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2. The biological function of the proto-oncogene Cot/tpl-2 (MAP kinase kinase kinase 8)

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Abstract. Cot, as well as its murine homologue tpl-2, was discovered in a COOH–terminal truncated form that unmasks the transformation capacity of the protein. The COOH-terminal domain of wt Cot contains an amino acid sequence that is a recognition signal for degradation via proteasome, besides, this domain of wt Cot is also an autoinhibitory domain of the specific activity of the wild type form. These data explain the transformation capacity of trunc-Cot/tpl-2, that when overexpressed is capable of activating several MAP kinases pathways as well as AP-1, NFAT, and NF-κB2 transcriptional activities. Earlier sobreexpression experiments lead to the proposal that Cot/tpl-2 could be involved in proliferative signalling, but the use of new technologies such as genetically modifies mice and interference RNA end up with the already accepted hypothesis that Cot/tpl-2 is involved in immune innate and adaptive processes. Cot/tpl-2 is activated in response to the activation of the TLR/IL-1 receptor superfamily as well as in response to the activation of some receptors of the TNF family. Independently of the cell system

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it is accepted that in resting cells Cot/tpl-2 forms a stable and inactive complex with p105 NF-κB among other proteins to protect it from degradation, adequate TLR/IL-1R stimulation induces the activation of the IKK complex that targets p105 NF-κB to be rapidly degraded by the proteasome pathway to p50 NF-κB, a subunit of the NF-κB transcription factor. Consequently Cot/tpl-2 is released from the complex and susceptible to transduce the activatory signal, leading to the activation of the MEK1-Erk1/Erk2 pathway. However, actually it is not completely understood all the requests that Cot/tpl-2 needs to be fully active and to this end it is also accepted that Cot/tpl2 requires to be phosphorylated. In addition the possibility that the requirements vary from cell system to cell system cannot be excluded. Physiologically, Cot/tpl-2 is involved in provoking innate immunity to establish adaptive immunity. In fact it is the unique MAP3K that activates Erk1/Erk2 when the TLRs/IL-1 receptors are activated and mediates the production of pro-inflammatory cytokines, such as TNFα, IL-1, or IL-6. More recently it has been shown that Cot/tpl-2 has the capacity to regulate the balance between Th1 and Th2 cytokines. All these data indicate that, although mutations in Cot gene result in the expression of a protein linked with cell malignancies, physiologically wt Cot/tpl-2 is involved in innate and adaptive immunity.

Characterization of Cot/tpl-2 protein

Cot/tpl-2 is a MAP kinase kinase kinase (MAP3K8) capable of activating the MEK1-Erk1/Erk2 pathway. The human Cot gene was identified in a 3´ rearrangement form that leads to the expression of a truncated/modified protein. The normal homologue has an open reading frame encoding 467 amino acids. The first 397 are identical to the truncated form (trunc-Cot), while the 69 amino acids from the COOH-terminal of wt Cot are replaced by 18 amino acids without sequence homology in the truncated form (1). The murine homologue of the human Cot gene is the tpl-2 gene. The tpl-2 gene was also identified in a similar truncated/modified form (2). The COOH-terminal domains of both wt Cot and wt tpl-2 are almost identical. The only difference between the last 77 COOH-terminal amino acids of the human wt Cot and the murine wt tpl-2 are the two conservative substitutions of the residues M437 for V and K439 for R. However, there is no similarity in the amino acids inserted in either trunc-Cot or trunc-tpl-2. These truncations/modifications unmask the transforming capacity of this MAP kinase kinase kinase, a property of the trunc-Cot/tpl-2 protein (Fig. 1).

Nevertheless, it has been shown that overexpression of the proto-oncogenic form is also capable of conferring a transformed phenotype to established cell lines (3-5).

