra: Secreted form of IL-1 Receptor Antagonist; LPS: Lipopolysaccharides; CESHUT: CHAPS Extract of Membranes Isolated from Stimulated HUT-78 cells; ERK: Extra-Cellular-Signal-Regulated Kinase; MEK: Mitogen-Activated Protein Kinase; PI3K: Phosphoinositide 3-Kinases

Introduction

Interleukin-1β (IL-1β) is a major proinflammatory cytokine which signals through ligation of the IL-1 receptor I (IL-1RI) and IL-1 receptor accessory protein (IL-1RAcP) [1]. The production of IL-1β is tightly controlled at several levels and required two signals, one activating gene transcription and leading to the expression of cytoplasmic pro-IL-1β, the other activating an inflammasome that activates caspase-1 and in turn the cleavage of pro-IL-1β into its mature form IL-1β [2,3]. The mechanism by which IL-1β is secreted is different from the canonical secretory mechanisms and remains elusive. One of the main natural inhibitors controlling mature IL-1β activity is the secreted form of IL-1 receptor antagonist (sIL-1Ra), which binds IL-1RI without inducing signal transduction [3,4]. Deregulation of the production of IL-1β and its natural inhibitor, sIL-1Ra, plays an important role in various chronic inflammatory diseases [5] including multiple sclerosis [6] and rheumatoid arthritis [7]. Relevant to these conditions direct cellular contact with stimulated T cells potently triggers cytokine production in human monocytes [8,9]. Direct cellular contact with stimulated T cells is now recognized a major pathway for the production of cytokines (e.g. IL-1β and tumor necrosis factor - TNF -) in monocytes/macrophages under chronic/sterile conditions [10-20]. Indeed, contact-mediated activation of monocytes/macrophages by stimulated T lymphocytes is as potent as optimal doses of LPS to inducing IL-1β and TNF production in monocytes [8,21]. We therefore assume that this mechanism is highly relevant to the pathogenesis and persistence of chronic/sterile inflammation in diseases with autoimmune etiology such as multiple sclerosis and rheumatoid arthritis in which auto reactive T cells play a major part.

An important challenge in therapeutic approaches in inflammatory diseases is to dampen inflammation without affecting the regular inflammatory responses to pathogens. To identify therapeutic targets specific to chronic inflammation we undertook to characterize signaling pathways that trigger the production of IL-1β and sIL-1Ra in human monocytes activated upon either chronic/sterile or infectious conditions.

In vitro, chronic/sterile inflammation was mimicked by contact with stimulated T cells. To obviate the complications of having the simultaneous presence of at least two viable cell types in culture, we developed strategies allowing only interactions between stimulated T cell molecules and monocytes, consisting in to use soluble extracts of plasma membranes isolated from T cells [22]. In parallel, LPS was used as a prototypical infectious stimulus. Using these models, we previously demonstrated that phosphatidylinositide-3 kinase δ (PI3Kδ) regulate IL-1β and sIL-1Ra expression in opposite ways in human monocytes activated by LPS or contact with T cells, dampening the production of pro-inflammarory cytokines in LPS-activated monocytes, but inducing it in contact-activated monocytes [23,24]. Therefore, inhibition of PI3Ks could exacerbate inflammatory response to pathogens.

