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TNFR1-induced NF-κB, but not ERK, p38MAPK or JNK activation, mediates TNF-induced ICAM-1 and VCAM-1 expression on endothelial cells

Zhigang Zhou, Michelle C. Connell, David J. MacEwan *

School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

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Abstract

Tumour necrosis factor (TNF) is a pro-inflammatory cytokine, whose primary targets include vascular endothelial cells. TNF-mediated adhesion molecule expression has been shown to play a central role in endothelial cells inflammatory responses and disorders such as atherosclerosis. However it is not fully understand how the TNF receptor subtypes, namely TNFR1 and TNFR2, regulate inflammatory responses in endothelial cells. The aim of this study was to elucidate the kinase signalling pathways that TNF receptors activate, and determine the pathways responsible for downstream expression of adhesion molecules, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in human endothelial cells. Using human umbilical vein endothelial cells (HUVEC), we demonstrated that TNF activates a range of mitogen-activated protein kinases (MAPKs), including the extracellular-regulated kinase (ERK) pathway and the p38MAPK and c-*Jun N*-terminal kinase (JNK) stress kinase pathways. Human endothelial cells express both TNF receptor subtypes at low levels, however using TNFR-specific agonistic agents, we uncovered that TNF acts through its TNFR1 receptor subtype to activate NF-κB transcriptional pathways. Further investigation revealed that ICAM-1 and VCAM-1 mRNA and protein are induced by TNFR1 (but not TNFR2) in a wholly NF-κB-dependent manner. These findings reveal for the first time that TNF stimulation of adhesion molecules ICAM-1 and VCAM-1 in human endothelial cells occurs through the TNFR1 subtype and is mediated by the NF-κB pathway, but not the ERK, p38MAPK or JNK kinase pathways.

Keywords: Cytokine; Receptor; Subtypes; Cell signalling; Endothelium; Adhesion molecule

1. Introduction

Tumour necrosis factor (TNF) is a pro-inflammatory cytokine that is predominantly produced by activated macrophages and monocytes. In response to allergen and pathogen invasion, endothelial cells also have the ability to produce TNF at localised focal inflammatory sites, forming part of an atherogenesis network [1]. TNF-induced inflammatory responses on endothelial cells are involved in a range of cardiovascular disorders, including atherosclerosis, coronary artery disease and congestive heart failure. The effects of TNF on vascular tissues include protein synthesis-independent changes in cell permeability and cell motility, leading to localised inflammatory responses, vascular leakage and oedema formation [2]. However the majority of the longer-term pro-inflammatory responses of TNF are through stimulus-induced expression of TNF's response genes, which then regulates functions of endothelial cells, such as leukocytes adhesion (via expression of adhesion molecules ICAM-1, VCAM-1 and selectins), chemokine expression, leukocyte activation and migration, and coagulation responses by expression of tissue factors and plasminogen

Abbreviations: caspase, cysteine-aspartate-directed proteases; $cPLA_2$, cytosolic phospholipase A_2; DD, death domain; ERK, extracellular signal-regulated kinase; hr-GFP, humanised renilla green fluorescent protein; HUVEC, human umbilical endothelial cells; ICAM, intracellular adhesion molecule; IκB, inhibitor of κB; JNK, c-*Jun N*-terminal kinase; MAPK, mitogen-activated protein kinase; MR2-1TNFR2-specific agonistic monoclonal antibody, PD98059, 2′-Amino-3′-methoxyflavone; SB203580, 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole; SP600125, anthra [1,9-cd]pyrazol-6(2H)-one 1,9-pyrazoloanthrone; NF-κB, nuclear factor-κB; R1-TNF, R32WS86T TNFR1-specific TNF; R2-TNF, D143NA145R TNFR2-specific TNF; TNF, tumour necrosis factor-α; TNFR, TNF receptor; TNFR1, Type I 55 kDa TNFR; TNFR2, Type II 75 kDa TNFR; TRAF, TNFR-associating factor; VCAM, vascular cell adhesion molecule.

* Corresponding author. Tel.: +44 1603 592005; fax: +44 1603 592003.
E-mail address: d.macewan@uea.ac.uk (D.J. MacEwan).

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activator inhibitor-1 (PAI-1) [3]. TNF co-ordinates this innate inflammatory cascade and the ensuing adaptive immune responses. The recruitment and transendothelial migration of circulatory inflammatory cells is controlled by TNF-stimulated endothelial cell surface adhesion molecules expression — a key response in the initiation of early phase atherosclerosis [4].

