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**ASTROGLIAL RESPONSE TO BILIRUBIN INVOLVES ACTIVATION OF THE TNF- $\alpha$  AND NF- $\kappa$ B PATHWAYS****A Fernandes, AS Falcão, C Gordo, MJ Gama, RFM Silva, MA Brito, D Brites.**

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**Background:** Nerve cell injury by unconjugated bilirubin (UCB) is a major complication of neonatal jaundice, occurring by mechanisms not entirely clarified. In previous studies, we have demonstrated that exposure of astrocytes to UCB leads to the release of TNF- $\alpha$  and interleukin (IL)-1 $\beta$ , while inhibiting the secretion of IL-6<sup>1</sup>. Cytokines play an important role in diverse forms of neurodegeneration<sup>2</sup>, and the pro-inflammatory cytokine TNF- $\alpha$  is known to induce the expression of genes involved in the inflammation and immune response. Activation may occur by action of TNF- $\alpha$  on its membrane receptor TNFR1, leading to the translocation of the inducible transcription factor NF- $\kappa$ B from the cytoplasm to the nucleus<sup>3</sup>.

**Aims:** This study aimed to: (i) evaluate the time point of maximum UCB-induced TNF- $\alpha$  release from astrocytes and whether the stimulation depends from TNF- $\alpha$  mRNA expression, (ii) investigate if NF- $\kappa$ B signaling is involved in the release of TNF- $\alpha$  elicited by UCB (iii) determine if activation of astrocytes involves the TNFR1 pathway.

**Methods:** Primary cultures of rat astrocytes were incubated with 50 and 100  $\mu$ M purified UCB and 100  $\mu$ M human albumin for 1, 2, 4 or 8 h, at 37°C. In another set of experiments, cells were pre-incubated for 1 h with 100  $\mu$ M of pyrrolidinedithiocarbamate (PDTC), an NF- $\kappa$ B activity inhibitor, and then treated with UCB for 4 h. Non-treated cells were used as control. Cytokines were assessed by ELISA (R&D Systems). TNF- $\alpha$  mRNA expression was determined by RT-PCR. TNFR1 levels were estimated by Western blot and NF- $\kappa$ B activation was evaluated by immunocytochemistry and slot blot analysis.

**Results:** Interaction of UCB with astrocytes led to a time-dependent release of TNF- $\alpha$  ( $p < 0.05$  from control, at 2 h time period) with maximal response between 4 h (50  $\mu$ M UCB, 63 pg/ml; 100  $\mu$ M UCB, 154 pg/ml) and 8 h incubation (50  $\mu$ M UCB, 97 pg/ml; 100  $\mu$ M UCB, 177 pg/ml). This stimulation was less relevant at the level of TNF- $\alpha$  mRNA expression which increase did not reach significance. Immunoreactivity and blotting for NF- $\kappa$ B was observed at the first hour mainly at the cytoplasmic level, while shifted to the nucleus from 2 h onwards, a finding consistent with the activation of this transcription factor. The involvement of NF- $\kappa$ B on the TNF- $\alpha$  secretion was further corroborated by the 60% PDTC-induced decrease in the release of this cytokine ( $p < 0.01$ ). In addition, the increase of ~90% in TNFR1 levels observed as early as 1 h after incubation, suggests that it precedes NF- $\kappa$ B in the cascade of events leading to the release of TNF- $\alpha$ .

**Conclusions:** Our results provide the first evidence that NF- $\kappa$ B has an important role in the immunostimulant effects of UCB and that TNFR1 is probably one of the first components to be activated in this signal transduction cascade. The identification of the signaling events involved in UCB-induced immunostimulation may open new avenues for preventing brain injury by hiperbilirubinemia.

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References

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