MEK1 and MEK2 are two Thr/Tyr dual mitogen-activated protein kinases (MAPK) whose activation is triggered by a wide variety of stimuli. Although their respective genes are located on different chromosomes, MEK1 and MEK2 display 80% sequence homology and are ubiquitously expressed in cells and tissues [25,26]. ERK1 and ERK2 are the only protein substrates of MEK1/2 that have been identified to date [25]. It is commonly assumed that MEK1 and MEK2 are functionally equivalent, whilst several lines of evidence indicate that they are regulated differentially and that each may fulfill non-redundant functions. Indeed, in contrast with $MEK2^{-/-}$ mice that show no phenotypic abnormalities [27], $MEK1^{-/-}$ mice display recessive lethality, their homozygous mutant embryos dying by day 10.5 of gestation [28]. More recently, it was shown that active MEK2 may function as a regulatory scaffold protein promoting a crosstalk between different transduction pathways [29], further demonstrating that MEK1 and MEK2 may display non-redundant functions in spite of identical downstream substrates. We also highlighted a non-redundant role of MEK1 and MEK2 in human monocytes activated by interferon-β. In the latter conditions, the expression of sIL-1Ra was controlled by a MEK2-PI3Kδ pathway, MEK1 being dispensable [30]. In contrast to other protein kinase inhibitors, inhibitors of MEK1 (PD98059) and MEK1/2 (U0126) do not target the ATP pocket (i.e. they are ATP-noncompetitive inhibitors), and thus are less prone to display off-target effects [31,32]. In the present study, by using U0126 and PD98059, we demonstrate that MEK1 is dispensable to IL-1β production and poorly involved in the induction of sIL-1Ra upon LPS-activation of monocytes whereas both MEK1 and MEK2 are required to IL-1β production under chronic/sterile inflammatory conditions. The present results identify MEK1 as a potential therapeutic target to dampen chronic/sterile inflammation without affecting inflammatory response to pathogens.

Materials and Methods

Materials

The following materials were purchased from the designated suppliers: FCS, streptomycin, penicillin, L-glutamine, RPMI-1640, and PBS free of Ca^{2+} and Mg^{2+} (Invitrogen); Lymphoprep (Axis-Shield); MEK1/2 inhibitor, U0126 and MEK1 specific inhibitor, PD98059 (LC Laboratories); lipopolysaccharides (LPS, Ultra-pure LPS-Ek, InvivoGen); Phaseolus vulgaris leucophytohemagglutinin (PHA, E-Y Laboratories Inc., San Mateo, CA); and phorbol myristate acetate (PMA, Sigma Chemicals Co., St. Louis, MO); Other reagents were of analytical grade or better.

Monocytes: Peripheral blood monocytes were isolated from buffy coats of blood of healthy volunteers provided by the Geneva Hospital Blood Transfusion Center (Switzerland) as previously described [33]. In accordance with the ethical committee of the Geneva Hospital, the blood bank obtained informed consent from the blood donors.

T Cells and Preparation of T cell Plasma Membranes: HUT-78, a human T cell line, was purchased from the American Type Culture Collection. HUT-78 cells were cultured and activated by PHA (1 μg/ml) and PMA (5 ng/ml) in RPMI 1640 medium supplemented with 10% heat activated FCS, 50 μg/ml streptomycin, 50 U/ml penicillin, 2 mM glutamine (complete RPMI medium) in a 5% CO_2-air humidified atmosphere at 37°C as described elsewhere [34]. Plasma membranes of stimulated HUT-78 cells were isolated and solubilized with CHAPS to obtain membrane CHAPS extract (CE_{sHUT}) as previously described [35]. The capacity of CE_{sHUT} to activate human monocytes was equivalent to living HUT-78 cells or primary human T lymphocytes (i.e., in cocultures), fixed T cells, or isolated membranes as previously determined [34-36]. Protein concentration was determined by the method of Bradford [37]. CE_{sHUT} was endotoxin-free as determined by the Endochrome-K LAL kit (Charles River Laboratories Inc.).

Western blot analysis

Human monocytes were resuspended at 6 x 10^6 cells/ml in complete RPMI medium and 500 μl was placed in 2-ml polypropylene tubes (Eppendorf) at 37°C for 1 h. Cells were preincubated for 45 min in the presence or absence of 5 μM of U0126 or PD98059 and then activated with 100 ng/ml LPS or 6 μg/ml CE_{sHUT}. At the indicated time, the activation was stopped by the addition of 800 μL of ice-cold PBS before centrifugation and cell lysis. Total cell lysates were prepared and subjected to Western blot analysis as described previously [38]. Nitrocellulose membranes were probed with rabbit anti-phospho-ERK1/2-p44/42 MAPK (Thr202/Tyr204), mouse anti-ERK1/2-p44/42 MAPK (Cell Signaling Technology), and mouse anti-β-tubulin (Sigma). Secondary IR700/800 conjugated goat anti-rabbit or goat anti-mouse antibodies (Rockland) were used, and antibody bound proteins were detected and quantified with an Odyssey system (Li-Cor).

