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Heterotrimeric complex of p38 MAPK, PKCδ, and TIRAP is required for AP1 mediated inflammatory response

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ABSTRACT

Inflammation could be described as a physiological response of the body to tissue injury, pathogen invasion, and irritants. During the inflammatory phase, cells of both the innate as well as adaptive immune system are activated and recruited to the site of inflammation. These mediators are downstream targets for the transcription factors; activator protein-1 (AP1), nuclear factor kappa-light-chain-enhancer (NF-κB), signal transducers and activators of transcription factors (STAT1), as well as interferon regulatory factors (IRFs), which control the expression of most immunomodulatory genes.

There is a significant increase in active p38 mitogen-activated protein kinase (p38MAK) immediately after lipopolysaccharide (LPS) stimulation, which results in the activation of AP-1 transcription factor and expression of proinflammatory cytokines, IL-12 and IL-23. We studied the novel mechanism of p38 MAPK activation through the formation of a heterotrimeric complex of Protein kinase C delta type (PKCδ), Toll-Interleukin 1 Receptor (TIR) Domain Containing Adaptor Protein (TIRAP), and p38 proteins. TIRAP serves as an adaptor molecule which brings PKCδ and p38 in close proximity. The complex facilitates the activation of p38MAPK by PKCδ.

Therefore, we propose that disruption of the heterotrimeric complex may be a good strategy to dampen the inflammatory response. Structure-based design of small molecules or peptides targetting PKCδ-TIRAP or TIRAP-p38 interfaces would be beneficial for therapy in AP1 mediated inflammatory diseases.

1. Introduction

The major role of the inflammatory response is to destroy the infection and tissue injury [1]. Innate immune cells occupying the tissues, such as macrophages, fibroblasts, mast cells, dendritic cells, as well as the circulating leukocytes, including monocytes as well as neutrophils, identify pathogen invasion or cell damage with intracellular or surface-expressed pattern recognition receptors (PRRs). These PRRs are responsible to identify the pathogen-associated molecular patterns (PAMPs) [2–4], which could be microbial nucleic acids, lipoproteins, and carbohydrates, or damage-associated molecular patterns (DAMPs). The DAMPs in turn are the endogenous molecules produced from internal injuries [5,6]. Activated PRRs then oligomerize and assemble large multi-subunit complexes that initiate signaling cascades which further trigger the activation of activator protein-1 (AP1), members of the interferon regulatory transcription factor (IRF) family like IRF3, signal transducer and activator of transcription (STAT) protein and nuclear factor-kappaB (NF-kappaB) by a complex molecular signaling [7,3,8].

Various intracellular proteins could initiate the inflammatory response. P38 proteins belong to a class of mitogen-activated protein kinases (MAPKs), crucial during inflammation, especially in macrophages [9–11]. P38 gets activated immediately in response to inflammatory and stress stimuli, including any biological, chemical, or physical agents and is involved in autophagy, apoptosis, and cell differentiation [12,13]. Accumulating evidence suggests that p38 plays an important role in ailments related to the inflammation of liver, kidney, brain, and lung and, hence is a critical player in inflammatory

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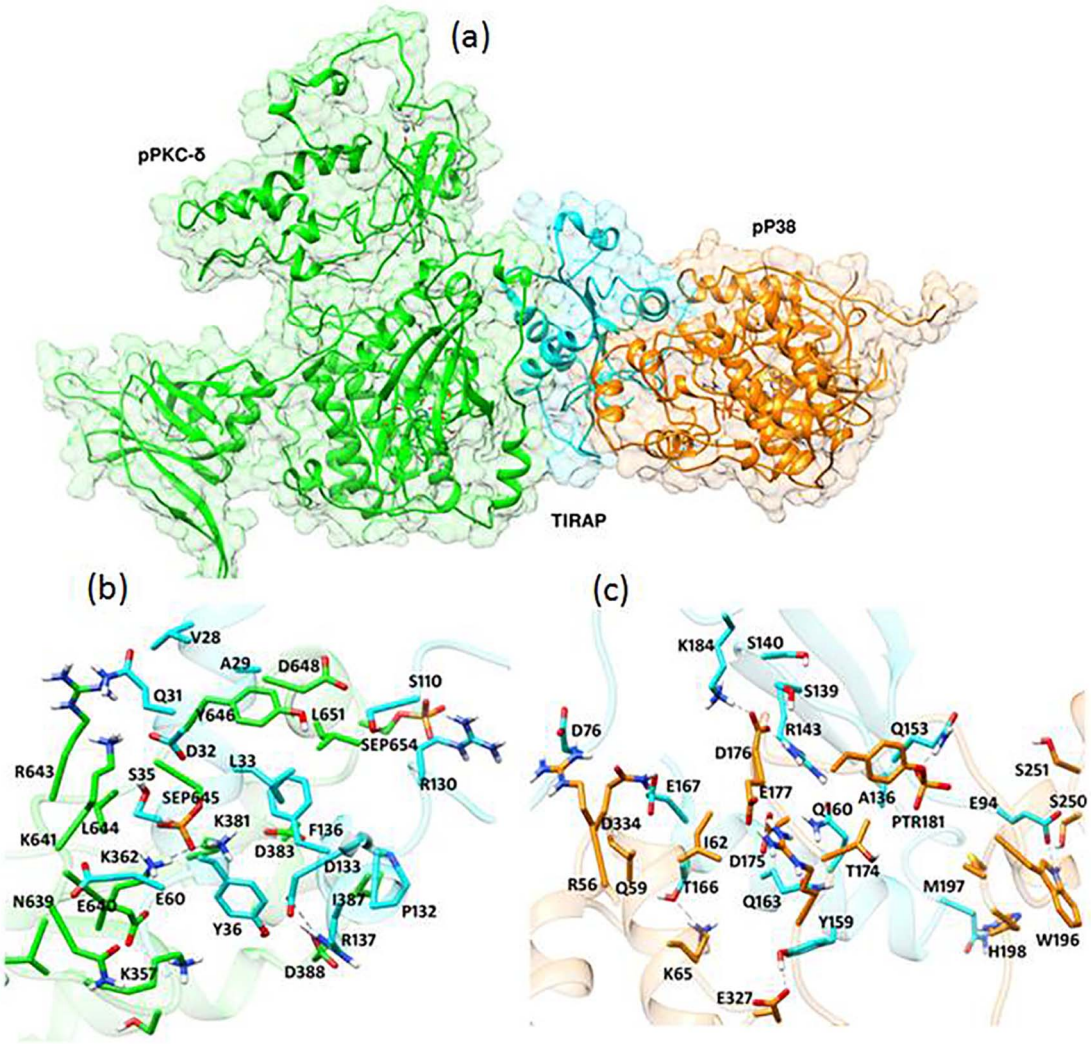