Intercellular adhesion molecule (ICAM) belongs to a subfamily of five members that are regulated by inflammatory stimuli. ICAM-1 is widely expressed at low basal levels and is up-regulated by inflammatory cytokines in leukocytes and endothelial cells, whereas ICAM-2 is present on leukocytes, platelets and endothelium. ICAM-3 is present in endothelial cells and leukocytes and is the only ICAM molecule on neutrophils. Each is capable of strong bonds with integrins and arresting inflammatory cells through firm adhesion [5]. Another major adhesion molecule is vascular cell adhesion molecule-1 (VCAM-1) which is not only expressed on endothelial cells but on other cell types such as macrophages, myoblasts

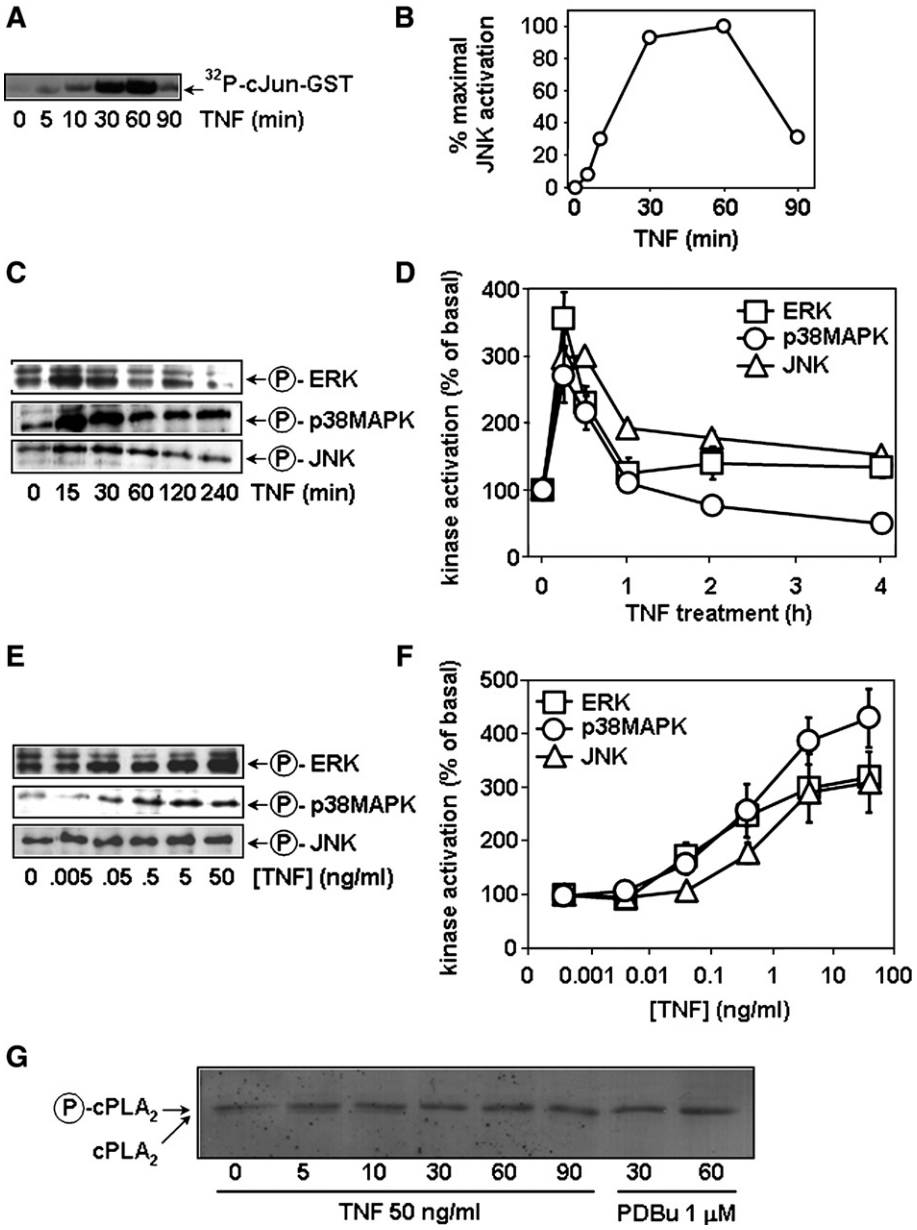

Fig. 1. Activation of MAPK kinase pathways by TNF in HUVEC. Kinase activation in HUVEC cells was measured (A) by c-*Jun*-GST activity assay as described in the Materials and methods section. Cells were stimulated with 50 ng/ml TNF for the indicated time. (B) Densitometric analysis was also performed. The data represents results from a single representative experiment repeated at least one other time with similar findings. (C–G) Western analyses of proteins from TNF-treated HUVEC as described in the Materials and methods section. ERK, p38MAPK and JNK time-course analyses (C,D) of 50 ng/ml TNF-treated cells, or concentration-response relationship (E,F) of cells treated with the indicated concentration of TNF for 30 min. Densitometric analyses are standardised to the immunoreactivity of non-phosphorylated MAPKs protein (not shown) to control for loading. The data represent means ± S.E.M., $n = 4$. (G) Western analysis of $cPLA_2$ in HUVEC treated with TNF or PDBu as indicated. Arrows indicate the non-phosphorylated and phosphorylated bands. The result is representative of at least two independent experiments with similar findings.