The COOH-terminal domain of wt Cot contains the EMLKRQRSLYIDLGALAGYFNL amino acid sequence that is a recognition signal for degradation via proteasome. This degron confers instability to other unrelated proteins and accounts for the 2.6-fold lower $t_{1/2}$ of wt Cot compared (35 min), to trunc-Cot (90 min). On the other hand, the deletion of the 44 amino acids of the COOH-terminal of wt Cot increases its specific activity in 3.8 fold, measured as MEK1-Erk1/Erk2 activation, indicating that the COOH-terminal domain of wt Cot is also an autoinhibitory domain of the specific activity of the wild type form (5) (Fig. 1). In fact, the COOH-terminal of tpl-2 is capable of inhibiting the kinase activity of trunc-tpl-2 “in vitro” (2). Moreover, phosphorylation of the residue S400, which is contained in the carboxi-terminal of the wt form but not in the truncated/modified form, by extracellular stimuli, increases the specific activity of the protein (6). In conclusion, all these data indicate that deletion of the carboxi-terminal region of the murine and human wt forms increases their kinase activity through two independent mechanisms that leads to an increase in the $t_{1/2}$ and an increase in the specific activity of the kinase, both potentiate each other in such a manner that equal amounts of trunc- and wt Cot mRNA results in a 10-fold increase in the activity of trunc-Cot.

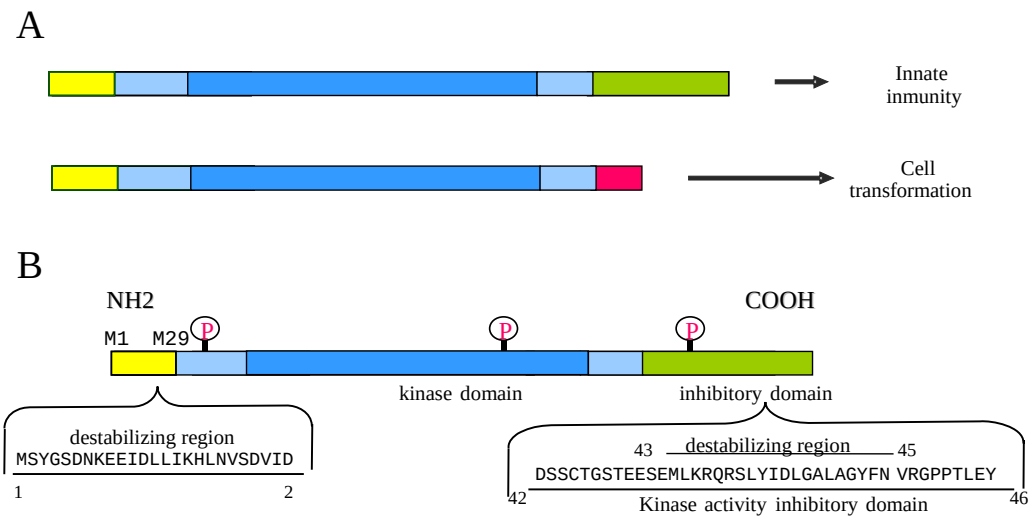

Figure 1. Schematic representation of Cot protein kinase.PartA compares the structures of the wt Cot (above) and trunc-Cot (below) proteins. The last 69 amino-acids of the carboxi-terminal region from the wt protein have been substituted by 18 unrelated amino-acids. The alternative translation start methionine (M29) is also indicated. Part B shows the different domains of the wt Cot protein. The amino-acid sequence of the destabilizing region located at the N terminus is shown. The C-terminus sequence include both a destabilizing region and a kinase activity inhibitory domain. The main phosphorylation sites (residues S62, T290, and S400) are indicated.

Two alternative translation initiation sites M(1) and M(29) has been described for wt Cot/tpl-2 (7). M(29) wt Cot does not contain the first 29 amino acids of M(1) wt Cot and exhibits a three -fold higher $t_{1/2}$ than M(1) wt Cot (7). This data suggests that the N-terminal of wt Cot contains another signal that triggers the protein to degradation. In fact antibodies against the C-terminal of Cot wt recognized two bands in Western blot analysis, being the one with higher mass more rapidly degraded (8-10), although the sequence of amino acids in the N-terminal of the protein involved in this process remains to be established.

Mutations in Cot/or tpl-2 gene are associated with tumorigenesis

Increased Cot/tpl-2 activity has been observed in different transformed cell types. In fact, different modifications in Cot/tpl-2 have been proposed to be responsible for cellular transformation (Table1). Cot was identified in a truncated/modified form that occurred as a consequence of a rearrangement in the 3´ terminal region of human Cot gene during transfection of the genomic DNA of a human carcinoma cell line into hamster SHOK cells; this rearrangement induced the transformation of the murine cells (1).