Fig. 1. Heterotrimeric complex of PKCδ-TIRAP-P38. a) The trimeric complex structure of PKCδ-TIRAP-pP38 is displayed as a ribbon representation along with 90% transparency of surface, where PKCδ is shown in green, TIRAP in cyan and P38 in orange colored ribbons. The molecular interactions at each of the interface are shown for b) PKCδ-TIRAP and c) TIRAP-P38 complexes. The interacting residues are shown in stick hetero atom type with color codes same as in the respective structure shown in (a). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Interface analysis of PKCδ-TIRAP-P38 complex. The information for total number of residues, interface area, solvation free energy gain, and total number of hydrogen bonds and salt bridges contributing to the formation of the stable trimeric complex is given.

ID	Structure 1			Structure 2			Interface area, Å^2	Δ^iG kcal/mol	N_{HB}	N_{SB}
	Mol	$^iN_{at}$	$^iN_{res}$	Mol	$^iN_{at}$	$^iN_{res}$				
Interfaces between pPKCδ and TIRAP										
1	pPKCδ	101	23	TIRAP	121	34	1066.9	− 11.3	18	13
Interfaces between TIRAP and pP38										
2	TIRAP	155	40	pP38	160	43	1558.6	− 14.6	21	8

$^iN_{at}$ number of atoms in interface area, $^iN_{res}$ number residues in interface area, Δ^iG—solvation free energy gain upon interface formation, N_{HB}—number of hydrogen bond and N_{SB}—number of salt bridges.

diseases mediated by macrophages [14–18]. P38 MAPK has been shown to involve in the AP1 and NF-kappaB transcriptional activity [19–21] which has an impact on a diverse range of events that are important in inflammation. Previous attempts to develop potent p38 MAPK inhibitors have not been successful as a result of unacceptable safety profiles due to off-target effects [22,23].

We have shown a novel mechanism of p38 MAPK activation and proinflammatory cytokine expression. This study would prompt the identification of novel therapeutic targets for the inhibition of p38 MAPK and hence suppression of inflammation. We have investigated the presence of heterotrimeric complex consisting of Protein kinase C delta type (PKCδ), Toll-Interleukin 1 Receptor (TIR) Domain Containing Adaptor Protein (TIRAP), and p38 MAPK. TIRAP serves as an adaptor molecule for bringing p38 MAPK and PKCδ in close proximity during the inflammatory response. Activation of protein kinase C (PKCδ) is one of the essential mechanisms of the inflammatory response [24], which might the results of activation of p38MAPK due to close proximity with PKCδ in the heterotrimeric complex and thus the activation of AP-1 and

Table 2
Interacting residue analysis of PKCδ-TIRAP-P38 complex. The residues contributing hydrogen bonds, salt bridges and hydrophobic interactions formation are listed.

Complex	PKCδ-TIRAP		TIRAP-P38	
	PKCδ	TIRAP	TIRAP	P38
Interacting residues	K357, K362, D383, K381, V384, I387, D388, D634, N639, E640, K641, R643, L644, Sep645, Y646, D648, L651	V98, A99, Q101, D102, L103, S105, Y106, E108,G109, S180, G181, R184, G199, R200,G201, P202, D203, G204, F206, R207	P71, T72, D76, K84, E94, Q135, A136, S139, S140, R143, Q153, D154, Y159, Q160, Q163, T166, E167	R56, Q59, I61, I62, K65, T174, D175, D176, E177, PTR181, W196, M197, H198, S250, S251, E327, D334

NF-kappaB transcription factor. We have determined the protein-protein interaction by Co-immunoprecipitation (Co-IP) as well as computational structural studies.

2. Methods

2.1. Tertiary structure prediction of PKCδ

To understand the structural interaction of PKCδ with TIRAP and p38, the full-length structure of PKCδ was modeled by using the homology modeling, threading and ab initio protocol based on the closest homologue in the sequence identity. The full-length PKCδ (UniProt ID: Q05655, 676aa) sequence was retrieved from the UniProt database (http://www.uniprot.org) and subsequently allowed for protein BLAST search against Protein Data Bank (PDB, http://www.rscb.org/pdb/) to find homologue templates. In the Blast search results, it was observed that the crystal structure of C2 domain of PKCδ was already available as PDB entry 1YRK [25]. Also the crystallographic structure of PKCβ-II (3PFQ) [26] and PKC-θ (1XJD) [27] shown homology with PKCδ was utilized for the modeling of catalytic subunit of the protein. Further, the region containing the zinc finger lobe was submitted to I-TASSER [28,29] and ROBETTA structure prediction server as a full-length sequence. The combination of structural information generated from the ROBETTA and I-TASSER threading methodology was utilized to construct the full-length model of PKCδ by using the multiple template script of the modeler program. Finally, the best model was selected based on their least discrete optimized protein energy (DOPE) and modeler objective function (MOF) score. The structural preparation for the docking studies was given in supplementary section.

