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Short communication

Toll-like receptor 2 signalling: Significance in megakaryocyte development through wnt signalling cross-talk and cytokine induction

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ABSTRACT

TLR2 is a toll-like receptor protein which is involved in innate immune responses. TLR2 recognize several virus, fungal and bacterial pathogens, upon their uptake cause internalization and cellular activation. During this process several cytokines participate including interleukins, IL6 and IL12. Interestingly, TLR2 is expressed on megakaryocytes (MKs) and platelets, which is crucial for immune mediated platelet activation. The role of TLR2 on MKs is not completely understood. We observed TLR2 induction leads to MK maturation and is involved in production of ROS which is essential for MK development. In Dami cells, TLR2 up-regulation causes increase in the cytokine production, particularly IL-6, which has been shown to stimulate CFU formation and CD41 expression. Additionally, TLR2 ligand induces wnt β-catenin signalling pathway components suggesting a cross talk between wnt and TLR pathway leading to maturation of MKs. This study shows TLR2 signalling induce cytokine production and regulate wnt signalling thereby cause maturation of MKs.

1. Introduction

Megakaryocytopoiesis is a unique process by which hematopoietic stem cells (HSCs) produce megakaryocytes (MKs) in the bone marrow. These cells mature to become MKs by successive rounds of endomitosis and polyploidy. Polyploidization is essential for the production of proteins necessary for the platelet formation and function, including membrane receptors such as CD41 and CD61 [1,2]. κ signalling pathways are regulating these developmental events of MKs and recently TLRs are reported to be expressed on MKs and platelets [3].

TLRs are toll-like receptors and are activated upon interacting with various pathogen associated molecular patterns (PAMPs). There are 13 TLRs that have been identified in humans and mice [4,5]. TLR2 recognize several virus, fungal and bacterial pathogens and their ligands such as lipoproteins, peptidoglycans, lipoteichoic acid from gram positive bacteria and zymosan from fungi [6]. Stimulation of TLR2 with these ligands induce NFκB signalling pathway which further results in increased expression and release of inflammatory cytokines such as interleukins and TNF-α [6,7]. Recently it has been shown that TLR1, TLR2, TLR4 and TLR6 are expressed on human MKs and platelets [3,8,9]. TLRs on MKs induce cytokines, among which IL-6 is the most prominent which enhances TPO effect on MKs [10]. It is known that ROS production in MKs is essential for MK maturation [3]. The significance of functional role of TLRs on MKs is not completely understood.

Earlier reports suggest that TLRs also regulate cell proliferation and survival by directing various signalling pathways. Interestingly in macrophages, TLR induction leads to the activation of wnt signalling pathway [11]. Also, wnt pathway has lately been explored to be operative in MK development and platelet production [12] but the relation between TLR2 and Wnt signalling pathway in MKs is not studied. Wnt signalling pathway is an evolutionarily conserved fundamental signalling system which governs organ development as well as homeostatic processes. Wnt proteins are insoluble secreted palmitoylated glycoproteins which acts as crucial signalling molecules in embryonic as well as adult tissue development by regulating cell fate determination, proliferation and polarity [13]. Wnt signalling is essentially divided into three types - β-catenin dependent canonical pathway, planar cell polarity pathway and Wnt/Ca2+ pathway. In the present study, TLR2 activation induced MK maturation by modulating wnt components in Dami cells showing a crosstalk between TLR and wnt signalling.

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2. Materials and methods

2.1. Cell culture

The human megakaryocyte cell line Dami were propagated in RPMI medium containing 10% fetal bovine serum (FBS) and 1% antibiotic ant-anti (Life Technologies, Inc.). The cells were grown in a humidified incubator at 37 °C, in the presence of 5% carbon dioxide. To induce the TLR2 signalling, Dami cells were treated with 1 μg/mL HKL (heat killed lacto bacillus, invivogen) as per the manufacturers guidelines.

2.2. Morphological analysis using microscopy

The human megakaryocyte cell line Dami were propagated in RPMI medium containing 10% fetal bovine serum (FBS) and 1% antibiotic ant-anti (Life Technologies, Inc.). To induce the TLR2 signalling, Dami cells were treated with zymosan (10 μg/ml) and 1 μg/mL HKL (heat killed lacto bacillus, invivogen) as per the manufacturers guidelines. LPS (500 ng/ml) was used as a negative control. Giemsa Stain was diluted 1:5 with deionized water. Cells were fixed on the coverslip with methanol and incubated for 5–7 min at room temperature. Fixed cells were stained with Giemsa for