The insertion of the Moloney Leukaemia Provirus into tpl-2 gene also induced a rearrangement in the 3´terminal region of tpl-2 gene. This tpl-2 gene reorganization plays an essential role in the progression of T lymphomas (11, 12). Similarly, the insertion of the Mouse Mammary Tumour Virus (MMTV) in the last intron of tpl-2 gene is associated with transformation

Table 1

Type of mutation	**Cell line/ tissue**	**Reference**
3´ rearrangement of Cot/tpl-2 gene	Hamster SHOK cells. In vitro transfection of human genomic DNA	Miyoshi (1991)
3´ rearrangement of Cot/tpl-2 gene	Murine T cell. Insertion of the Moloney Leukemia provirus	Patriotis (1993)
3´ rearrangement of tpl-2 gene	Mammary gland. Insertion of Murine Mammary provirus	Erny (1996)
3´ rearrangement of Cot gene	Primary human adenocarcinoma	Clark (2004)
Genomic locus amplification	Human breast cancer cells	Sourvinos (1999)
Postraductional modifications	Infection of the human T cell leukemia virus type-I	Babu (2006)
Unknown	Human large granular lymphocyte proliferative disorder	Christoforidou (2004)
Unknown	Epstein-Barr virus infection	Eliopoulos (2002)

of mammary gland cells (13). More recently, in some human adenocarcinoma primary tumors a modification in the 3´ region of Cot gene has been confirmed (14). The deletion of the 3´ region of the Cot and tpl-2 genes provoke not only the expression of a truncated/modified protein, as explained above, but also the deletion of the 3´ untranslated region of both genes that harbor three copies of the RNA destabilizing sequence AUUUA. This, results in the higher expression levels of their corresponding mRNAs (12, 13). On the other hand, the human Cot genomic locus, without modifications in the coding sequence of Cot gene, is amplified in some human breast cancers (15). Based on these data it has been proposed that Cot could be a prognostic factor in breast cancer development (16). Moreover, a correlation between high Cot mRNA levels and human large granular lymphocyte proliferative disorder has been also proposed (17). Besides the infection of the human T cell leukemia virus type-I (HTLV-I) induces high steady state expression levels of the M(29) wt Cot, that as previously mentioned, exhibit a high stability, resulting in high levels of Cot activity (18). Cot is also commonly highly expressed in Epstein Barr virus -associated malignancies, such as nasopharyngeal carcinoma and gastric cancer (19). All these data, indicate that increased Cot/tpl-2 activity plays a role in cell transformation, besides more recently it has been also proposed that Cot could also participate in cell invasion processes (20, 21).

However in contrast to the established role of Cot/tpl-2 as an oncogene, it has been also reported that wt Cot/tpl-2 can function as a tumour suppressor gene, since CD8+ T cell lymphomas are developed in old Cot/tpl-2 KO mice (22). In these cells the Cot/tpl-2 downregulation decreases the expression of the T cell co-receptor CTL4, which negatively regulate de signals transducer through the T cell receptor (TCR).

Regulation of Cot/tpl-2 activity by extracellular signals

Cot and tpl-2 genes were identified in an oncogenic form; thereby for many years, it was expected, that the proto-onco Cot/tpl-2 protein could be involved in the intracellular proliferative signals pathways. However, the fact that the tpl-2 knock-out mice was resistant to LPS/D-Galactosamine-induced pathology, due to the low production of TNFα among other cytokines, indicated that, physiologically, endogenous Cot/tpl-2 would be involve in innate and adaptive immunity rather in proliferative signals (23).