2.2. Protein-protein interaction studies

Trimer complex formation was created based on the protein-protein interaction studies of TIRAP with PKCδ [30] and p38 by using the high Ambiguity Driven Bio-molecular Docking (HADDOCK), a flexible docking program [31,32] in combination with Crystallographic and NMR System (CNS v1.2) which serves as a structure calculating device [33]. As an initial step, the pPKCδ, TIRAP and pP38 protein structure were prepared by assigning the hydrogen atoms and followed by short minimization using Maestro (Schrödinger 2014-2). The TIRAP (V98, D012, Y106, S180, G181, R200, D203 and R207) interacting region of pPKCδ (K357, K362, D388, D634, D648 and L651) was used as an active site region for our docking purpose to form a hetero dimer complex of PKCδ-TIRAP. In case of TIRAP and P38 interacting residues were identified based on the blind pre-docking results of Cluspro server. The docking protocol of HADDOCK consists of three stages: complex generation based on rigid body energy minimization of the structure for 1000 steps followed by a structural refinement in torsional space, from which the best 200 structures were selected based on their intermolecular energy. Finally, the best structures were refined using the 8 Å explicit solvent layer (TIP3) [34]. The best cluster was selected based on the HADDOCK score and RMSD cut-off of 7.5 Å, and used for interface analysis using the PISA (Protein Interfaces, Surfaces and Assemblies) web server. Finally, the timer complex was made by superimposing both the complexes by keeping the TIRAP as the scaffolding molecule. The PDBePISA interface [35,36] analysis was carried out to investigate the binding free energy of the docked complexes.

2.3. Macrophage culture

Bone Marrow-Derived Macrophages (BMDMs) were extracted from C57/BL6 mice, as described previously [37]. BMDMs were prepared by culturing single-cell suspensions of bone marrow (BM) for 5–7 d in Dulbecco's minimal essential medium (DMEM, Invitrogen) by addition of 10% heat-inactivated FBS (Atlanta Biologicals or Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin (CellGro, Mediatech) or 1% (vol/vol) Antibiotic-Antimycotic mix (Life Technologies), with 10% L929 cell-conditioned (ATCC) complete DMEM medium. In order to get TIRAP/Mal-silenced BMDMs, TIRAP shRNA Plasmid (m) was transfected using Lipofectamine™ LTX (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Silenced Mal expression was confirmed by immunoblotting. Macrophages were washed and detached by incubating with 1 mM EDTA in 1 × PBS from an 18-gauge needle (BD), and further plated for experiments 1–3 d later. The p38 MAPK inhibitor SB203580 was obtained from Sigma (St. Louis, MO, U.S.A.).

2.4. Immunoblotting

For immunoblot analysis, BMDMs were treated with LPS (100 ng/ml: *Escherichia coli*, 0111:B4; Sigma-Aldrich) for indicated time points. Cells were washed in phosphate-buffered saline (PBS) and lysed in RIPA buffer (Life technologies), containing protease and phosphatase inhibitor tablet (Invitrogen). Total protein was isolated from cell lysates by centrifugation at 4 °C, 13,000 rpm for 15 min. Protein was resolved on 10% SDS-PAGE, blotted onto nitrocellulose membrane (Life technologies) and probed with antibodies for SOCS1, TIRAP, and β-actin from Cell Signaling Technology. P38, pP38, PKCδ and secondary antibodies were obtained from Santa Cruz.

2.5. Immunoprecipitation

For immunoprecipitation experiment, cells were lysed in NP40 lysis buffer (20 mM Tris HCL, pH 8.0, 137 mM NaCl, 10% glycerol, 1% NP40, 2 mM EDTA) with protease inhibitors. Protein concentrations was calculated using the BCA assay (Bio-Rad Laboratories). 100 μg of protein lysate was incubated with the PKCδ antibody (Santa Cruz Biotechnology Inc.) along with plus protein A/G agarose beads (Santa Cruz Biotechnology Inc.) overnight on a rotary shaker. Beads were then washed with 4 × lysis buffer and protein expression was analyzed by immunoblotting with target protein antibody p38 (Santa Cruz Biotechnology Inc.) and TIRAP (Cell Signaling Technology).

2.6. RNA isolation, c-DNA synthesis, and quantitative real-time RT-PCR

Total cellular RNA was extracted from BMDMs using TRIzol reagent (Sigma) following the manufacturer's instructions. The mRNA based cDNA was synthesized using cDNA synthesis kit (Biorad) followed by q-PCR using

