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Analysis of the interplay between Toll-like receptor and TGF-beta signalling pathways in human primary macrophages under metabolic conditions

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Diabetes is one of the most common metabolic diseases worldwide, characterized by hyperglycaemia (HG) and dyslipidemia. Macrophage-mediated chronic inflammatory responses accelerate the progression of type 2 diabetes and its complications. The activation of TLR and TGFβ1 signalling pathways in macrophages is closely associated with the development of chronic inflammation. TLR-mediated inflammation is linked to cardiovascular events caused by dyslipidaemia, while TGFβ1 pathway dysregulation triggers complications like diabetic nephropathy, posing challenges for targeted diabetes therapy due to their complex signalling. The crosstalk between TLR and TGFβ1-mediated pathways in macrophages in metabolic conditions is an open question. A deeper understanding of how HG impacts these pathways in macrophages is crucial for improving diabetes and its vascular complications treatment. Aims of the thesis project included: 1) to identify the effect HG on the TLR system in macrophages; 2) to analyse the effects of dyslipidaemic components on macrophages in HG conditions; 3) to investigate the impact of HG and dyslipidaemia on the TGFβ1/SMAD signalling pathway in M(IL4); 4) to investigate the transcriptional response induced by TGFβ1 and TLR ligand PAM3CSK4 in macrophages under hyperglycemic conditions. Primary monocytes were isolated from buffy coats and cultured in SFM medium supplemented with M-CSF (5 ng/ml) under NG(5mM glucose) and high glucose (HG, 25 mM) conditions for 6 days. Stimulation with 1 µg/ml of LPS and 10 ng/mL of PAM3CSK4was performed on day 6 of macrophage cultivation for 24 h. 10 ng/ml of TGFβ1 was added for 3 h. RT-PCR showed that HG enhanced expression of TLR1 and TLR8 in M(NC), TLR 2 and 6 in M(IFNγ), and TLR4 and TLR5 in M(IL4). The expression level of TLR4 in HG/M(IL4) was significantly upregulated. HG amplified the response of M(IL4) to LPS by significantly enhancing IL1β and modestly suppressing IL10 production. HG in combination with PAM3CSK4, amplified expression of TLR4, and production of IL1β. In HG conditions, the phosphorylation process of SMAD2/3 but not SMAD1/5 was inhibited. The level of p-SMAD1/5 was decreased significantly after pre-treatment with PAM3CSK4, while p-SMAD2/3 was not affected. The candidate mechanism for the PAM3CSK4/ TLR1/2 mediated suppression of pSMAD1/5 found in this study is the Smad6-dependent ubiquitination and degradation of the adapter protein MyD88. NGS data showed that the addition of PAM3CSK4 reverses the anti-inflammatory function of TGFβ1 and promotes the expression of CXCL9, CXCL12 and IL32, making the inflammatory response of M(IL4) more severe. KEGG enrichment analysis and Western Blot results both showed that the combination of PAM3CSK4 and TGFβ1 significantly promoted the activation of the NF-κB pathway. In summary, HG was identified as a sensitizer of tolerogenic macrophages to the dyslipidaemic ligands by increasing expression of TLRs. TGFβ1 promoted the pro-inflammatory effect of the dyslipidaemic ligand in hyperglycaemic macrophages. TLR and TGFβ1 pathway can cross-talk in M(IL4) via competition for the Smad6, affecting Smad6-dependent MyD88 ubiquitination and degradation. Identification of the pro-inflammatory crass-talk between TLR and TGFβ1 signalling pathways in macrophages opens perspective for the identification of new molecular targets in diabetic vascular complications.