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INSIGHTS

Sensing danger through a "finger"

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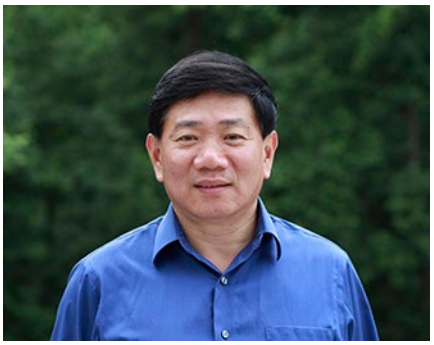
In this issue of *JEM*, the study by Chen et al. (https://doi.org/10.1084/jem.20181031) reveals a previously unrecognized role of cellular nucleic acid–binding protein (Cnbp) as a novel transcriptional regulator of interleukin-12β (IL-12β) transcription and IL-12–driven, Th1-mediated immune responses, which has important implications for both host defense and inflammatory disease.

Cnbp, also called zinc-finger protein 9 (ZNF9), is a highly conserved zinc-finger protein with seven tandem repeats of CCHC zinc-finger knuckles and one arginine-glycine-glycine (RGG) box. This DNA- and RNA-binding protein with broad sequence specificity has been associated with diverse cellular functions, including transcription and translation (Chen et al., 2013; Margarit et al., 2014; Benhalevy et al., 2017), and is linked to embryonic cranial facial development and age-related inflammatory disease sporadic inclusion body myositis. However, the role of Cnbp in immunity has been suggested but not definitively demonstrated and delineated.

In this study, Chen et al. generated mice lacking Cnbp and found that in Cnbp-deficient, bone marrow–derived macrophages exposed to diverse microbial pathogens and pathogen-derived products, surprisingly, the expression of only a restricted subset of genes was altered: IL-12β was the most affected. A concomitant increase in IL-10 production was also observed, which was proven to be inconsequential to the suppressed IL-12β expression by using IL-10R blocking or IL-10–neutralizing antibody. Cnbp-deficient macrophages induced canonical NF-κB/Rel signaling normally but were diminished in their ability to facilitate the nuclear translocation and target gene promoter binding activity of c-Rel, a key driver of IL-12β transcription. To note, Cnbp deficiency in both macrophages and dendritic cells also negatively impacted LPS-induced mRNA expression of IL-12α and IL-23α, which are the dimerization partners of IL-12β to form IL-12 and IL-23, respectively. This is compatible with the observation of c-Rel's preferential and critical role in IL-12α and IL-23α gene transcription (Grumont et al., 2001; Carmody et al., 2007). Consistent with its crucial role in IL-12 production by APCs, Cnbp-deficient mice were more susceptible to acute toxoplasmosis associated with reduced levels of IL-12, as well as an impaired Th1-mediated IFN-γ response essential to controlling parasite replication. Collectively, these findings identify Cnbp as an important regulator of c-Rel–dependent IL-12α and β gene transcription in APCs, IL-12 production, and IL-12–driven T helper type 1 (Th1) cell immunity against an intracellular pathogen and possibly other related microbes.

The synergism between Cnbp and c-Rel on IL-12β gene transcription shown in this study by Chen et al. (2018) was quite remarkable, suggesting close cooperation between the two nuclear factors. The data presented support a model whereby Cnbp facilitates c-Rel nuclear translocation. Cnbp's own nuclear localization requires its phosphorylation and dimerization (Lee et al., 2017). Multiple possible mechanisms are proposed to account for how Cnbp promotes c-Rel's nuclear import. The investigators favor one of the scenarios that CNBP influences the nuclear translocation of c-Rel by indirect mechanisms because of their observation that, although Cnbp and c-Rel could physically interact, only a modest fraction of c-Rel is stably associated with CNBP. In this context, calmodulin (CaM), a highly conserved, ubiquitously expressed Ca(2+) binding protein that serves as a key mediator of intracellular Ca(2+) signals, has been shown



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to differentially regulate the activation of NF-κB/Rel proteins following stimulation. Specifically, CaM binds c-Rel and RelA (p65) after their release from IκB and can inhibit nuclear import of c-Rel while letting RelA translocate to the nucleus and act on its target genes. CaM binding–deficient mutants of c-Rel exhibit increases in both nuclear accumulation and transcriptional activity on the IL-2 and GM-CSF promoters in the presence of a Ca(2+) signal (Antonsson et al., 2003). It is a tantalizing thought that Cnbp may somehow negatively influence CaM's expression or function to facilitate c-Rel's nuclear transport in preference over p65. It is interesting to note in the present study that nuclear localization of p65 was significantly increased in Cnbp-deficient cells 30-min following LPS stimulation (see Fig. S4 E in Chen et al., 2018).