Now it is accepted that Cot/tpl-2 is activated in response to the activation of the TLR/IL-1 receptor superfamily as well as in response to the activation of some receptors of the TNF family (24-26) and that Cot/tpl-2 plays a unique role in inflammatory processes. The TLRs, included in the TLR/IL-1R

superfamily, contain an extracellular leucine-rich repeat region and are sensors of the infection by pathogens since they recognized the different *pathogen-associated molecular patterns* (PAMPS): bacterial lipopeptides (TLRs1, 2, and 6) lipopolysaccharide (TLR4), flagellin (TLR5) and nucleic acids (TLRs3,7,8, and 9). The IL-1 receptor subfamily, with an Igg like extracellular region, includes receptors for IL-1 (IL-1R), IL-18 (IL-18R, and IL-33 (T1/ST2 R). However, despite their different extracellular structure, both TLRs and IL-1Rs contain in their intracellular region the TIR domain and activate similar intracellular pathways including the recruitment to the receptor of some adaptors like MyD88, the activation of the kinases IRAK, and the ubiquitination of the TRAF proteins, resulting in the activation of the IKK complex (reviewed in 27-29).

Independently of the cell system, it is accepted that in resting cells, Cot/tpl-2 forms a stable and inactive complex with p105 NF-κB among other proteins to protect it from degradation. The interaction between p105 NF-κB and wt Cot/tpl-2 was first described as a result of a yeast two-hybrid screen (30) and was further corroborated, by the fact that p105 NF-κB knock-out macrophages, show normal tpl-2 mRNA levels but tpl-2 protein, could be only detected when cells are treated with proteasome inhibitors (8,9). Studies performed with overexpressed p105 NF-κB and wt Cot/tpl-2, have indicated that Cot/tpl-2 protein interacts with p105 NF-κB trough two different domains (31). One interaction takes place through the COOH-domain of wt Cot/tpl-2, where a proteasome degradation signal is located. This interaction explains why the interaction between p105 NF-κB and Cot/tpl-2 protects Cot/tpl-2 from degradation. Another interaction between the kinase domain of Cot/tpl-2 and p105 NF-κB has been also proposed (31). This interaction does not allow the substrates of Cot/tpl-2, like MEK1, to interact with the kinase and to be subsequently phosphorylated (32). Besides, cotransfection experiments have also demonstrated that ABIN2 (A20-binding inhibitor of NF-κB2) also interacts with wt Cot/tpl-2 in addition to p105 NF-κB (33) (Fig. 2).

This interaction has been also further confirmed in knock-out ABIN2 macrophages where, as occurred in p105 NF-κB knockout macrophages, endogenous wt Cot/tpl-2 protein is neither detected due to its rapid degradation. But, in contrast to p105 NF-κB, the interaction of ABIN2 with Cot/tpl-2 does not block its kinase activity (34, 35). All these data indicate that both p105 NF-κB and ABIN2 are necessary to form a complex with wt Cot/tpl-2 to protect the kinase from the proteasome degradation. Besides ABIN2 and p105 NF-κB it has been also reported that a member of the kinase suppressor family of RAS (KSR2) interacts with Cot/tpl-2 when both

