Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Bruton's tyrosine kinase regulates TLR7/8-induced TNF transcription via nuclear factor-κB recruitment

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ARTICLE INFO

Article history:
Received 5 March 2018
Accepted 19 March 2018
Available online xxx

Keywords:
Macrophages
Bruton's tyrosine kinase
Toll-like receptors-7/8
R848
NFκB

ABSTRACT

Tumour necrosis factor (TNF) is produced by primary human macrophages in response to stimulation by exogenous pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) via Toll-like receptor (TLR) signalling. However, uncontrolled TNF production can be deleterious and hence it is tightly controlled at multiple stages. We have previously shown that Bruton's tyrosine kinase (Btk) regulates TLR4-induced TNF production via p38 MAP Kinase by stabilising TNF messenger RNA. Using both gene over-expression and siRNA-mediated knockdown we have examined the role of Btk in TLR7/8 mediated TNF production. Our data shows that Btk acts in the TLR7/8 pathway and mediates Ser-536 phosphorylation of p65 RelA and subsequent nuclear entry in primary human macrophages. These data show an important role for Btk in TLR7/8 mediated TNF production and reveal distinct differences for Btk in TLR4 versus TLR7/8 signalling.

1. Introduction

TNF production is precisely regulated at both the gene and protein expression level [1]. Toll-like receptors (TLRs), by recognising ligands as diverse as bacterial cell wall components and nucleic acids, are important inducers of TNF production in disease. In addition, recognition of endogenously derived damage-associated molecular patterns (DAMPs) makes them key players in the induction and maintenance of autoimmune inflammation [2].

Non-receptor tyrosine kinases play a major role in TLR signalling [3–5], and in particular, Bruton's Tyrosine Kinase (Btk), a member of the Tec family of non-receptor protein tyrosine kinases (PTKs), is a crucial regulator of TLR induced TNF production [6,7]. In humans, a lack of functional Btk leads to X-linked agammaglobulinemia (XLA), a condition characterised by both B cell deficiency and ineffective immune responses to bacterial and viral challenge [8]. XLA patient monocytes show reduced production of TNF and IL-1β in response to TLR2 and TLR4 ligands [9,10] and stimulation of XLA-derived dendritic cells with siRNA results in significantly decreased production of both TNF and IL-6 [11]. Btk deficiency in B cells reduces TLR9-induced production of IL-10, leading to elevated levels of TNF, IL-6 and IL-12p40 [12,13] a finding that may explain the increased levels of cytokines present in XLA serum [14].

In HEK293 cells Btk physically interacts with the cytoplasmic Toll/IL-1 receptor (TIR) domains of TLRs 4, 6, 8 and 9 as well as the adaptor molecules Myd88 and Myd88-adapter-like (Mal) [15]. Following stimulation, TLR receptors (except TLR3) recruit Myd88 via its cytoplasmic Toll/IL-1 receptor (TIR) domain. Various other molecules including IL-1 receptor-associated kinases 1 and 4 (IRAKs 1 and 4), TNF receptor associated factor (TRAF) 6, TAB2/3 and TAK1 then associate with the receptor complex. IκB is phosphorylated by the TAK1-activated IκB kinase (IKK) complex, ubiquitinated and degraded by the 26S proteasome. Following NFκB release from the inhibitory IκB complex, p65RelA is phosphorylated on a number of serines to regulate p65RelA nuclear translocation and gene transactivation [16]. NFκB is considered to be essential for TNF transcription, and over-expression of IκBα decreases TNF production from LPS-stimulated human primary macrophages [17].