Cytokine production

Isolated monocytes (5 x 10^4 cells/200μl/well) were preincubated for 45 min in the presence or absence of the indicated concentration of kinase inhibitor in complete RPMI medium and then activated for 24 h with 100 ng/ml of LPS or 6 μg/ml of CE_{sHUT}. All conditions were conducted in triplicate. After supernatant harvesting, cells were lysed in 200 μl/well PBS containing 1% Nonidet P40. Culture supernatants and/or cell lysates were tested for the production of IL-1β and sIL-1Ra by commercially available enzyme immunoassay kits (eBioscience).

mRNA quantification

Monocytes (2 x 10^6 cells/2ml/well) were cultured in 6-well plates for 45 min in the presence or absence of 5μM of U0126 or 5 μM of PD98059 and then activated by LPS (100 ng/ml) or CE_{sHUT} (6 μg/ml proteins) for 3 h. Preparation of total RNA was carried out with Nucleo Spin RNA II kit (Macherey-Nagel) and quantitative real-time duplex PCR analysis was conducted as described previously [38]. The levels of mRNA expression were normalized with the expression of a housekeeping gene (18S). Cytokines and 18S probes were purchase from Applied Biosystems. All measurements were carried out in triplicates.

Statistical analysis

When required, significance of differences between groups was evaluated using Student's *t*-test.

Results

Production of IL-1β and sIL-1Ra in human monocytes

Previous works demonstrated that CE_{sHUT} and LPS induce IL-1β and sIL-1Ra production in human monocytes [23,24]. Since the levels of cytokine production varied between different monocyte preparations (i.e., blood donors), the results below are presented as percentages of the cytokine production measured in the absence of inhibitor. Table 1 shows the mean production of IL-1β, pro-IL-1β and sIL-1Ra observed in the presented experiments. CE_{sHUT} induced lower IL-1β production but higher production of sIL-1Ra as compared with LPS. However, the total production of IL-1β and pro-IL-1β (i.e., extra- and intracellular IL-1β) triggered by CE_{sHUT} or LPS was similar reaching 3102 ± 1038 pg/ml and 3217 ± 1068 pg/ml, respectively, suggesting that LPS was more efficient in inducing IL-1β secretion. CE_{sHUT} was more potent than LPS to trigger sIL-1Ra production, confirming previous data [24].

MEK/ERK pathway activation by CE_{sHUT} and LPS

To assess MEK1/2 activation in monocytes upon different inflammatory conditions, monocytes were activated by CE_{sHUT} or LPS and phosphorylation of ERK1/2 (i.e., the canonical substrates of MEK1/2) was measured by Western blot. The maximum ERK1/2 phosphorylation in monocyte was observed after 15-20 min activation independently of the stimulus (Figure 1A). The phosphorylation of ERK1/2 was inhibited to a higher extent in the presence of U0126 (MEK1/2 inhibitor) than in the presence of PD98059 (MEK1 inhibitor) independently of the stimulus (Figure 1B). These results demonstrate that MEK inhibitors decreased ERK1/2 phosphorylation and that their efficiency was independent of the stimulus.

IL-1β and sIL-1Ra production is differentially regulated by MEK1 and/or MEK2 upon chronic/sterile and acute/infectious inflammatory conditions

To elucidate the implication of MEK1 and/or MEK2 in chronic/sterile and infectious inflammation, the effects of the MEK1 inhibitor, PD98059, and the MEK1/2 dual inhibitor, U0126, were tested in monocytes activated by CE_{sHUT} or LPS. In monocytes activated by CE_{sHUT}, the production of IL-1β and sIL-1Ra was inhibited by U0126 and PD98059 in a dose dependent manner (Figures 2A and 2B). As expected, the inhibition of both MEK1 and MEK2 (U0126) was more efficient to decrease IL-1β and sIL-1Ra production than the inhibition of MEK1 only (PD98059) suggesting that the activation of MEK1 and MEK2 was required for optimal induction of cytokine production. In contrast with the production of sIL-1Ra (Figure 2B), CE_{sHUT}-induced IL-1β production was exquisitely sensitive to U0126 which diminished the production by more than 60% at a concentration as low as 1 μM. Although inhibited, the secretion of sIL-1Ra was decreased to a lower extent than that of IL-1β in the presence of MEK inhibitors and reached only 44 ± 5% and 19 ± 13% at the highest concentration of kinase inhibitors used in this study (Figures 2A and 2B). These results indicate that MEK1 and MEK2 do not represent a major regulator of sIL-1Ra production in chronic/sterile inflammatory conditions. In LPS-activated human monocytes, the production of cytokines was differentially regulated by MEK1 and MEK2 (Figures 2C and 2D). IL-1β production was strongly inhibited in the presence of U0126 reaching a plateau at 62 ± 14% inhibition at 2.5 μM U0126. In contrast, IL-1β levels remained unaffected by PD98059 even at high concentrations (Figure 2C), suggesting that MEK1 was dispensable to IL-1β production upon LPS-activation of human monocytes. sIL-1Ra production was regulated by both MEK1 and MEK2, even thought it was less sensitive to PD98059 than to