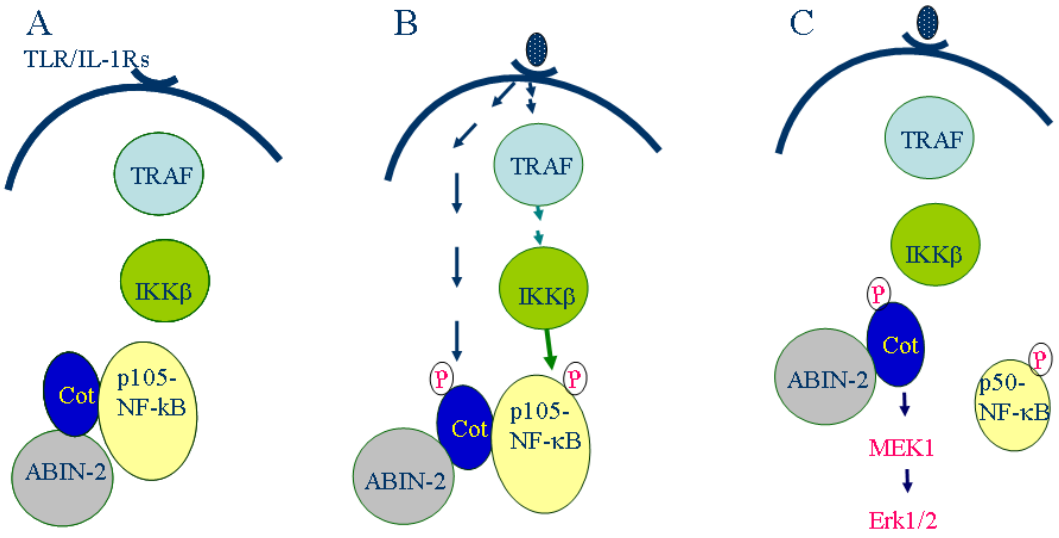

Figure 2. Mechanisms of Cot/tpl-2 activation. The figure shows a schematic representation of the mechanisms involved in the activation of Cot/tpl-2 by extracellular stimuli (TLRs/IL-1Rs). A) Cot protein status in basal conditions. In unstimulated cells Cot/tpl-2 protein is forming a complex with at least two proteins (p105 - NF–κB and ABIN-2), this association maintains Cot/tpl-2 inactive and stable. B) Upon ligand binding, activated IKKβ phosphorylates p105 tagging it for proteosoma degradation. Other signals, not clearly understood yet, allow Cot phosphorylation in different residues C) Cot is then released from its interaction with p105 and phosphorylated Cot/tpl-2 activates Erk1/2 pathway which is subsequently rapidly degraded by the proteasome.

proteins are overexpressed. The co-expression of KSR2 with wt Cot/tpl-2 significantly and specifically reduces Erk1/Erk2 and NF-κB activation by Cot/tpl-2 (36); however the physiological role of this interaction remains to be established. Further studies have to be performed to determine if other proteins are part of the p105 NF-κB-ABIN2-Cot/tpl-2 complex (37).

Adequate TLR/IL-1R stimulation induces the activation of the IKK complex; active IKKβ kinase phosphorylates p105 NF-κB in different residues (38-40). These phosphorylations target p105 NF-κB to be rapidly degraded by the proteasome pathway to p50 NF-κB, a subunit of the NF-κB transcription factor (38-41). Consequently Cot/tpl-2 is released from the complex and susceptible to transduce the activatory signal (8, 9, 31, 42). Once Cot/tpl-2 is dissociated from the complex is rapidly degraded just upon activation, by phosphorylation, of MEK1 (8,9). However, actually it is not completely understood all the requisites that Cot/tpl-2 needs to be fully active and the possibility that the requirements vary from cell system to cell system can not be excluded. In one hand, overexpression of TRAF6, an adaptor that mediates the activation of the IKK complex, is sufficient to induce Cot/tpl-2

activation in MEF cells (43), but not in HeLa cells (10). Overexpression of TRAF6 in HeLa cells induces IκBα degradation, indicating that TRAF6 overexpression induces the activation of the IKK complex. However, the overexpression of this adaptor is not sufficient to induce Cot/tpl-2 activation, although TRAF6 is required to activate Cot in response to IL-1 stimulation (10). Similarly, in macrophages other TRAF protein, TRAF2, participates in the intracellular signalling to activate Cot/tpl-2 in response to TNFα but as in the case of HeLa it is not sufficient to induce the activation of the kinase (44). All together these data provide evidence that at least two intracellular signals are required to induce Cot activation (Fig. 2). One of which, is the dissociation of Cot from the inactive complex, being this intracellular pathway TRAF dependent, but others signal(s) is (are) required to fully activate the kinase. In this context it has been reported that upon cellular stimulation Cot/tpl-2 becomes phosphorylated in different residues, but the exact role of each of these phosphorylations remains to be elucidated (Fig. 1). It has been reported by several groups that in response to extracellular stimuli like LPS, TNFα or IL-1, Cot/tpl-2 is phosphorylated in the activation loop of the kinase, on residue T290 (45-47). This T290 phosphorylation, seems also to play a role in the dissociation of Cot/tpl-2 protein from the NF–κB p105 complex and it is essential for the activation of Erk1/Erk2 by Cot/tpl-2 (45, 48). Once Cot/tpl-2 is phosphorylated on residue T290, it phosphorylates itself in S62, this autophosphorylation enhances the capacity of Cot/tpl-2 to activate the Erk1/Erk2 pathway (45). On the other hand, a phosphorylation on the S400 of Cot/tpl-2 by LPS stimulation, is also required for a full Cot/tpl-2 activation (6). AKT is capable of phosphorylating this residue “in vitro” (49), but does not plays any role in the phosphorylation of this residue “in vivo” and the identity of this kinase is still unknown (6). All these data indicate that in general to activate physiologically Cot/tpl-2 two requirements are necessary, dissociation of the inactive complex and an adequate phosphorylation state of the protein.