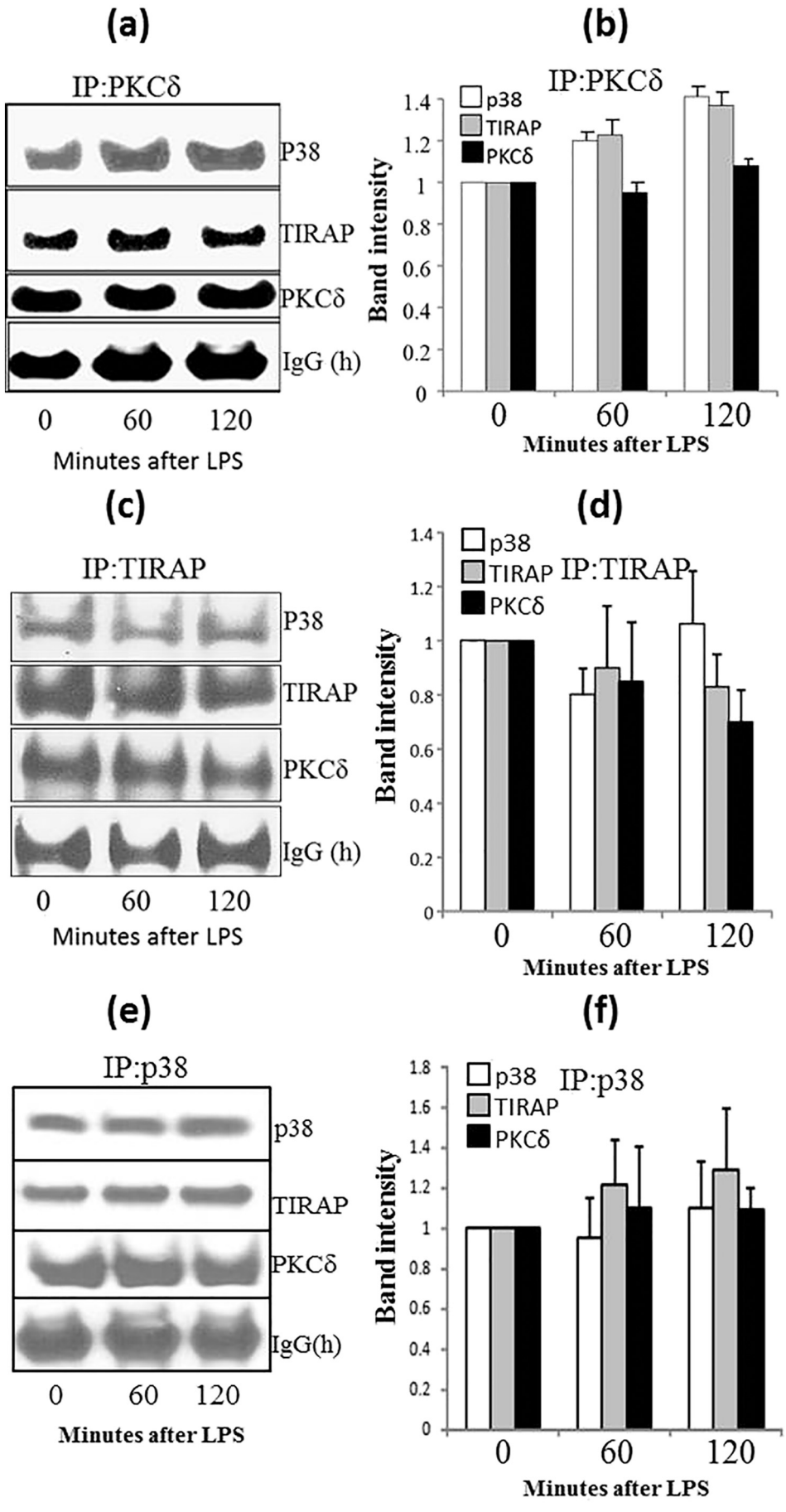

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Fig. 2. Interactions among PKCδ, TIRAP, and p38 in BMDMs. Reciprocal IPs were performed from LPS (100 ng/ml) stimulated BMDMs cell lysates using PKCδ, TIRAP, and p38-specific antibodies and IgG as a control. Immunoprecipitation of PKCδ, TIRAP, and p38 was confirmed by Anti- PKCδ, -TIRAP and -p38 antibodies for immunoblot (IB) detection. The average band intensity of the respective bands from three independent experiments was measured. This was done using the imaging software ImageJ (https://imagej.nih.gov/ij/). All data are representative of three independent experiments; all are presented as mean ± SD. P values were determined by Student's *t*-test; *P < 0.05.