Table 1: Production of IL-1β, pro-IL-1β and sIL-1Ra by human monocytes.

Stimulus	IL-1β (pg/ml)	Pro-IL-1β (pg/ml)	sIL-1Ra (pg/ml)
CE_{sHUT} (6 μg/ml)	654 ± 245	2,528 ± 1,469	18,376 ± 2,287
LPS (100 ng/ml)	1,367 ± 737	1,851 ± 534	6,058 ± 602

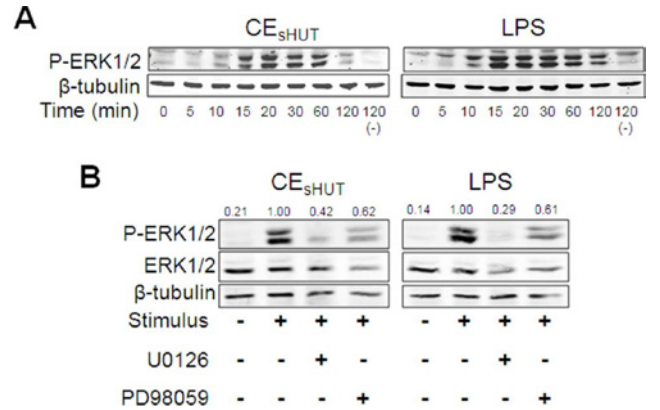

Figure 1: Induction of ERK1/2 phosphorylation by CE_{sHUT} and LPS and inhibition by U0126 and PD98059.

A) Isolated human monocytes (3 x 10^6 cells) were activated for the indicated time with either CE_{sHUT} or LPS. Cells were lysed and subjected to Western blot analysis as described in Materials and Methods. (-) monocytes incubated in the absence of stimulus for 120 min.

B) Monocytes were activated for 20 min in the presence or absence of 5 μM of the indicated inhibitor. Western blots were immunostained for phosphorylated ERK1/2 (P-ERK1/2), total ERK1/2 and β-tubulin as indicated. Immunoblotting data are representative of 3 separate experiments. ERK1/2 phosphorylation quantified by the Odyssey system (Li-Cor) is mentioned above the blot, the ratio of phosphorylated ERK1/2 to total ERK1/2 being considered as 1.0 in monocytes activated in the absence of inhibitor.

U0126 inhibition (Figure 2D). In the presence of PD98059, sIL-1Ra production was only slightly inhibited, reaching a maximum inhibition of 31 ± 6% at 10 μM PD98059. Together these results suggest that both MEK1 and MEK2 activation was required to IL-1β and sIL-1Ra optimal production in monocytes activated by CE_{sHUT}, i.e., upon sterile inflammatory conditions, but only MEK2 was required to IL-1β production in LPS-activated monocytes. Since only mature IL-1β is released in the extracellular space, we assessed whether MEK1 and/or MEK2 controlled IL-1β production at the transcriptional or the maturation/secretion level, and measured the effects of inhibitors on the production of pro-IL-1β, i.e., the production of intracellular IL-1β. As shown in Figures 2E and 2F, the inhibition of pro-IL-1β expression by U0126 in CE_{sHUT}- and LPS-activated monocytes was comparable to that observed for the production of IL-1β. These data suggest that MEK1/2 pathway control the expression of both IL-1β and, to a lower extent, sIL-1Ra upstream protein translation independently of the stimulus and that MEK1 is dispensable to IL-1β production in LPS-activated monocytes.