The specific kinases that phosphorylate both residues T290 and S400 on Cot/tpl-2 protein remain to be identified. However, it has been reported, that in response to IL-1 and TNFα stimulation Cot/tpl-2 activation requires the activity of a tyrosine kinase of the SRC family (10, 44), thereby it can not be excluded the possibility that a SRC tyrosine kinase activity, mediates the phosphorylation on residues T290 or S400 or both. By using immobilized metal affinity chromatography (IMAC) and a linear trap mass spectrometer analysis, other phosphorylation sites on Cot/tpl-2 proteins have been also identified, but the role of these phosphorylations on Cot/tpl-2 protein are still unknown (50).

More recently, it has been proposed that Cot/tpl-2 also mediates the activation of Erk1/Erk2 in response to the activation of the proteinase activating factor 1 (PAR-1) (21) and the adiponectin receptor (51). These two receptors contain in their structure the classical seven transmembrane domains, but not the TIR domain. Thereby, it is unlikely that these receptors could activate Cot/tpl-2 through the Myd88 adaptor. All these data open the possibility that Cot/tpl-2 take also part in other type of intracellular signaling pathways.

Signal pathways activated by Cot/tpl-2

Both overexpressed wt Cot/tpl-2 and trunc Cot/tpl-2 are capable of increasing the activity of several MAP kinases pathways. Overexpression of wt or trunc-Cot/tpl-2 induce an increase in the MAP kinases pathways Erk1/Erk2, JNK (52), p38γ**,** and Erk5 (4). Cot/tpl-2, partially as a consequence of the up-regulation of the different MAP kinases pathways, induces the activation of several transcription factors, such as AP-1, NFAT and NF-κB.

Overexpression of Cot/tpl-2 induces AP-1 activity in different cell systems (53, 54). This activation is probably mediated by the capacity of Cot to stimulate c-Jun and c-Fos activities (4, 55). In this context, it has been proposed that the ability of Cot/tpl-2 to induce cellular transformation is dependent on its ability to activate these two transcription factors (4, 55).

Overexpressed Cot/tpl-2 also up-regulates the NFAT transcriptional activity (54). In fact, a physical interaction among both overexpressed Cot/tpl-2 and NFATc2 has been also demonstrated (56). In one hand, Cot/tpl-2 potentiates NFAT-induced transactivation (57), on the other one it has been also reported that Cot/tpl-2 induces an NFAT accumulation in the nucleus (58, 59). Since the NFAT transcription factors are regulated at two different levels, firstly at the level of subcellular localization and secondly at the level of the intrinsic DNA binding activity, it remains to be established, whether the reported accumulation of NFAT by Cot/tpl-2 in the nucleus, could be a consequence of the increased transactivation of NFAT triggered by the kinase, resulting in higher nuclear retention, or Cot/tpl-2 could also have an active role in the modulation of the NFAT in/out shuttling of the nucleus.

Different groups have also shown that overexpressed Cot/tpl-2 induces the activation of the NF-κB, although the mechanism it still unclear. It has been claim that Cot/tpl-2 induces an increase in p65 NF-κB transcription factor activity by phosphorylating the residues S276 (60), S311(61), or S536

(62), or both S468 and 536 (63). Several groups have stated that the NF-κB activation by Cot/tpl-2 is through the activation of the IKK complex (64-70), due to the capacity of Cot/tpl-2 to activate NIK (63, 65), a kinase that mediates the activation of the IKK complex (71). Besides, it has been also proposed that Cot/tpl-2 is required to induce p105 NF-κB degradation (19, 30) by phosphorylating p105 NF-κB in a different site than the IKK complex (32).