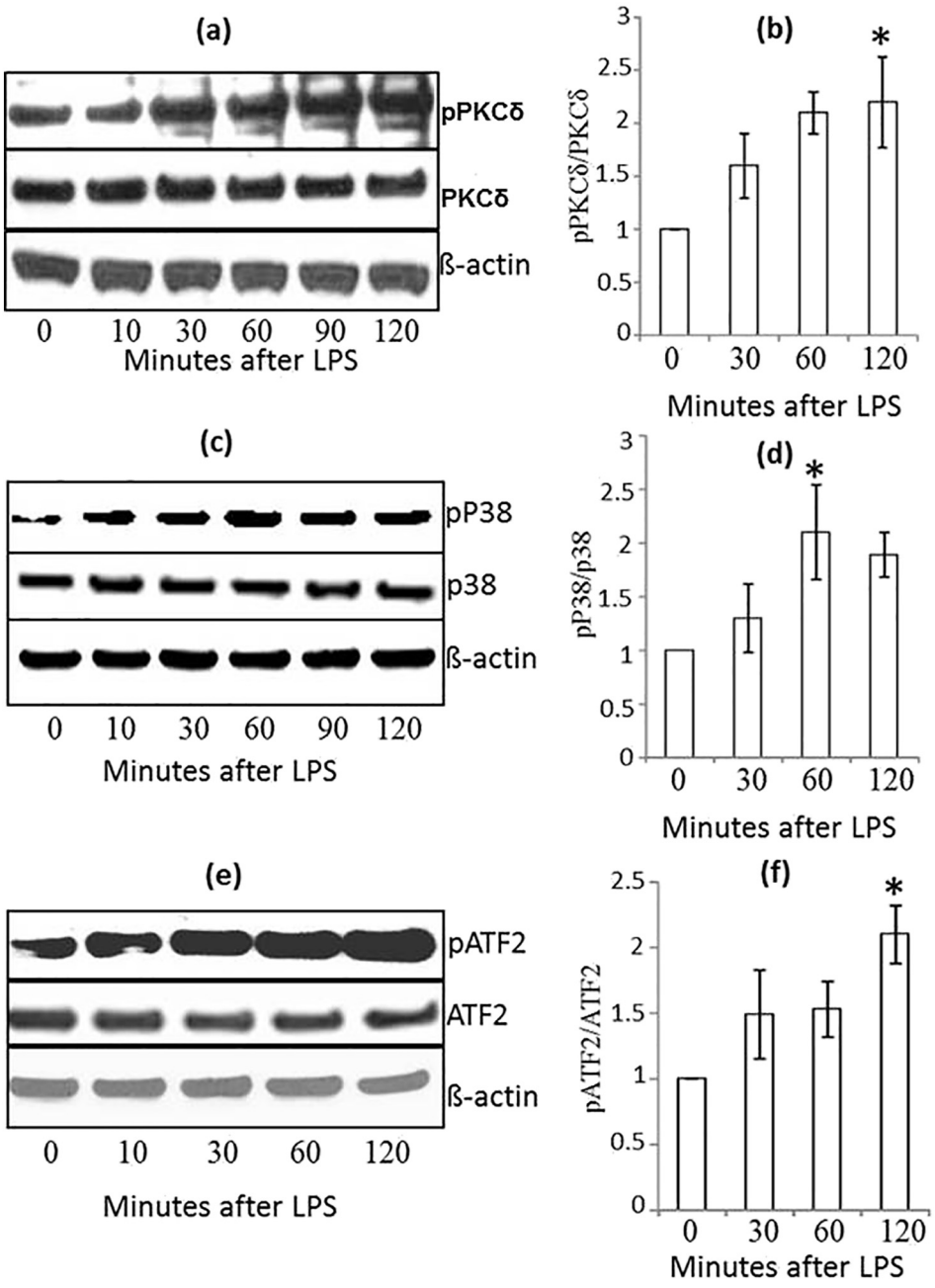

Fig. 3. Kinetics of PKCδ, p38 and ATF2 phosphorylation in response to LPS in wild-type BMDM. (a) PKCδ phosphorylation in wild-type BMDM challenged with LPS for 2 h (b) densitometry quantification of PKCδ phosphorylation. (c) p38 phosphorylation in wild-type BMDM challenged with LPS for 2 h (d) densitometry quantification of p38 phosphorylation (e) ATF-2 phosphorylation in wild-type BMDM challenged with LPS for 2 h (g) densitometry quantification of ATF2 (AP1 subunit) phosphorylation. The average band intensity of the respective bands from three independent experiments was measured. The band intensity was quanitfied using the imaging software ImageJ (https://imagej.nih.gov/ij/). All data are representative of three independent experiments; all are presented as mean ± SD. P values were determined by Student's *t*-test; *P < 0.05, **P < 0.005.

the SYBR Green PCR master Mix (Applied Biosystems). CT values of target gene compared to that of the housekeeping gene (GAPDH) were used to quantify gene expression in each sample. The following primers were used: IL12, forward 5′-AAGAGCAGTAGCAGTTCCCC-3′ and reverse 5′-GTTGGGCAGGTGACATCCTC-3′; IL23, forward 5′-ACCAGCGGG ACATATGAATCT-3′ and reverse 5′-AGACCTTGGCGGATCCTTTG-3′; GAPDH, forward 5′-GCACAGTCAAGGCCGAGAAT-3′ and reverse 5′-GCCTTCTCCATGGTGGTGAA-3′. All quantitations were normalized to GAPDH. All relative quantitation measurements were performed using the $\Delta\Delta C_t$ method according to the manufacturer's instructions.

3. Results

3.1. Heterotrimeric complex formation of PKCδ, TIRAP, and P38

TIRAP is an adapter protein which forms trimer complex with PKCδ

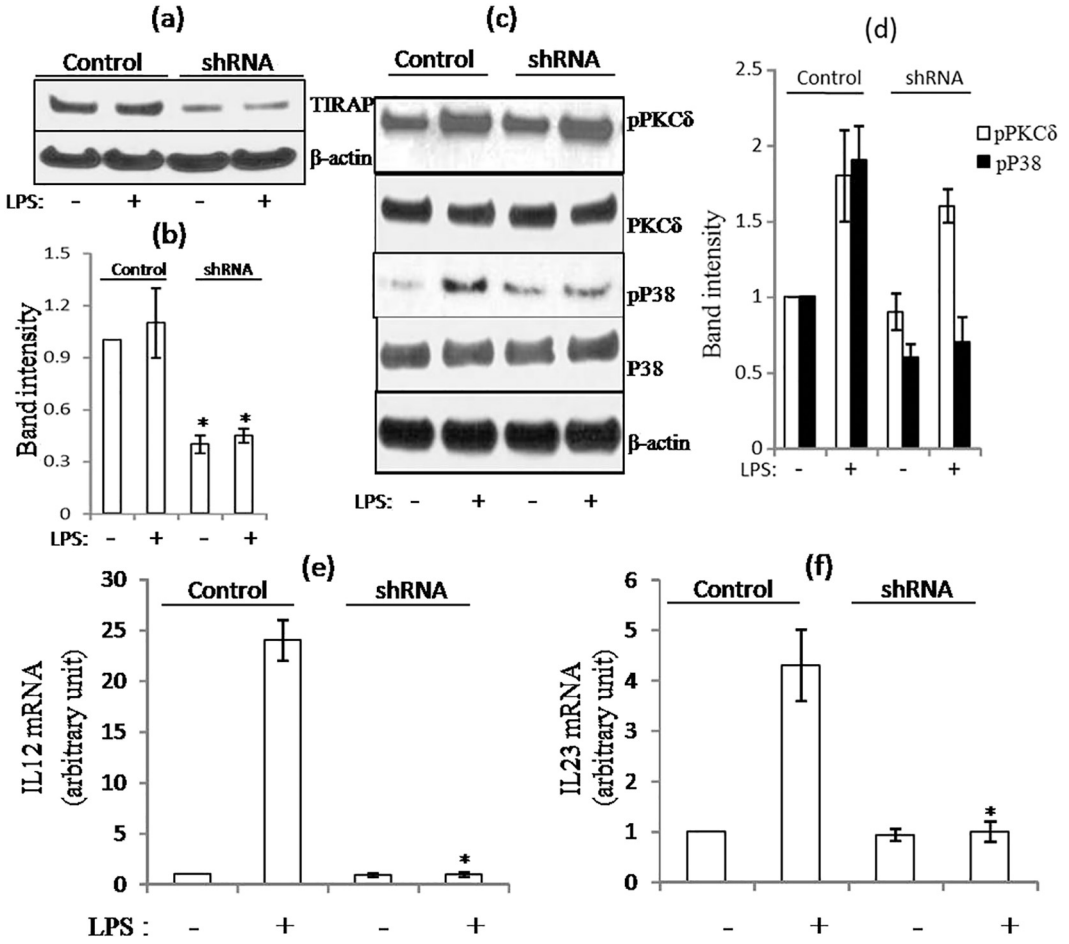

Fig. 4. TIRAP regulates PKCδ, p38 and ATF2 phosphorylation in wild-type BMDMs. (a) BMDMs were transfected with TIRAP shRNA construct, then cells were stimulated with LPS (100 ng/ml). Total TIRAP protein was detected by immunoblotting control and shTIRAP rasfected BMDMs. (b) Densitometry quantification of TIRAP was done using the imaging software ImageJ. (c) TIRAP silencing effect was monitored in wild type and shTIRAP transfected BMDMs. Phosphorylation of PKCδ and p38 was determined shTIRAP Trasfected BMDMs in presence and absesce of LPS. (d) mRNA expression was analyzed by RT-PCR for (a) IL12 (b) IL23 in wild type and shTIRAP transfected BMDMs before and after LPS treatmen. Data are mean ± SEM (n = 3). All data are representative of three independent experiments; all are presented as mean ± SD. P values were determined by Student's *t*-test; *P < 0.05, **P < 0.005.

