RIP2 mediates LPS-induced IL-12 p40 production in human monocyte-derived dendritic cells *

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INTRODUCTION

IL-12, the critical factor for the generation of the Th1 type immune response, is produced by dendritic cells (DCs) upon stimulation with LPS. Different signal pathways mediate LPS-induced expression of IL-12 and involve PI3K, MAPKs and the transcription factor NF-κB. Here we investigated the signalling events of DCs treated by LPS and asked whether the activation of the Raf/RIP2 pathway is involved in the expression of IL-12.
Using specific inhibitors of the MAPK pathways we investigated the gene regulation of the IL-12 subunit p40 and analysed the effects of LPS on the phosphorylation and activation of p38 MAP kinase, ERK1/2, IκBα and RIP2 in activated human DCs. In order to assess the role of RIP2 in LPS-induced IL-12 gene regulation we used small interfering RNA to knock down RIP2

Raf but not MEK is involved in LPS-induced IL-12 p40 gene expression

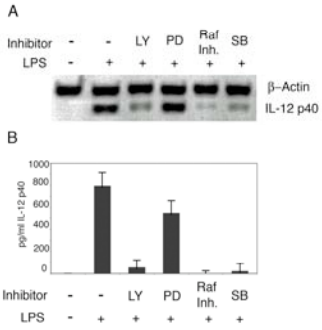

Fig. 1: Effect of different protein kinase inhibitors on IL-12 p40 expression. A, DCs were pretreated for 30 minutes with the inhibitors as indicated and then stimulated with LPS for 6 h. Total RNA was extracted using the TRIzol method. RT-PCR was performed to assess the expression of IL-12 p40 and β-actin. B, Secretion of IL-12 p40 into the cell culture medium was measured by ELISA after treatment with inhibitors for 30 min and activation with LPS for 16 h.

Raf mediates LPS-induced phosphorylation of p38 MAPK and IκBα

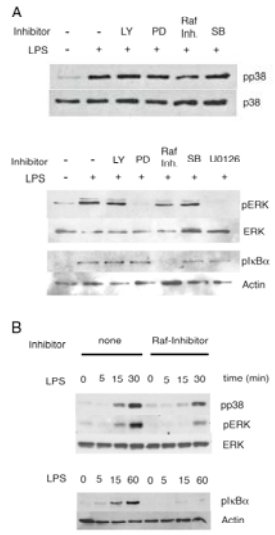

Fig. 2: Raf mediates phosphorylation of p38, ERK, and IkBα in DCs stimulated with LPS. A, DCs were pretreated with inhibitors for 30 min, and then stimulated with LPS for 30 min (upper part) or 60 min (lower part), respectively. Total cell lysates were immunoblotted with phospho-specific antibodies against p38, IkBa and ERK. For loading control membranes were reprobed with antibodies against p38, actin, and ERK. B, DCs were pretreated with the Raf-Inhibitor and stimulated with LPS for the indicated time. Total cell lysates were blotted with phospho-specific antibodies for p38, ERK and IkBα. As loading control the membrane was reprobed with anti-ERK and anti-actin.

Raf mediates LPS-induced autophosphorylation of the kinase RIP2

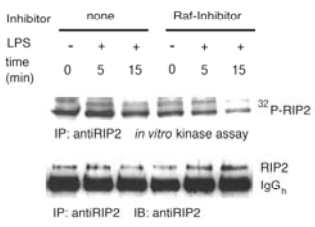

Fig.3: LPS-induced RIP2 kinase activity is regulated by Raf. DCs were pretreated with or without the Raf-Inhibitor and stimulated with LPS for the time indicated. Endogenous RIP2 protein was affinity-purified with anti-RIP2 antibody and in vitro kinase assays were performed. Reaction products were run on a SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane was exposed with a PhosphoImager to detect autophosphorylation of RIP2 (upper panel) and probed with anti-RIP2 antibody for RIP2 protein level (lower panel).

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Knock-down of RIP2 inhibits IL-12 p40 gene expression

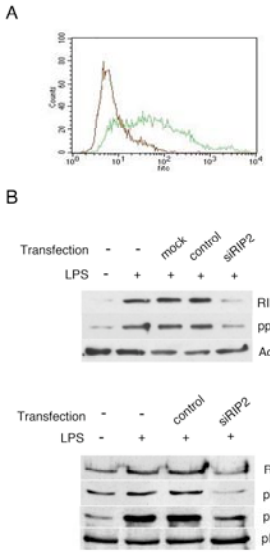

Fig. 4: Knockdown of RIP2 in DCs. (A) DCs were transfected with unlabelled (red curve) or with FITC-labelled control siRNA (green curve). 48 h after transfection DCs were starved for 2 h and then treated for 6 h with LPS – the assay conditions used in the following experiments. Transfection efficiency with labelled-siRNA was assessed by flow cytometric analysis. (B) DCs were transfected with 150nM of siRNA-RIP2 (siRIP2), 150nM of an irrelevant siRNA (control), transfection agent alone (mock) or left untreated (-). 48 h after transfection DCs were starved for 2 h and activated with LPS for 6 h. Cell lysates were analysed for expression of RIP2 and phosphorylation of p38 MAPK, ERK and IkBa. Level of actin and PI3K expression served as loading control.

Knockdown of RIP2 inhibits IL-12 p40 gene expression

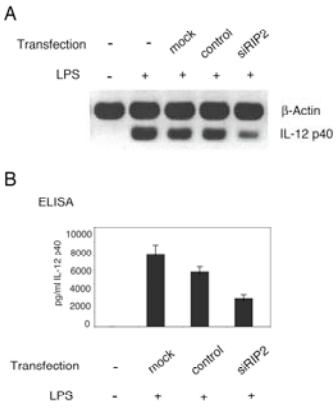

Fig. 5: Knockdown of RIP2 inhibits IL-12 p40 expression. (A) DCs were transfected with siRNA as indicated and stimulated for 6 h with LPS. Total RNA was extracted by TRIzol and RT-PCR was performed to monitor expression of IL-12 p40 and β-actin. (B) To test for the amount of secreted IL-12 p40 the cell culture medium was collected 16 h after treatment with LPS and secreted IL-12 p40 was measured by ELISA.

Model for LPS-induced IL-12 production in human DCs via RIP2

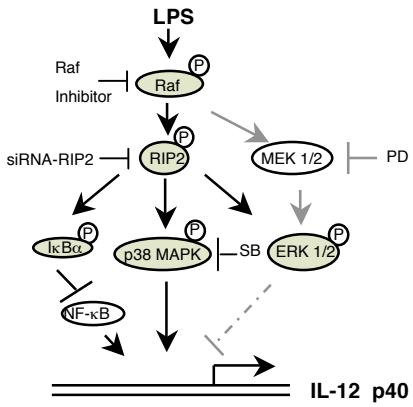

Fig. 6: LPS activates the kinase Raf and its effector RIP2. RIP2 is a branchpoint that regulates different signal pathways involved in stimulation of the expression of IL-12 p40. Raf also phosphorylates ERK via MEK, but this pathway is not involved in the expression of IL-12. ERK has no effect or even mediates a negative feedback. Inhibitors used in this study are indicated.

CONCLUSION

We demonstrate that the Raf effector, RIP2, is a main regulator for signal pathways involved in IL-12 production of DCs and therefore is important to mediate innate immunity. Similar mechanisms may be involved in the expression of other cytokines. Apart from its function in DCs RIP2 also plays an essential role in proliferation and differentiation of T cells . Further characterization of RIP2 and its direct upstream and downstream elements may help to find methods for immune intervention.