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TBK1: A key regulator and potential treatment target for interferon positive Sjögren's syndrome, systemic lupus erythematosus and systemic sclerosis

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ABSTRACT

Objective: Upregulation of type I interferons (IFN-I) is a hallmark of systemic autoimmune diseases like primary Sjögren's syndrome (pSS), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). Expression of IFN-I is induced by three different receptor families: Toll-like receptors (TLRs), RIG-like receptors (RLRs) and DNA-sensing receptors (DSRs). TANK-binding kinase (TBK1) is an important signaling hub downstream of RLRs and DSRs. TBK1 activates IRF3 and IRF7, leading to IFN-I production and subsequent induction of interferon stimulated genes (ISGs). The objective of this study was to explore the potential of BX795, an inhibitor of TBK1, to downregulate IFN-I activation in pSS, SLE and SSc.
Methods: TBK1, IRF3, IRF7 and STAT1 were determined by RT-PCR in PAXgene samples and phosphorylated-TBK1 (pTBK1) was analyzed by flowcytometry in plasmacytoid dendritic cells (pDCs) from IFN-I positive (IFNpos) patients. Peripheral blood mononuclear cells (PBMCs) of pSS, SLE and SSc patients and TLR7 stimulated PBMCs of healthy controls (HCs) were cultured with the TBK1 inhibitor BX795, followed by analysis of ISGs.
Results: Increased gene expression of TBK1, IRF3, IRF7 and STAT1 in whole blood and pTBK1 in pDCs was observed in IFNpos pSS, SLE and SSc patients compared to HCs. Upon treatment with BX795, PBMCs from IFNpos pSS, SLE, SSc and TLR7-stimulated HCs downregulated the expression of the ISGs MxA, IFI44, IFI44L, IFIT1 and IFIT3.
Conclusions: TBK1 inhibition reduced expression of ISGs in PBMCs from IFNpos patients with systemic autoimmune diseases indicating TBK1 as a potential treatment target.

1. Introduction

In systemic autoimmune diseases like primary Sjögren's syndrome (pSS), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) upregulation of type I interferons (IFN-I) is a hallmark [1–3] and potential treatment target. Systemic upregulation of IFN-I is present in 50–90% of the patients with pSS, SLE and SSc as determined by various methods in different cohorts of patients [1–5]. Plasmacytoid dendritic cells (pDCs) produce IFN-I in response to RNA- and DNA-containing immune complexes (ICs) activating the endosomal toll-like receptors (TLR) 7 and 9. IFN-I expression can also be induced by RIG-like receptors (RLRs) and DNA-sensing receptors (DSRs) upon activation by cytosolic nucleic acids (RNA/DNA). A dysregulated expression of the RLRs RIG-I and MDA5 in IFN-I positive (IFNpos) pSS patients was previously described by us [6]. In lupus nephritis and glands of pSS patients, the expression of endogenous nucleic acids encoded by a virus-like

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element correlated with IFN-I activation, indicating a contribution of RLRs and DSRs to IFN-I activation [7]. Gain of function mutations in the nucleic acid-sensing routes in interferonopathies like Aicardi-Goutières also support a role for nucleic acids-sensing receptors in systemic IFN-I activation [8,9].

Tumor necrosis factor (TNF) receptor-associated factor NF-κB activator (TANK)-binding kinase 1 (TBK1) is a kinase downstream of the RLRs and DSRs. TBK1 is a non-canonical IκB kinase (IKK) which requires, just like its closely related structural homologue IKKε, phosphorylation at Ser^{172} to become activated. Activated TBK1 and IKKε phosphorylate interferon regulator factor (IRF) 3 and 7 followed by translocation to the nucleus and subsequent induction of transcription and production of IFN-I. IFN-I can then bind to the receptor of IFN-I (IFNAR), which is present on immune cells, and via the JAK-STAT pathway lead to induction of interferon stimulated genes (ISGs) [10,11]. Interestingly, among those ISGs are RLRs and DSRs indicating a close interplay between the various IFN-I inducing pathways (Fig. 1A). Additionally, IKKε has been implicated to be involved in inducing STAT1 phosphorylation downstream of the IFNAR [12].

Currently, trials targeting the IFNAR in SLE show encouraging results and support the pathogenic role of IFN-I [13]. Blocking more upstream the actual transcription of IFN-I by inhibition of TBK1, as a signaling hub irrespective of the route of activation, might potentially be a better treatment target. Interestingly BX795, a molecule which inhibits TBK1 and IKKε, has recently been shown to inhibit IFN-I production and signaling in human PBMCs with a mutation-induced interferonopathy [8]. Here we hypothesize that in IFNpos autoimmune diseases like pSS, SLE and SSc, phosphorylation of TBK1 (pTBK1) is upregulated due to activation of RLRs and/or DSRs. Inhibition of TBK1 activity could downregulate IFN-I production.