Cytokine transcript expression is differentially regulated by MEK1 and/or MEK2 upon chronic/sterile and acute/infectious inflammatory conditions

To determine whether MEK1 and/or MEK2 modulated the expression of IL-1β and sIL-1Ra mRNA as suggested by results of Figure 2, we determined the expression of IL-1β and sIL-1Ra transcripts in monocytes in the absence or presence of MEK inhibitors. As shown in Figure 3, the expression of IL-1β and sIL-1Ra mRNA reflected the results obtained at the protein level independently of the stimulus. Indeed, IL-1β mRNA and sIL-1Ra mRNA expression was inhibited by MEK1 and MEK1/2 inhibitors in CE_{sHUT}-activated monocytes, with higher inhibition observed with U0126. In LPS-activated monocytes sIL-1Ra mRNA was inhibited by U0126 and PD98059, whereas only U0126 inhibited IL-1β transcript expression (Figure 3B) further suggesting that MEK1 was dispensable to the induction of IL-1β expression upon infectious inflammation. Together these results demonstrate that the MEK-ERK1/2 pathway regulates the IL-1β system in both sterile and infectious conditions, but only MEK2 is required to optimally triggering IL-1β production in LPS-activated monocytes.

Discussion

The present results demonstrate that MEK1 activation is dispensable to signal transduction triggered by LPS that leads to IL-1β production. This contrasts with signal transduction triggered by CE_{sHUT} (i.e., direct cellular contact) which requires the activation of both MEK1 and MEK2 to optimally inducing IL-1β expression. The expression of sIL-1Ra is only slightly regulated by MEK1 and MEK2, independently of the stimulus. The low extent of sIL-1Ra inhibition observed with U0126 or PD98059 suggests that the MEK-ERK pathway does not play a major role in the regulation of sIL-1Ra production.

LPS signaling through ligation of the toll-like receptor 4 (TLR4) leads to NFκB activation and transcription of numerous genes including *IL1B* and *IL1RA*. It is well known that in addition

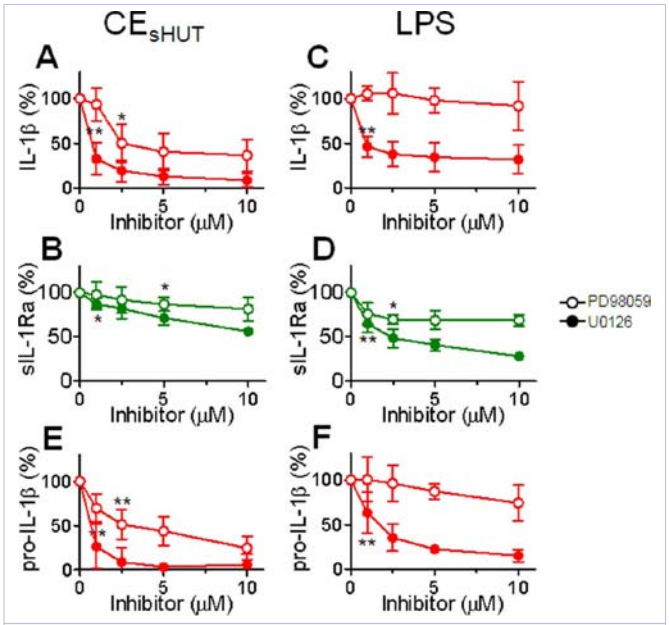

Figure 2: MEK1 and MEK2 differentially regulate IL-1β downstream CE_{sHUT}- and LPS-activation of monocytes. Isolated human monocytes were treated with the indicated concentration of U0126 (closed circles) or PD98059 (open circles) for 45 min before activation by CE_{sHUT} (A, B, and E) or LPS (C, D, and F). The production of IL-1β (A and C, red symbols), sIL-1Ra (B and D, green symbols) and pro-IL-1β (E and F, red symbols) was measured in cell lysates and supernatants harvested after 24h. Results obtained from at least 3 different donors are presented as mean ±SD of percentage of cytokine production observed in the absence of inhibitor. The lowest inhibitor concentration which induced a significant inhibition of cytokine production is indicated: (*) $p \leq 0.05$ and (**) $p \leq 0.01$ as determined by Student's *t*-test.