Another potential connection of the Cnbp and c-Rel relationship is through the Wnt–β-catenin signaling pathway. In *Xenopus* development, maternal Wnt/β-catenin signaling establishes a program of dorsal-specific gene expression re-

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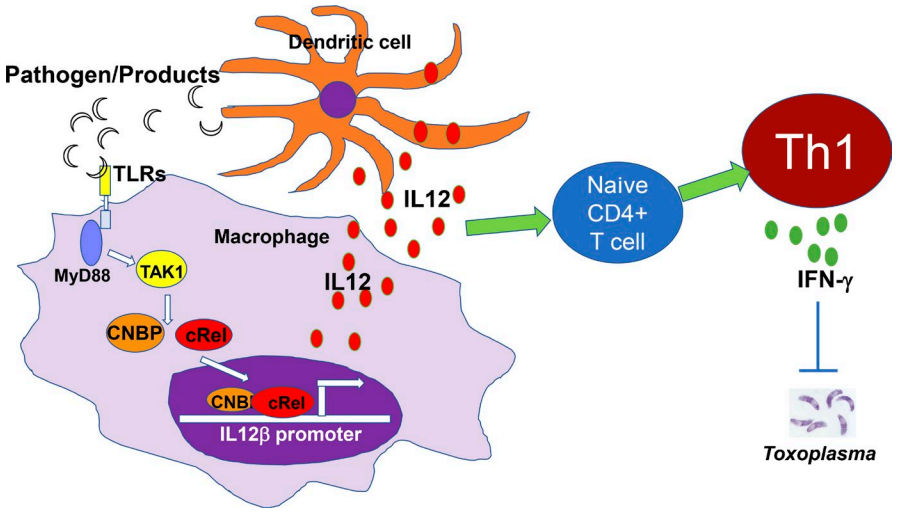

Cnbp regulates IL-12β gene transcription, IL-12 production, and Th1 immunity. Cnbp resides in the cytosol of macrophages and translocates to the nucleus in response to diverse microbial pathogens and pathogen-derived products through the TLR–MyD88–IRAK–TAK1 signaling pathway. Cnbp has a selective ability to control the activation of c-Rel, a key driver of IL-12β gene transcription. The nuclear translocation and DNA binding activity of c-Rel require Cnbp. Furthermore, Cnbp itself binds the IL-12β promoter. Principally through its IL-12–augmenting activity in APCs, Cnbp enhances the Th1-mediated IFN-γ response to acute toxoplasmosis to control parasite replication.

quired for axial patterning. A subset of dorsally expressed genes depends not only on Wnt/β-catenin stimulation, but also on a MyD88-dependent TLR-IL1-R signaling pathway (Armstrong et al., 1998). These two signal transduction cascades converge in the nucleus to coactivate gene transcription in blastulae through a direct interaction between β-catenin and NF-κB proteins (Armstrong et al., 2012). Margarit et al. (2014) used Cnbp single-stranded DNA-consensus binding sequence to identify putative Cnbp target genes present in the human, mouse, chicken, *Xenopus*, and zebrafish genomes. Most of the identified genes are associated with embryonic developmental processes, particularly the Wnt signaling pathway, which might explain Cnbp's requirement for craniofacial development. Further, it has been reported that Wnt5a signaling increases IL-12 secretion by human dendritic cells and enhances IFN-γ production by $CD4^+$ T cells (Valencia et al., 2014). More definitive evidence is needed to establish the convergence of the two important pathways (Wnt and NF-κB) in cell proliferation, apoptosis, inflammation, and immunity.

An analysis of Cnbp expression in normal human tissues at steady state reveals that immune cells in fact express this gene the most, particularly B and T lymphocytes (BioGPS, 2018). This begs for further study of the direct role of Cnbp in these antigen-specific immune effectors. Given the investigators' evidence that Cnbp deficiency has little impact on immune cell development at steady state (Chen et al., 2018), the attention is rationally placed on its influence on lymphocyte functions. Emerging evidence suggests that at least in some aspects of B lymphocyte functions, Cnbp may play a role in connection with c-Rel. c-Rel–deficient mice generate a normal mature B cell repertoire but are impaired in the formation of germinal centers upon T-dependent immunization in a B cell–intrinsic fashion (Pohl et al., 2002). Likewise, in T cell lymphocytes, certain genes essential for immune function such as IL-2 and Foxp3 are directly regulated by c-Rel, which is crucial for optimal IL-2 production and expression of IL-2Rα (CD25; Köntgen et al., 1995). c-Rel complexes are mainly bound to IκBβ, and stimulation via CD28 leads to degradation of IκBβ and activation of c-Rel signaling pathway. Thus, c-Rel–deficient T cells cannot respond robustly to T cell activation signals (Visekruna et al., 2012). It is conceivable and likely that Cnbp has similar activities on B and T lymphocytes in a cell-intrinsic manner during immune responses against certain pathogens. This possibility requires definitive proof using conditional gene deletion approaches.