The cis-elements of these transcription factors modulated by Cot/tpl-2 overexpression (AP-1, NFAT, and NF-κB) are conserved in many promoter regions, thereby, it should be expected that Cot/tpl-2 overexpression, would induce the transcription of many different genes (54, 57, 58, 72). However, more recent studies performed in cells; where the Cot/tpl-2 protein is knock-out suggest that in a physiological context Cot/tpl-2 may be more specific than when overexpressed. This is in part due to the fact that Cot/tpl-2 share substrates with other kinases. Indeed, overexpression of the Cot/tpl-2 dead kinase mutant sequesters MEK-1, that physiologically is also phosphorylated by other kinases, such as c-RAF.

Involvement of Cot innate and adaptive immunity

The physiological activation of Cot/tpl-2 has been extensively studied in macrophages after LPS stimulation. Cot/tpl-2 is the unique MAP3K that activates Erk1/Erk2 in response to the activation of TLR4 by LPS in macrophages (9, 23, 73, 74) (Fig. 2) without modulating the activation of p38, JNK, Erk5, and NFκB (23). In TNFα- or LPS-stimulated macrophages, in CD-40- stimulated-macrophages or B cells, as well as in IL-1-stimulated HeLa cells, Cot/tpl-2 does not modulated JNK and p38 activation (10, 23, 43). However in tpl-2-/- MEF cells not only Erk1/Erk2, but also JNK, and NF-κB activation are defective when stimulated with TNF whereas only Erk1/Erk2 activation is impaired when these cells are stimulated with IL-1. Thereby, it has been proposed that the signalling role of endogenous Cot/tpl-2 is stimulus and cell type specific (60).

The ablation of Cot/tpl-2 expression in LPS-stimulated macrophages partially blocks TNFα (23) and IL-10 secretion (24), and reduces the expression of COX-2 (74). Cot/tpl-2 activation is also required to induce Erk1/Erk2 activation when bone marrow derived macrophages are activated with peptidoglycan, double-stranded RNA or loxoribine, that are ligands for TLR2, TLR3, and TLR7 respectively (24, 26). Cot/tpl-2 is also the sole MAP3K that activates Erk1/Erk2 pathway in B cells in response to anti-CD40 and participates in the secretion IgE (43). In carcinoma Hela cells

Cot/tpl-2- MEK1-Erk1/Erk2 pathway is required to produce IL-8 and MIP-1β in response to IL-1 stimulation (10). On the other hand, and although Cot/tpl-2 is involved in the intracellular signalling generated by the activation of TLR9 by CpG-DNA, it is not clear whether other MAP3K, besides Cot/tpl-2, upregulates the Erk1/Erk2 pathway in response to CpG-DNA stimulation (26, 75, 76).

The role of Cot in inflammation is not only confirmed by experiments performed in intact cells due to its capacity to modulate the production of cytokines/chemokines involved in this process, but it also has been assessed "in vivo". In fact Cot/tpl-2 KO mice have a reduction in caerulein-induced pancreatic inflammation upon tpl-2 ablation (77) and it have been also shown that Cot/tpl-2 plays a role in the onset of Crohn's disease (78)(Fig.3) Besides, experiments performed with the Cot/tpl-2 KO mice, as well as with intact cells were Cot/tpl-2 is down modulated, have shown that this kinase modulates the strict balance between Th1 and Th2 cytokines. It has been reported that Cot/tpl-2 is an important negative regulator of Th1-type adaptive response due to its capacity to downregulate IL-12 production (75) (Fig. 3).

Antigen presenting cells from Cot/tpl-2 KO mice stimulated with CpG, an activator of TLR9, showed an increase in the IL-12 production (75, 76), and this has been confirmed "in vivo" by performing studies with the whole animal (75). More recently, it has been also shown, that antigen presenting cells from Cot/tpl-2 KO mice stimulated with LPS or CpG, showed not only an increase in IL-12 but also in the production of IFNβ, being concomitant with a reduction in the levels of IL-10, a typical Th2

