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Short communication

TLR7-mediated activation of XBP1 correlates with the IFNα production in humans

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ABSTRACT

The transcription factor X-box binding protein 1 (XBP1) represents a key component of the endoplasmatic reticulum (ER) stress response and is required for the production of several pro-inflammatory cytokines. XBP1 is furthermore essential for the development and survival of plasmacytoid dendritic cells (pDCs), and has recently been suggested to be involved in toll-like receptor (TLR) 2/4 signaling. Activation of TLR7 on pDCs results in an upregulation of pro-inflammatory cytokines, such as type I interferons (IFN-I), and has been implicated in several autoimmune and inflammatory diseases, but the role of XBP1 in this process remains unknown. Here, we show that signaling via TLR7 leads to an upregulation of XBP1 and IFNα-production. XBP1 mRNA expression levels positively correlated with the production of IFNα, while blocking of XBP1 mRNA splicing significantly reduced mRNA transcripts of IFNα. In conclusion, these data suggest a central role of XBP1 in TLR7-induced IFNα production and identify XBP1 as a potential novel therapeutic target in IFNα-driven autoimmune and inflammatory diseases.

1. Introduction

X-box binding protein 1 (XBP1) plays an important role in the development of plasmacytoid dendritic cells (pDCs) [1]. Furthermore, XBP1 represents a central transcription factor of the endoplasmatic reticulum (ER) stress response. ER stress primes cells to respond to innate immune stimuli leading to the transcriptional activation of type I IFN genes [5]. Recent studies showed that toll-like receptor (TLR) 2 and TLR4 signaling specifically activate the inositol requiring kinase 1 α (IRE1α) – XBP1 pathway, which in turn increases the expression of interleukin (IL) 6, tumor necrosis factor (TNF) α and interferon (IFN) β [2–4]. The role of XBP1 in the induction of inflammatory responses by pDCs remains unknown. Stimulation of pDCs through TLR7 triggers a variety of cellular responses, including the production of type I IFNs. In many inflammatory diseases, e.g. systemic lupus erythematosus, TLR7-mediated IFNα production has been shown to lead to chronic inflammation [6]. The better understanding of signaling events downstream of TLR7 might therefore enable the identification of novel targets for the manipulation of TLR7-mediated IFNα production and IFNα-driven autoimmune and inflammatory diseases. Here we investigated the involvement of XBP1 in the TLR7 signaling pathway and the subsequent production of IFNα in humans.

2. Material and methods

2.1. Study subjects

Healthy participants were recruited at the University Hospital Hamburg-Eppendorf, Germany. The study was approved by the ethical commission of the *Ärztekammer Hamburg*. A total number of 22 participants were enrolled into this study. The mean age was 30.8 years. 50% of participants were female. All subjects gave informed consent prior to enrollment.

2.2. Stimulation

EDTA-blood was processed within one hour after venipuncture to prevent loss of responsiveness to stimulation. Isolated cells were stimulated in the following conditions for 2 h at 37 °C: 1 μg/mL of TLR7 ligand CL097 (Invivogen, San Diesgo, CA, US), 1 μg/mL of TLR2

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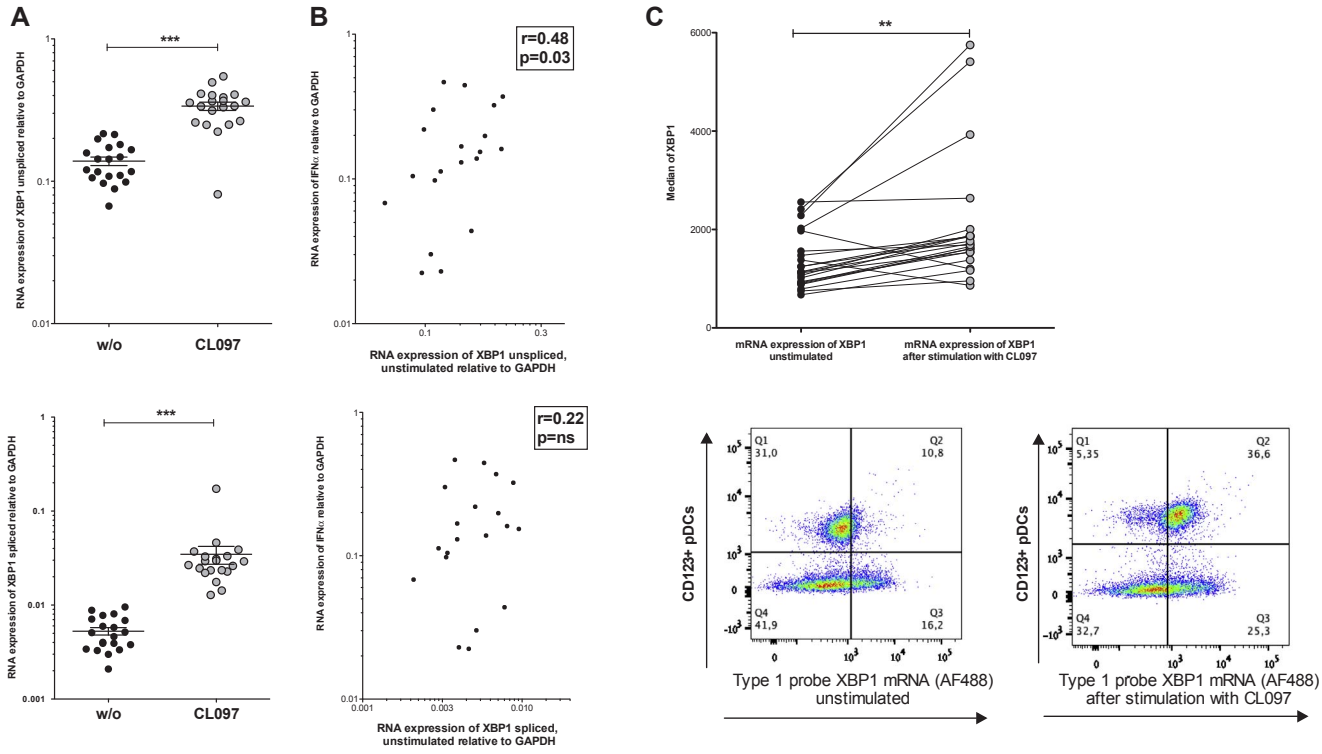

