ADIPONECTIN SECRETED BY TUBULAR RENAL CELLS MEDIATES THE CELLULAR INFLAMMATORY DAMAGE LPS-INDUCED

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BACKGROUND AND AIM

Most studies report elevated levels of adiponectin (ADPN) in patients with inflammatory and immune-mediated pathologies, although the exact mechanisms leading to this increase and the specific role of ADPN in the pathophysiology of these conditions remain to be elucidated. Studies performed using animal models, demonstrate that ADPN deficiency protects against acute kidney injury. We reported that human tubular renal cells (HK-2) secrete ADPN which levels increase upon lypopolisaccaride (LPS)-exposure. Therefore the aim of the present study is to investigate the role of ADPN secreted by HK-2 cells in the inflammatory damage exerted by LPS.

MATERIALS AND METHODS

In HK-2cells, untreated and treated with LPS (10μg/ml) ADPN, TNFa, IL-6 and MCP-1 mRNA levels were evaluated by real time PCR (RT-PCR); ADPN, NFkB, total and phosphorylated JNK protein levels by Western blot analysis (WB); cells vitality by MTT assay and apoptosis by ladder-assay.

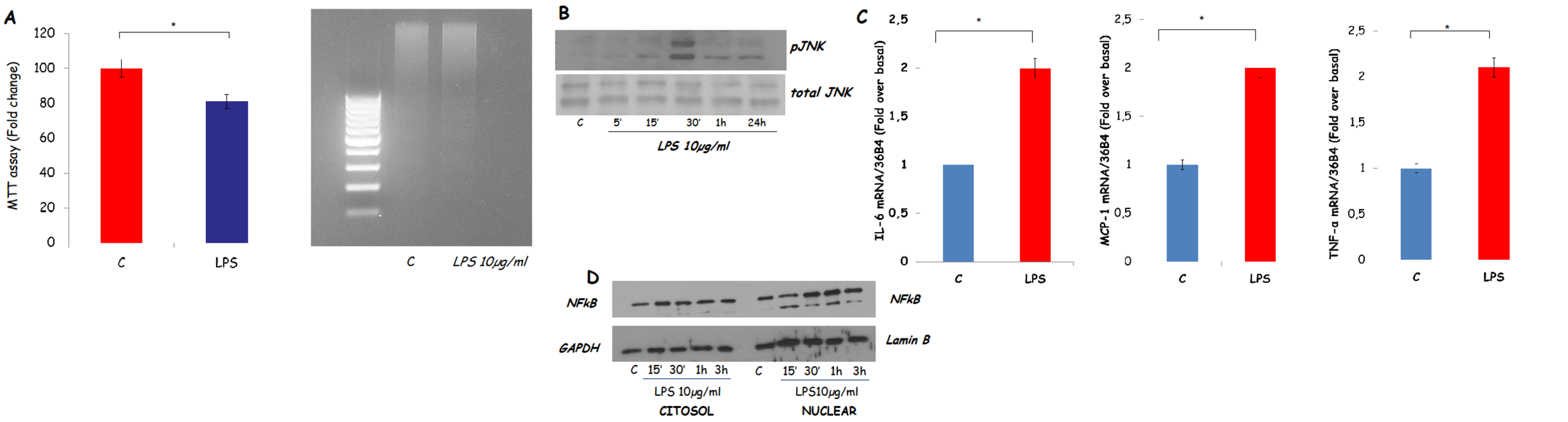

Fig 1 In HK-2 cells LPS exposure increases gene expression of pro-inflammatory chemokines and cytokines together with a nuclear translocation of NFkB: (A) MTT growth assay (Left) and DNA-ladder assay (Right) in HK-2 cells untreated (C) or treated for 72 h with LPS 10μg/ml. **(B)** Levels of phosphorylated (p) JNK (Thr183/Tyr185) and total non-phosphorylated protein (total JNK) were measured by WB in cellular extracts obatained from HK-2 cells untreate (C) or treated at the indicated time with LPS 10μg/ml. β-Actin was used as loading control. (C) IL-6, MCP-1 and TNF-a mRNA expression in HK-2 cells untreated (c) or treated for 24 h with LPS 10μg/ml. The histograms show the quantitative representation of data (mean ± SD) of three independent experiments after correction for 36B4 mRNA expression. (D) All the experiments were executed in triplicate and one of three similar experiments is presented. *p<0.005 versus control.