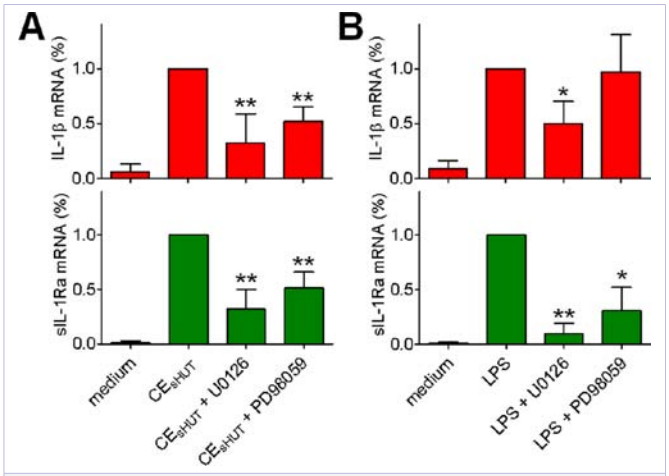

Figure 3: MEK1 is dispensable to IL-1β induction by LPS. Isolated human monocytes (2 x 10^6 cells) were activated for 3 h with CE_{sHUT} (A) or LPS (B) as described in Materials and Methods in the presence or absence of 5 μM of the indicated inhibitor. IL-1β (red) and IL-1Ra (green) transcripts were analyzed by quantitative real-time PCR. Results obtained from at least 3 different donors are presented as mean ± SD of percentage of cytokine production observed in the absence of inhibitor. Significance was assessed by Student's *t*-test (*) $p \leq 0.05$ and (**) $p \leq 0.01$.

to the LPS-TLR4-NFκB pathways other signal transduction pathways are activated by LPS such as PI3K-AKT and MAPK pathways. Cross-talk between the canonical LPS-TLR4-NFκB pathways and MAP kinase pathways was described, although mechanisms were not fully elucidated [39]. However, it is likely that MEK1/2-ERK1/2 pathway is activated downstream IL-1 receptor associated kinase (IRAK) 1/2 and IκBα kinase (IKK) β which activate tumor progression locus (Tpl) 2 and in turn MEK1/2 [40]. The differential activation of MEK1 and MEK2 downstream LPS-TLR4 ligation may represent a fine-tuning process controlling infectious inflammation with induction of dampening signals such as the PI3Kδ-AKT-GSK3 pathway [24]. This type of control is not set-up by CE_{sHUT} that activates both MEK1/2 leading to deregulated IL-1β production.

The present study establishes that MEK1 activity is dispensable to the optimal induction of IL-1β in LPS-activated monocytes. This is reminiscent of results in the murine monocyte/macrophage cell line J774A.1 which demonstrated that the induction of IL-1β expression by LPS depended on the activation of MAPKs including ERK1/2 [41]. In agreement with the present results, the latter study showed that IL-1β production was not inhibited in the presence of 10 μM PD98059 although ERK1/2 phosphorylation was inhibited by very high concentration of PD98059 (50 μM). Together with our results, this suggests that MEK1 was hardly involved in IL-1β induction by LPS in murine and human monocytes/macrophages.

The mechanism by which MEK2 controls the production of IL-1β downstream LPS stimulation remains to be determined. Although MEK1 and MEK2 activities are rarely distinguished in the literature, growing evidence indicates that MEK1 and MEK2 may be differentially regulated and exert non-redundant functions [42-51]. That MEK1 and MEK2 play different roles in the regulation of several cellular processes implies that either substrate other than ERK1/2 might be differentially phosphorylated by MEK1 and MEK2, or MEK1/2 functions that do not directly require kinase activity, or both [29,52]. However, ERK1/2 is still the only substrates of MEK1/2 identified to date. Noticeably, in studies revealing different functions of MEK1 and MEK2, MEK2 is often characterized as an important element in cross-talk between two pathways involving direct interaction between active MEK2 and other signaling molecules [29,30,53]. It has also been described that MEK2 may exert activity independently of ERK1/2 phosphorylation [29,30,53]. In the latter studies, MEK2 is likely to display protein scaffold activity promoting Pin1 binding to BPGAP1 and membrane localization of PI3Kδ, respectively. In the study by Pan et al. [29] the scaffold activity of MEK2 aimed at diminishing the activation of ERK1/2. The present study does not prove a role of regulatory scaffold for MEK2, it only establishes that there is a pathway initiated by MEK2 that leads to IL-1β production in LPS-activated human monocytes.