IL-12 and IL-23, as well as their respectively instigated Th1- and Th17-mediated responses, are prominent targets for clinical intervention in the treatment of certain inflammatory diseases and cancer. This work by Chen et al. (2018) unveils a new target Cnbp which may provide a unique way to specifically restrain the excessive activities. For example, in humans, there are two forms of the degenerative disorder myotonic dystrophy (DM), which is caused by a triplet repeat expansion in the noncoding region of either the *DMPK* (DM1) or *CNBP* (DM2) gene. The early appearance of cataract is a feature of both diseases. Rhodes et al. (2012) found that the up-regulated genes in DM1 and DM2 were highly enriched in both IFN-regulated genes and genes associated with the response to double-stranded RNA and the innate immune response. The characteristic fingerprint of IFN-regulated genes and the signaling pathways identified in diseased lens cells point to a role for double-stranded RNA activation of the innate immune response in the pathology of DM and forms the basis for a novel hypothesis to explain the complex mechanism of the disease involving DMPK or Cnbp. It is intriguing to note that the human *CNBP* gene is also often amplified in prostate, lung squamous, ovarian, cervical, esophagus, and head and neck cancers (The Cancer Genome Atlas data analysis; data not shown). All these cancers have in common an inflammatory underpinning; IL-6 is a major factor promoting it in the tumorigenic processes and their progression. Perhaps not surprisingly, CNBP has been shown to be a transcriptional regulator of IL-6 in inflammatory responses (Lee et al., 2017).

In summary, this study by Chen et al. (2018) helps open a new direction to explore more broadly the role of a previously underappreciated nuclear protein that dynamically integrates several important signaling pathways in embryonic development, cellular proliferation, differentiation, apoptosis, tissue inflammation, tumorigenesis, and host response to infectious assaults. It also has implications for novel therapeutic interventions targeting various Cnbp-related signaling components active in these diseases.

Antonsson, A., et al. 2003. *Mol. Cell. Biol.* 23:1418–1427. https://doi.org/10.1128/MCB.23.4.1418-1427.2003

Armstrong, N.J., et al. 1998. *Mech. Dev.* 71:99–105. https://doi.org/10.1016/S0925-4773(98)00003-3
Armstrong, N.J., et al. 2012. *PLoS One.* 7:e36136. https://doi.org/10.1371/journal.pone.0036136
Benhalevy, D., et al. 2017. *Cell Rep.* 18:2979–2990. https://doi.org/10.1016/j.celrep.2017.02.080
BioGPS. 2018. CNBP (CCHC-type zinc finger nucleic acid binding protein). http://biogps.org/#goto=genereport&id=7555 (accessed October 23, 2018).
Carmody, R.J., et al. 2007. *J. Immunol.* 178:186–191. https://doi.org/10.4049/jimmunol.178.1.186
Chen, S., et al. 2013. *Biochim. Biophys. Acta.* 1830:4769–4777. https://doi.org/10.1016/j.bbagen.2013.06.007
Chen, Y., et al. 2018. *J. Exp. Med.* https://doi.org/10.1084/jem.20181031
Grumont, R., et al. 2001. *J. Exp. Med.* 194:1021–1032. https://doi.org/10.1084/jem.194.8.1021
Köntgen, F., et al. 1995. *Genes Dev.* 9:1965–1977. https://doi.org/10.1101/gad.9.16.1965
Lee, E., et al. 2017. *Nucleic Acids Res.* 45:3280–3296. https://doi.org/10.1093/nar/gkx071
Margarit, E., et al. 2014. *Biochim. Biophys. Acta.* 1839:1151–1160. https://doi.org/10.1016/j.bbagrm.2014.08.009
Pohl, T., et al. 2002. *Proc. Natl. Acad. Sci. USA.* 99:4514–4519. https://doi.org/10.1073/pnas.072071599
Rhodes, J.D., et al. 2012. *Hum. Mol. Genet.* 21:852–862. https://doi.org/10.1093/hmg/ddr515
Valencia, J., et al. 2014. *Immunol. Lett.* 162:188–199. https://doi.org/10.1016/j.imlet.2014.08.015
Visekruna, A., et al. 2012. *Clin. Dev. Immunol.* 2012:239368. https://doi.org/10.1155/2012/239368