2. Patients and methods

2.1. Patients and controls

Healthy controls (HCs) and patients with a positive diagnosis for pSS according to 2002 American-European Consensus Group

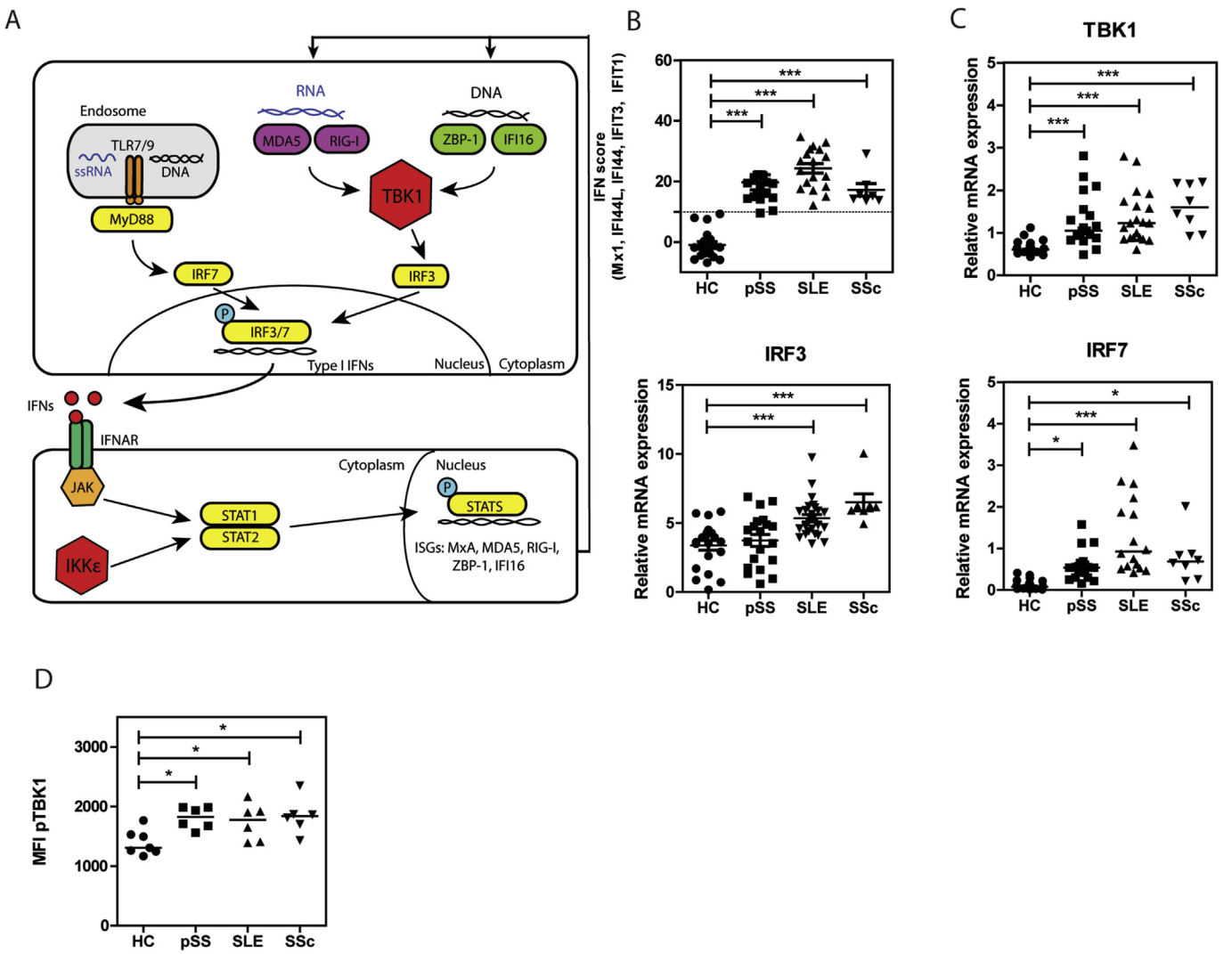

Fig. 1. Systemic activation of TBK1 in IFN type I positive autoimmunity. **(A)** Simplified scheme of the IFN type I inducing pathways and the signaling hub TBK1/IKKε (in red), which can be targeted by the inhibitor BX795. **(B)** IFN scores of IFN type I signature positive pSS, SLE, SSc patients and healthy control (HC) tested in this study. Line indicates cut-off value between IFN positive and negative. **(C)** Gene expression of TBK1, IRF3 and IRF7 was determined in IFN type I signature positive pSS (n = 20), SLE (n = 20), SSc (n = 8) patients and healthy controls (n = 20). **(D)** Protein expression of phosphorylated-TBK1 (pTBK1) in blood-derived plasmacytoid dendritic cells of pSS (n = 6), SLE (n = 6), SSc (n = 6) patients and healthy controls (n = 7). Expression of pTBK1 was calculated as 'pTBK1-specific staining (MFI)'-'isotype control (MFI)'. For three or more group comparisons Kruskal-Wallis was used. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

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