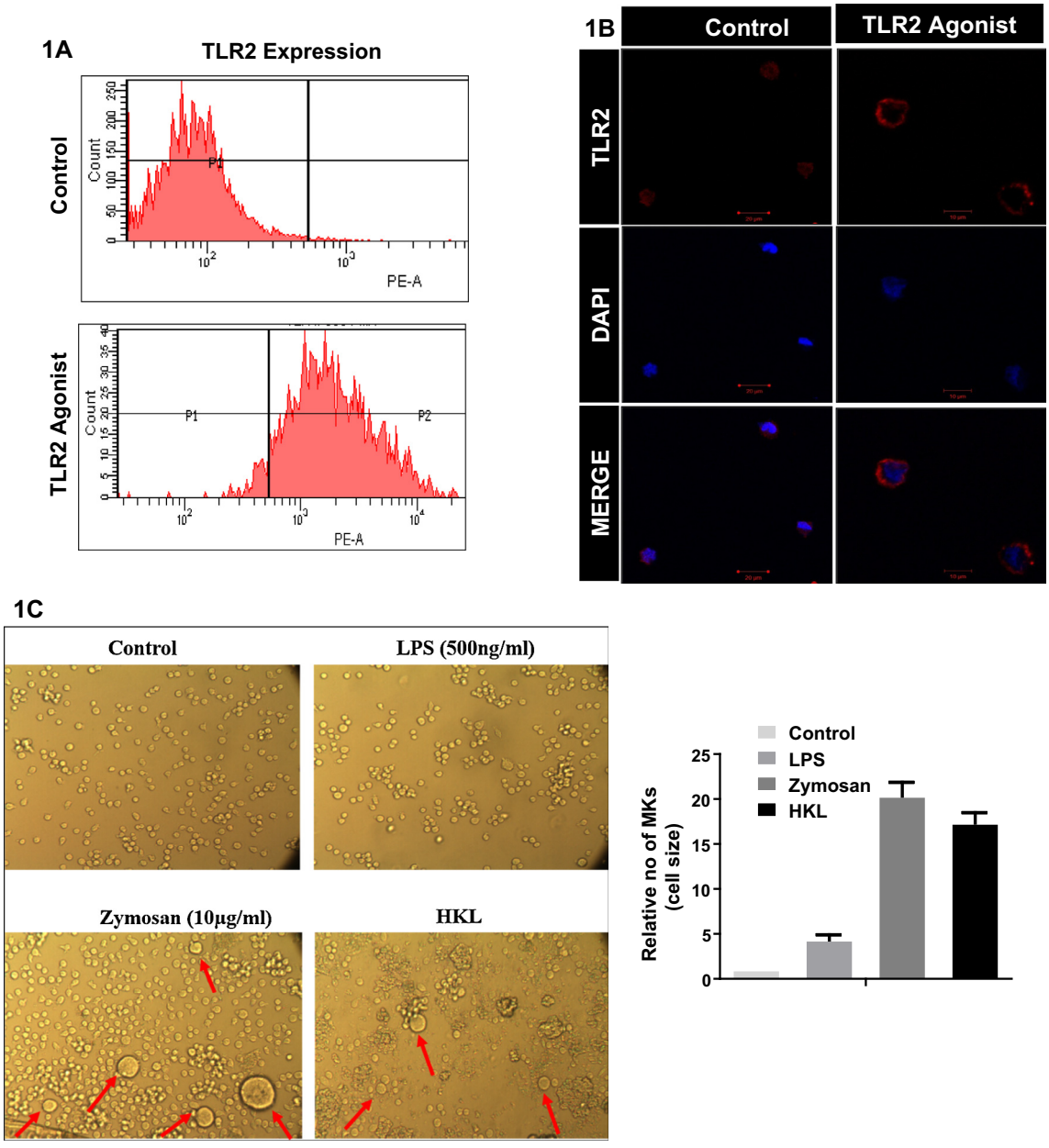

Fig. 1. TLR2 activation lead to increase in TLR2 expression, ROS production and megakaryocyte maturation. Dami cells were treated with HKL and after 24 h TLR2 gene expression was studied by (A) flow cytometry, (B) confocal microscopy, (C) cell morphology (cell size) by TLR2 stimulation studies by HKL and Zymosan using microscopy. (D) Nuclear morphology (nuclear size) by TLR2 stimulation studies by HKL and Zymosan using Giemsa staining. (E) Megakaryocyte differentiation markers CD41 and CD61 expression was quantified by qPCR, (F) TLR2 increases ROS production shown by higher staining with 2′,7′-dichlorofluorescein triacetate for the detection of release of ROS in HKL treated cells.