Here we provide evidence for Btk in TLR7/8 signalling in human primary macrophages. Btk regulates TLR7/8-induced TNF production at early time points via the 3'enhancer region of the TNF gene. Moreover, we show that Btk controls the initiation of TNF

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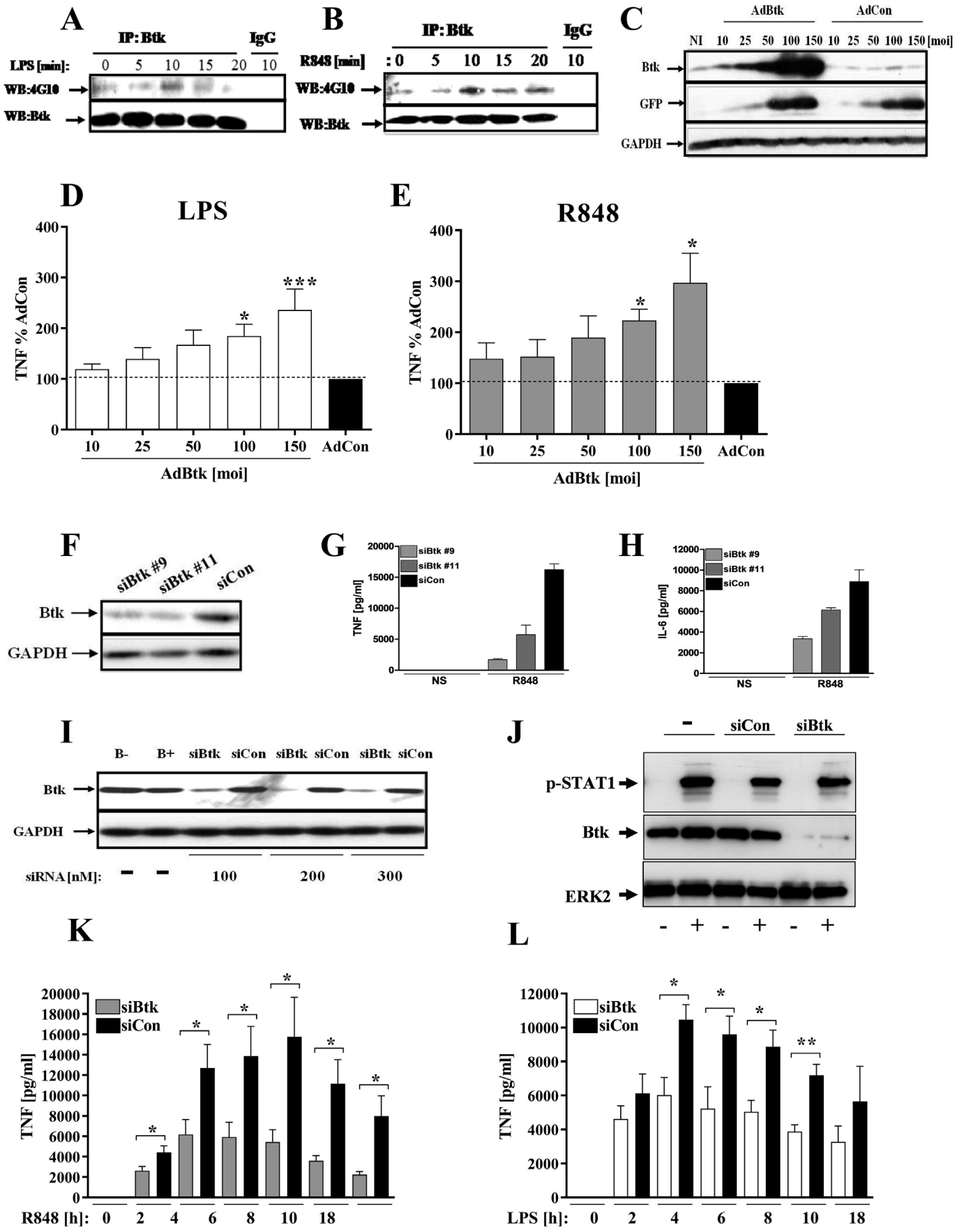
https://doi.org/10.1016/j.bbrc.2018.03.140



Please cite this article in press as: T.H. Page, et al., Bruton's tyrosine kinase regulates TLR7/8-induced TNF transcription via nuclear factor-κB recruitment, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.140