Ruprecht-Karls-Universität Heidelberg
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Dissertations-Kurzfassung

The role of nuclear factor of activated T cells 5 (NFAT5) in inflammation and the potential use bifunctional enzyme triggered carbon monoxide releasing molecule (CORMs) in treatment of systemic inflammation

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Tonicity-Responsive Enhancer Binding Protein (TonEBP), also known as nuclear factor of activated T cells 5 (NFAT5), was initially identified as a major transcription factor for osmoregulation. More recently, the role of TonEBP /NFAT5 has been extended as it also regulates the expression of pro-inflammatory cytokines. Hence, TonEBP /NFAT5 may protect cells from shrinking in a hyperosmolar environment but at the same time may evoke inflammation. Even though a number of studies have unequivocally demonstrated that hyperosmolar stress of renal murine inner medullar collecting duct (miMCD) cells leads to nuclear TonEBP /NFAT5 accumulation and induction of TNFα and CCL2 expression, it has not been well studied if in miMCD cells pro-inflammatory mediators likewise initiate nuclear translocation of TonEBP /NFAT5. In the present study we not only attempted to clarify this issue but also studied the possibility of inhibiting iNOS expression by making use of so called bifunctional CO-releasing molecules (CORM). In particular we addressed the following questions:

1. Is TonEBP/NFAT5 activated by mediators of inflammation, i.e. LPS and TNFα in miMCD cells and does this result in regulation of TonEBP/NFAT5 target genes at the mRNA and protein level?
2. Is TonEBP/NFAT5 activation involved in regulation of inflammatory mediators (cytokines and chemokines) in miMCD cells, murine Macrophage (Raw264.7 cell line) and isolated spleen cells from wild-type and TonEBP/NFAT5-/- mice?
3. Does carbon monoxide (CO) regulate TonEBP/NFAT5 activation in Raw264.7 cells? Does it influence iNOS and HO-1 expression?
4. Is there synergy between CO and monomethyl fumarate (MMF) in suppressing inflammation?

This study in essence has shown that 1) TonEBP/NFAT5 regulates inflammatory mediators but is also activated by LPS. 2) Apart from miMCD cells also in murine macrophage TonEBP/NFAT5 regulates the expression of TNFα and CCL2 3) CO effectively inhibits LPS mediated cytokine production in macrophages. This is accompanied by inhibition of iNOS and induction of HO-1. 4) The concurrent application of MMF and CO as so called bifunctional CORM acts synergistic in inhibiting inflammation, iNOS expression and HO-1 induction. In conclusion, this study revealed that TonEBP/NFAT5 is an essential mediator of inflammation in medullar collecting duct cells under hyperosmolar conditions. Also under septic (LPS) conditions TonEBP/NFAT5 is activated and may thus perpetuate local inflammatory responses. The use of bifunctional CORMs, i.e. MMF-CORM, is of potential clinical relevance in the treatment of sepsis as it not only inhibits systemic inflammatory cytokines, but also inhibits iNOS expression. The latter might also be important for preventing urinary concentration problems in septic patients.