and P38 for its subsequent signaling mechanism. To understand the binding mode of these protein partners, the full-length protein structure of PKCδ was modeled based on homology and abinitio calculation whereas the crystallographic structures of TIRAP and P38 was retrieved from PDB database [38,39]. The detailed structural information of these proteins is given in the supplementary section. Further, protein-protein docking was carried out using the HADDOCK software. The resulting docked trimeric complex of PKCδ-TIRAP-P38 is shown in Fig. 1a. The binding interface of PKCδ and TIRAP complex is covered by the interface area of about 1066.9 Å2, constituted by 23 residues of PKCδ and 34 of TIRAP with −11.3 kcal/mol solvation free energy (Table 1). Both polar and charged residues forming 18 hydrogen bonds and 13 salt bridges enhance the binding interactions of the PKCδ-TIRAP complex (Table 2, Fig. 1b). The N-lobe residues (K357, K362, D383, K381, I387 and D388) and the AGC-kinase C-terminal domain residues (D634, N639, E640, K641, R643, L644, S645, Y646, D648, L651 and Sep654) of PKCδ form hydrogen bonds, salt bridges and hydrophobic interactions with the residues of αA helix (V98, A99, Q101, D102, L103, S105 and Y106), AB loop (E108 and G109) which is proven to interact with MyD88, the loop connecting βD-αD (S180, G181 and R184) and the loop which connects αE and αE helical residues G199, R200, G201, P202, D203, G204, F206 and R207 of TIRAP (Supplementary Table 1). Based on the literature comparison with Rehan et al. studies, the residues of PKCδ [K357, K362, D388, D634, D648 and L651] and those of TIRAP [V98, D012, Y106, S180, G181, R200, D203 and R207] have been shown to interact stably and hence further support our data and validate our docking results. In case of TIRAP and pP38 interaction, twenty one hydrogen bonds and 8 salt bridges with the interface solvation energy of −14.6 kcal/mol (Table 1, Fig. 1a) constitutes the complex formation. Here, the N-terminal coil region (P71, T22, D76 and K84), the loop connecting (LC) βA-αA (E94), βB-βC (Q135, A136, S139, R143 and S140), βC-αC (Q153 and D154) and αC (Y156, Q160, Q163 and T166) forms stable interaction with residues of P38 protein R56, Q59, I61, I62, K65 (LC β5-α1 and α1), T174, D175, D176, E177 and Ptr181 (Activation loop), W196, M197 and H198 (LC α4-α5), S250 and S251 (LC α7-α8) and E327, D334 (LC α11 and α11) (Supplementary Table 2, Fig. 1c). TIRAP act as an adapter molecule via the proposed interaction mechanism with pPKCδ on one side and pP38 on the other, thus collectively constituting a trimer complex. The identified interface residues from the trimer complex suggest a potential pharmacophore map which could be utilized to find potential lead molecules as novel inhibitors.

3.2. Co-immunoprecipitations (Co-IP) show interactions between PKCδ, TIRAP, and p38 in the heterotrimeric complex

The interaction of PKCδ, TIRAP, and p38 was further determined by reciprocal Co-immunoprecipitation (Co-IP) to confirm the computational results. We did the reciprocal Co-IP assay in macrophages in the presence and absence of LPS with PKCδ, TIRAP, and p38 antibodies and

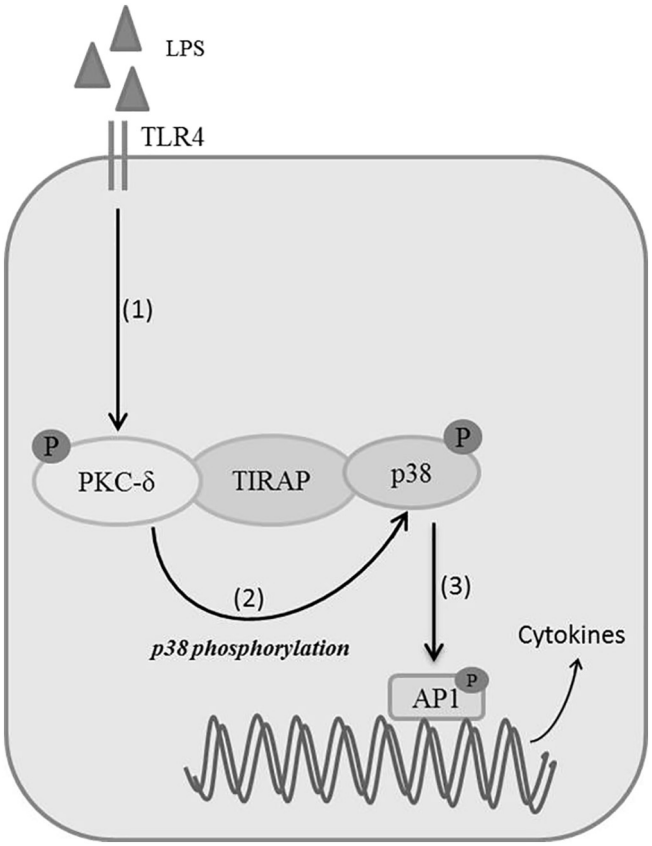

Fig. 5. Heterotrimer complex of PKCδ-TIRAP-p38 mediated inflammatory response in macrophages. (1) LPS from Gram-negative bacteria stimulates TLR4 receptor in macrophages; (2) This induces the activation of the AP1 signaling pathway via heterotrimer complex of PKCδ-TIRAP-p38; (3) LPS stimulation activates PKCδ, which in result phosphorylates p38 MAPK (4, 5) p38 MAPK activation induces AP1 transcriptional activity and expression of inflammatory cytokines such as IL-12, IL-23.

immunoblotting (IB) was performed using protein-specific antibodies and IgG as a control. The reciprocal Co-IP data clearly indicates that PKCδ, TIRAP, and p38MAPK proteins interact and serve as a complex, which supports downstream signaling during inflammation (Fig. 2). Our data suggests that heterotrimeric complex is present even in the absence of LPS. Further, this complex supports in downstream inflammatory signaling after endotoxin shock.