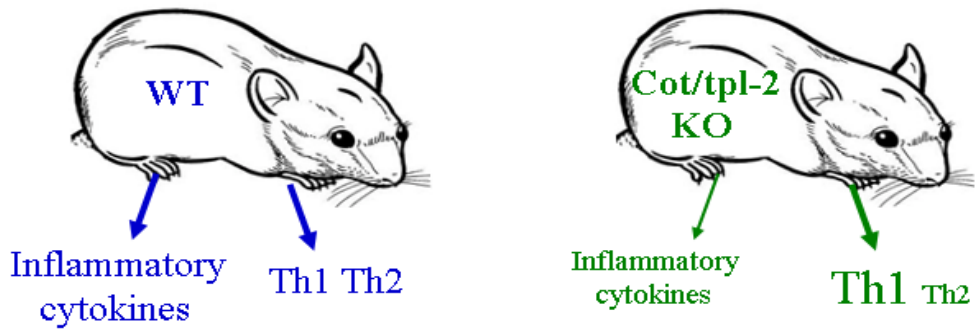

Figure 3. Cot/tpl-2 modulates Th1/Th2 response and inflammation. In the figure are summarized some data from the bibliography using mice null for the Cot/tpl-2 gene. The Cot/tpl-2 KO mice shows a decreased production of inflammatory cytokines such as TNFα or IL-1β and an increased production of cytokines characteristic of the Th1 response like IL-12, IFNβ and decreased production of IL-10.

cytokine (76). However, further studies have to be carried on to determine the exact role of Cot/tpl-2 in polarizing the immune response to type Th1, since IL-12-stimulation of CD4+ T lymphocytes of Cot/tpl-2 KO mice do not to produce IFNγ, another key cytokine in the Th1 response (79).

Cot/tpl-2 a target of anti-inflammatory drugs

Cot/tpl-2 and RAF isoforms are capable of activating the MEK1-Erk1/Erk2 pathway, being Erk1/Erk2 a MAP kinase that phosphorylates multiple substrates. However RAF-MEK1-Erk1/Erk2 pathway is activated in response to proliferative signals, whereas Cot/tpl-2 mediates the intracellular signalling of PAMPs and pro inflammatory cytokines, indicating that Cot/tpl-2 is involved in innate and adaptive immunity and thereby in inflammation. Besides, the Cot/tpl-2 physiological role can not be replace by any other protein, indicating that this protein is a very good target to develop new and improved anti-inflammatory drugs (80, 81). In fact, several groups and companies, have focused in the development of small organic compounds to specifically block the kinase activity by occupying the active center of Cot/tpl-2. To date, 1,7-napthyridine-3-carbonitriles, quinoline-3-carbonitriles and thieno[2,3-*c*]pyridines, have been reported as Cot/tpl-2 kinase inhibitors (82, 85). It has been shown, that the 7-amino substituted thieno[2,3-*c*]pyridines are capable to inhibit activation of Erk1/Erk2 in bone marrow derived macrophages in response to LPS stimulation as well as TNFα secretion in human whole blood (85). Among the quinoline-3-carbonitriles, it has been proposed that the 8-bromo-4-(3-chloro-4-fluorophenylamino)-6-[(1-methyl-1H-imidazol-4-yl)met hylamino]quinoline-3-carbonitrile compound is capable to inhibit, at an oral dose of 50 mg/kg, the production TNF-α in a LPS-induced rat model (83).

More recently, it has appeared the possibility to obtain a specific ribozyme to degrade the Cot mRNA. This catalytic RNA is an adenine-dependent hairpin ribozyme that specific cleaves “in vitro” the mRNA of Cot/tpl-2 between bp +225 and +266 bp (86, 87). Further studies are required to determine whether this type of compounds or other improved ones will be useful to inhibit specifically the expression of Cot/ tpl-2 “in vivo”.

Conclusion

Cot gene was identified in 1991, and since then we are beginning to understand its physiological role in biological processes. Today, there is also a very clear link between inflammation and cancer and Cot/tpl-2 is a good example of how a protein that is required to control immune responses

properly, playing a role in cell transformation when des-regulated. Now, it is also clear that this gene has a unique role in the development of the immune responses and in inflammation and thus Cot/tpl-2 is emerging as a very interesting target to arise new anti-inflammatory drugs. However, and as it has been discussed here, there are still many questions unresolved before to complete the picture of Cot/tpl-2 activation and function.

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