As shown in scheme of Figure 4, the triggering of IL-1β system in human monocytes is tightly controlled downstream LPS with induction of dampening signals such as the PI3Kδ-AKT-GSK3 pathway. This is not observed with the pathologic induction of IL-1β by CE_{sHUT} which triggers pathways that all converge to activate IL-1β production. In contrast with the inhibition of PI3K pathway that might have pernicious effects potentially leading to exacerbated inflammatory response to pathogens, the inhibition of MEK1 may restore a balanced production of IL-1β and sIL-1Ra in chronic/sterile inflammatory conditions without affecting responses to infectious pathogens.

Conclusion

Our results and previous studies [24,40] demonstrate that the induction of IL-1β expression upon chronic/sterile inflammatory conditions escape regulatory mechanisms that usually control it upon inflammatory response to infectious agents represented here by LPS (Figure 4). This further identifies contact with stimulated T cells as an uncontrolled, deregulated mechanism triggering pathogenic inflammation. That MEK1 is dispensable to induction of IL-1β by LPS designates this kinase as a potential therapeutic target to dampen detrimental inflammation without affecting protective inflammatory response to pathogens. There is currently no cure for autoimmune diseases with chronic/sterile inflammation. Patients are usually treated with immunosuppressive drugs aiming at diminishing overall inflammatory responses. Usage of protein kinase inhibitors has become an attractive class of drugs among which MEK1 and

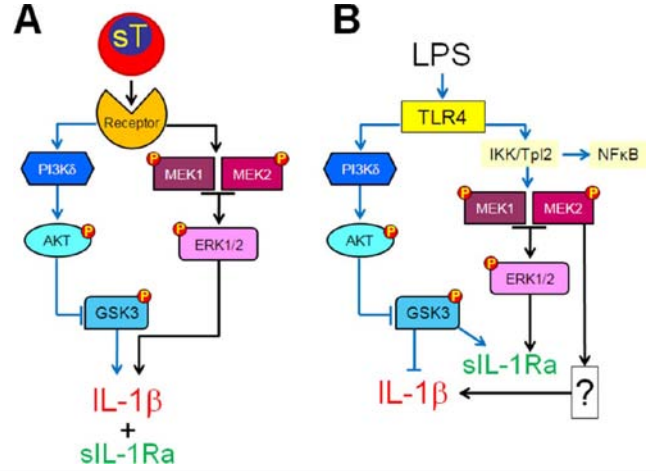

Figure 4: Control of IL-1β and sIL-1Ra production upon chronic/sterile and infectious conditions.

A) Upon chronic/sterile condition (contact with stimulated T cells), MEK1 and MEK2 are activated and contribute to the induction of IL-1β and sIL-1Ra production in human monocytes, and both MEK-ERK and PI3Kδ-AKT-GSK3 pathways trigger cytokine expression.

B) Upon infectious conditions (LPS), MEK1 and MEK2 are activated. Both MEK1 and MEK2 contribute to the induction of sIL-1Ra expression whereas only MEK2 is needed to the induction of IL-1β production. The PI3Kδ-AKT-GSK3 pathway dampens IL-1β production but triggers sIL-1Ra expression. Black lines and arrows represent results described in the present study, blue lines and arrows were previously established by us and others elsewhere (24,40). IKK, IκBα kinase; Tpl2, tumor progression locus 2; PI3Kδ, phosphatidylinositide-3 kinase δ; AKT, protein kinase B; GSK3, glycogen synthase kinase 3; TLR4, toll-like receptor 4.

MEK1/2 inhibitors are currently assessed in clinical trials mainly in cancer patients [54]. However, as recently stated by P. Cohen [40], whether safe drugs that modulate protein kinase activities can also be developed for the treatment of chronic diseases, where they may need to be taken for decades, is an issue that is still unresolved.

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