3.3. Hetrerotrimer complex is required for AP1-mediated inflammatory response

Signal transduction through TLR4 ligand is mediated by adaptor protein TIRAP that binds to protein PKCδ [40]. Inhibition of PKCδ has been shown to severely diminish p38 activation, suggesting that p38 is a direct phosphorylation target of PKCδ. Activation of p38 MAP kinase by PKCδ has been reported in the downstream activation of pro-inflammatory cytokine expression leading to the onset of inflammatory outburst. We have seen a significant increase in phosphorylation of PKCδ, p38 MAP, and AP1 transcriptional factor after LPS stimulation in BMDMs (Fig. 3). To confirm the role of TIRAP in heterotrimeric complex formation and in the activation of PKCδ, p38 MAP, and AP1, we used shTIRAP to silence TIRAP in BMDMs. TIRAP silencing inhibits p38MAPK phosphorylation but not PKCδ phosphorylation (Fig. 4b). Further, p38 inhibition attenuates IL12 and IL23 proinflammatory cytokine expression (Fig. 4e & f). These findings indicate that the PKCδ-TIRAP-p38 complex in macrophages enhances the inflammatory response by PKCδ mediated phosphorylation of p38 MAPK (Fig. 5).

4. Discussion

As TLRs are key checkpoints in the immune response, a complete understanding of their signaling is required to control the downstream events regulating both innate and acquired immunity. We examined the presence of heterotrimeric complex (PKCδ-TIRAP-p38) down- stream to TLR4 signaling in bone marrow-derived macrophages (BMDMs). TIRAP has been identified as an intermediate molecule and brings PKCδ and p38MAPK in close proximity. Further, PKCδ mediated phosphorylation of p38MAPK induces proinflammatory cytokines expression and hence determines severity of inflammation.

Computational modeling and docking studies were performed to investigate the interaction between PKCδ-TIRAP and TIRAP-p38 in the heterotrimeric complex (Fig. 1). More direct evidence of heterotrimeric complex (PKCδ-TIRAP-p38) formation has been provided by reciprocal Co-Immunoprecipitation (Co-IP), which indicated the interaction between PKCδ, TIRAP, and p38MAPK (Fig. 2). Earlier, it has been reported that TLR4 activation increases PKCδ phosphorylation in macrophages. Toll-like receptors have been shown to recognize pathogen-associated molecular patterns and initiate innate immunological responses after an interaction with infectious agents. The effect of PKCδ specific inhibitor, rottlerin was investigated on TLR4-mediated signaling in murine microglia stimulated with lipopolysaccharide. We identified an increase in phosphorylation of PKCδ, p38MAPK, and ATF2 after LPS stimulation (Fig. 3). We identified that TIRAP serves as an adaptor molecule which brings PKCδ and p38MAPK in close proximity leading to the activation of p38MAPK by PKCδ.

To confirm the role of TIRAP in the formation of the heterotrimeric complex we further silenced it using shTIRAP (Fig. 4a). TIRAP silencing significantly inhibited activation of p38MAPK but not PKCδ (Fig. 4b). This supports that p38MAPK activation is regulated by PKCδ through TIRAP. Further IL-12 and IL-23 proinflammatory cytokines expression was monitored in wild-type BMDMs as well as shTIRAP transfected BMDMs. This data supports that regulation of proinflammatory cytokine expression is dependent on the heterotrimeric complex where TIRAP stabilize the complex formation. To confirm the p38MAPK mediated signaling mechanism we used p38 inhibitor and determined the IL-12 and IL-23 expression (Fig. 5). IL12 and IL23 expression can be inhibited by p38 specific inhibitor (SB203580). Collectively, we find that PKCδ-TIRAP-p38 heterotrimeric complex determines the severity of inflammatory response in macrophages after LPS stimulation. These observations may have important implications for the development of therapeutic entity (small molecule or peptide) based on the interface site of PKCδ-TIRAP and TIRAP-p38 for various inflammatory diseases.

Our key findings provide novel insights into the mechanism of action of heterotrimeric complex (PKCδ-TIRAP-p38) in proinflammatory cytokine expression, which controls the development of the inflammatory trigger in stimulated macrophages (Fig. 5). TIRAP facilitates the heterotrimeric complex formation and downstream signaling during the inflammatory signaling cascade, which could have crucial implications for the development of therapeutic entity (small molecule or peptide) based on the interface site of PKCδ-TIRAP and TIRAP-p38 for various inflammatory diseases.

Conflict of interest

The authors declare no conflict of interest with the contents of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.intimp.2017.04.028.