Fig. 1. mRNA expression levels of XBP1 and IFNα in PBMCs and pDCs stimulated with CL097. **(A)** Higher expression levels of unspliced XBP1 mRNA relative to GAPDH in PBMCs after stimulation with CL097 (n = 20, p = 0.0001, Wilcoxon matched-pairs signed rank test). Higher expression levels of spliced XBP1 mRNA relative to GAPDH after stimulation with CL097 (n = 20, p = 0.0001, Wilcoxon matched-pairs signed rank test). **(B)** IFNα mRNA expression in PBMCs follows stimulation with CL097 significantly correlated with the expression levels of unstimulated, unspliced XBP1 mRNA (n = 20; p = 0.03, Spearman rank-based correlation). IFNα mRNA expression follows stimulation with CL097 did not significantly correlate with the expression levels of unstimulated, spliced XBP1 mRNA (n = 20; p = ns, Spearman rank-based correlation). **(C)** Higher expression of *in situ* XBP1 mRNA in pDCs using a PrimeFlow® RNA Assay. Upper row: unstimulated XBP1 mRNA expression versus XBP1 mRNA expression after two hours of stimulation with CL097 (n = 22; p = 0.001; Wilcoxon matched-pairs signed rank test). Lower row: Representative dot plot of *in situ* XBP1 mRNA expression (type1 probe AF488) in pDCs assessed by flow cytometry. Left picture: unstimulated XBP1 mRNA expression (Q2, 10.8%); right picture: XBP1 mRNA expression after two hours of stimulation with CL097 (Q2, 36.6%).

ligand heat killed *Staphylococcus aureus* (Invivogen, San Diego, CA, US), 1 µg/mL of TLR2/4 ligand lipopolysaccharide (LPS) (Sigma-Aldrich Chemie GmbH, Munich, Germany), 500 µM of TLR9 ligand CpG ODN2216 (Invivogen, San Diego, CA, US) or 1 µg/mL IFNα (PeproTech, Hamburg, Germany), as indicated. Cells were maintained in RPMI1640 supplemented with 10% heat-inactivated FBS.

2.3. Blocking of XBP1

The splicing of XBP1 mRNA was blocked by the IRE1α endoribonuclease inhibitor 4µ8C (TOCRIS Bioscience, United Kingdom). 4µ8C was dissolved in dimethylsulfoxid (DMSO) according to manufacturer's instruction. Two million PBMCs per milliliter were incubated for two hours at 37 °C in the presence of either 60 µM 4µ8C, 1 µg/mL CL097, 60 µM 4µ8C with 1 µg/mL CL097 or left untreated. DMSO only was used as a negative control.

2.4. In situ XBP1 mRNA expression assay by flow cytometry

Five million PBMCs were plated and surface stained for 30 min on ice. Cells were subjected to the QuantiGene FlowRNA assay (affymetrix, eBiosciences, San Diego, CA) as per manufacturer's instruction and already published [7] with type6-IFNα probe, type1-XBP1 probe and type4-*B2M* probe (probes were all from affymetrix, eBiosciences). The bacterial *DapB* probes were used as a control. pDCs were identified as CD3/CD14/CD19/CD11c^{neg}HLA-DR/CD123^{bright} cells. Samples were run in duplicates and acquired on the BD LSRFortessa within two hours after staining. The median of XBP1, IFNα and B2M probes was analyzed using FlowJo software.

2.5. Real-time PCR

RNA was purified using TRIzol (Life Technologies, Carlsbad, CA; US)/chloroform ultrapure (Applichem, Darmstadt, Germany) and RNeasy Mini kit (Qiagen, Hilden, Germany) according to manufacturer's instruction. cDNA was prepared using qScript™ cDNA SuperMix (Quanta BioSciences, Gaithersburg, Maryland, US) according to manufacturer's instruction. PCR was performed using Quanti fast SYBR green (Qiagen). Samples were run in duplicate on the LightCycler® 480 System (Roche, Basel, Switzerland) and values were calculated in relation to housekeeping gene GAPDH.

2.6. Western blot analysis

PBMCs were stimulated for 20 h at 37 °C in absence or presence of CL097 (1 µg/mL). SDS-PAGE and immunoblotting were performed from 30 µg of extracted proteins. XBP1s protein levels were assessed, using anti-XBP1s antibody (Biolegend, San Diego, USA) and normalized to tubulin expression.

2.7. Statistical analyses

We used two-tailed paired Wilcoxon rank tests to determine statistical significance between the groups. Linear regression was calculated with Spearman rank-based correlation. A p-value < 0.05 was considered as significant.

3. Results and discussion

Recent studies described that signaling via TLR2 and TLR4 can

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