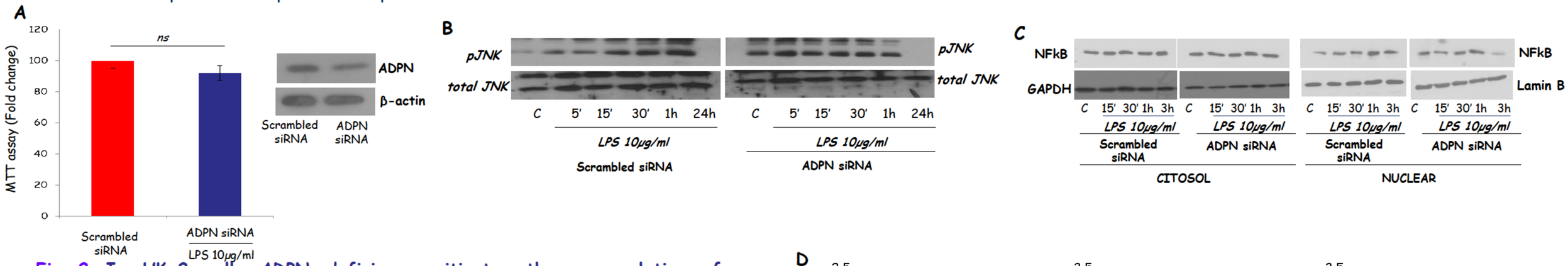

Fig 2 In HK-2 cells ADPN deficiency mitigates the up-regulation of pro-inflammatory cytokines LPS-induced (A) MTT growth assays in HK-2 cells transfected with scrambled siRNA (Sc siRNA) or with targeted human ADPN mRNA sequence (ADPN-siRNA) and untreated (C) or treated for 72 h with LPS 10μg/ml. **(B)** Levels of phosphorylated (p) JNK (Thr183/Tyr185) and total non-phosphorylated protein (total JNK) were measured by WB in cellular extracts obatained from HK-2 cells transfected with scrambled siRNA (Sc siRNA) or with ADPN-siRNA and untreated or treated with LPS 10μg/ml at the reported time. (**C**) Levels of NFkB citosol (left) and nuclear (right) protein were measured by WB in cellular extracts obatained from HK-2 cells transfected with scrambled siRNA or with ADPN-siRNA and untreated or treated with LPS 10μg/ml at the reported time. GAPDH and Lamin B were used as loading control. (**D**) TNF-a, MCP-1 and IL-6 mRNA expression in HK-2 cells transfected with scrambled siRNA (Sc siRNA) or with ADPN-siRNA and untreated or treated with LPS 10μg/ml for 24 h. The histograms show the quantitative representation of data (mean ± SD) of three independent experiments after correction for 36B4 expression. All the experiments were executed in triplicate and one of three similar experiments is presented. *p<0.005 versus control; ns= not significant.

RESULTS

MTT assay revealed that LPS exposure decreases HK-2 cells vitality but not induces apoptosis as demonstrated by DNA-laddering assay. WB and RT-PCR shows that LPS-treatment induces the up-regulation of pJNK and TNFa, IL-6 and MCP-1 increased-mRNA levels. WB performed in cytosolic and nuclear proteins extract from cells treated with LPS revealed the NFkB nuclear translocation. Interestingly, in cells treated with LPS the chemical inhibition of ADPN by silencing approaches (siRNA) reversed the reduced cells vitality together with the phosphorylation status of JNK. In addition we observed that in HK-2 cells knocked-down for ADPN gene, the up-regulation of the cytokines and chemokine LPS-induced and the NFkB nuclear translocation were reversed.

CONCLUSIONS

Our results suggest that ADPN secreted by HK-2 cells during LPS exposure, mediates the inflammatory cellular damage increasing IL-6, TNFa and MCP-1 mRNA levels, via NFkB.

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