References

[1] K. Newton, V.M. Dixit, Signaling in innate immunity and inflammation, Cold Spring Harb. Perspect. Biol. 4 (2012) 1–19.
[2] S. Akira, K. Takeda, Toll-like receptor signalling, Nat. Rev. Immunol. 4 (2004) 499–511.
[3] S. Akira, S. Uematsu, O. Takeuchi, Pathogen recognition and innate immunity, Cell 124 (2006) 783–801.
[4] R. Medzhitov, C. Janeway, Innate immunity, N. Engl. J. Med. 343 (2000) 338–344.
[5] A. Banerjee, S. Gerondakis, Coordinating TLR-activated signaling pathways in cells of the immune system, Immunol. Cell Biol. 85 (2007) 420–424.
[6] M. Chang, W. Jin, S.-C. Sun, Peli1 facilitates TRIF-dependent Toll-like receptor signaling and proinflammatory cytokine production, Nat. Immunol. 10 (2009) 1089–1095.
[7] R. Medzhitov, T. Horng, Transcriptional control of the inflammatory response, Nat. Rev. Immunol. 9 (2009) 692–703.
[8] A. Hoffmann, A. Levchenko, M.L. Scott, D. Baltimore, The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation, Science 298 (2002) 1241–1245.
[9] G.L. Schieven, The biology of p38 kinase: a central role in inflammation, Curr. Top. Med. Chem. 5 (2005) 921–928.
[10] Y. Jiang, et al., Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta), J. Biol. Chem. 271 (1996) 17920–17926.
[11] Z. Wang, et al., The structure of mitogen-activated protein kinase p38 at 2.1-A resolution, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 2327–2332.
[12] S.J. O'Keefe, et al., Chemical genetics define the roles of p38alpha and p38beta in acute and chronic inflammation, J. Biol. Chem. 282 (2007) 34663–34671.
[13] J. Branger, et al., Anti-inflammatory effects of a p38 mitogen-activated protein kinase inhibitor during human endotoxemia, J. Immunol. 168 (2002) 4070–4077.
[14] A. Risco, et al., p38γ and p38δ kinases regulate the Toll-like receptor 4 (TLR4)-induced cytokine production by controlling ERK1/2 protein kinase pathway activation, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 11200–11205.
[15] A.M. Badger, et al., Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function, J. Pharmacol. Exp. Ther. 279 (1996) 1453–1461.
[16] J.M. Olson, A.R. Hallahan, p38 MAP kinase: a convergence point in cancer therapy, Trends Mol. Med. 10 (2004) 125–129.
[17] G. Ramesh, W.B. Reeves, p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice, Am. J. Physiol. Ren. Physiol. 289 (2005) F166–F174.
[18] J.F. Schindler, J.B. Monahan, W.G. Smith, p38 pathway kinases as anti-inflammatory drug targets, J. Dent. Res. 86 (2007) 800–811.
[19] B.L. Slomiany, A. Slomiany, Involvement of p38 MAPK-dependent activator protein (AP-1) activation in modulation of gastric mucosal inflammatory responses to Helicobacter pylori by ghrelin, Inflammopharmacology 1 (2013) 67–78.
[20] N. Sakai, T. Wada, K. Furuichi, Y. Iwata, K. Yoshimoto, K. Kitagawa, S. Kokubo, M. Kobayashi, S. Takeda, H. Kida, K. Kobayashi, N. Mukaida, K. Matsushima, H. Yokoyama, p38 MAPK phosphorylation and NF-κB activation in human crescentic glomerulonephritis, Nephrol. Dial. Transplant. 6 (2002) 998–1004.
[21] R.M. Guo, W.M. Xu, J.C. Lin, L.Q. Mo, X.X. Hua, P.X. Chen, K. Wu, D.D. Zheng, J.Q. Feng, Activation of the p38 MAPK/NF-κB pathway contributes to doxorubicin-induced inflammation and cytotoxicity in H9c2 cardiac cells, Mol. Med. Rep. 2 (2013) 603–608.
[22] J.C. Lee, J.T. Laydon, P.C. McDonnell, et al., A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, Nature 372 (1994) 739–746.
[23] A. Cuenda, J. Rouse, Y.N. Doza, et al., SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1, FEBS Lett. 364 (1995) 229–233.
[24] H. Kim, R. Zamel, X.H. Bai, M. Liu, PKC activation induces inflammatory response and cell death in human bronchial epithelial cells, PLoS One 8 (5) (2013) e64182.
[25] H. Sondermann, J. Kuriyan, C2 can do it, too, Cell 121 (2005) 158–160.
[26] T.A. Leonard, B. Różycki, L.F. Saidi, G. Hummer, J.H. Hurley, Crystal structure and allosteric activation of protein kinase C βII, Cell 144 (2011) 55–66.
[27] Z.-B. Xu, et al., Catalytic domain crystal structure of protein kinase C-theta (PKCtheta), J. Biol. Chem. 279 (2004) 50401–50409.
[28] A. Roy, A. Kucukural, Y. Zhang, I-TASSER: a unified platform for automated protein structure and function prediction, Nat. Protoc. 5 (2010) 725–738.
[29] Y. Zhang, Template-based modeling and free modeling by I-TASSER in CASP7, Proteins 8 (Suppl. 69) (2007) 108–117.
[30] M. Kubo-Murai, K. Hazeki, N. Sukenobu, K. Yoshikawa, K. Nigorikawa, K. Inoue, T. Yamamoto, M. Matsumoto, T. Seya, N. Inoue, O. Hazeki, Protein kinase Cdelta binds TIRAP/Mal to participate in TLR signaling, Mol. Immunol. 44 (9) (2007) 2257–2264.
[31] S.J. de Vries, et al., HADDOCK versus HADDOCK: new features and performance of HADDOCK2.0 on the CAPRI targets, Proteins 69 (2007) 726–733.
[32] C. Dominguez, R. Boelens, A.M.J.J. Bonvin, HADDOCK: a protein-protein docking approach based on biochemical or biophysical information, J. Am. Chem. Soc. 125 (2003) 1731–1737.
[33] A.T. Brunger, et al., Acta Crystallogr. D Biol. Crystallogr. 54 (Pt. 5) (1998) 905–921.
[34] N.S. Topna, M. Kannan, R. Krishna, Interacting mechanism of ID3 HLH domain towards E2A/E12 transcription factor – an insight through molecular dynamics and docking approach, Biochemistry and Biophysics Reports, 1 2016, pp. 180–190.
[35] E. Krissinel, Crystal contacts as nature's docking solutions, J. Comput. Chem. 31 (2010) 133–143.
[36] E. Krissinel, K. Henrick, Inference of macromolecular assemblies from crystalline state, J. Mol. Biol. 372 (2007) 774–797.
[37] M.S. Baig, S.V. Zaichick, M. Mao, A.L. de Abreu, F.R. Bakhshi, P.C. Hart, et al., NOS1-derived nitric oxide promotes NF-κB transcriptional activity through inhibition of suppressor of cytokine signaling-1, J. Exp. Med. 212 (2015) 1725–1738.
[38] R. Diskin, M. Lebendiker, D. Engelberg, O. Livnah, Structures of p38alpha active mutants reveal conformational changes in L16 loop that induce autophosphorylation and activation, J. Mol. Biol. 365 (2007) 66–76.
[39] Z. Lin, J. Lu, W. Zhou, Y. Shen, Structural insights into TIR domain specificity of the bridging adaptor Mal in TLR4 signaling, PLoS One 7 (2012) e34202.
[40] Daniel J. Loegering, Michelle R. Lennartz, Protein kinase C and Toll-like receptor signaling, J. Enzym. Res. (2011), http://dx.doi.org/10.4061/2011/537821.