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PANX1 in inflammation heats up: New mechanistic insights with implications for injury and infection

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ABSTRACT

A new study by Yang and colleagues has revealed that TNF-alpha regulates PANX1 levels through an NF-kB-dependent mechanism in human endothelial cells. PANX1 modulates Ca^{2+} influx contributing to IL-1beta production independent of purinergic signaling. These novel findings expand our understanding of TNF-alpha-mediated upregulation of IL-1beta with implications for responses to tissue injury and infection.

Tumor necrosis factor (TNF)-alpha triggers inflammation in endothelial cells via the upregulation of pro-inflammatory cytokines, including interleukin (IL)-1beta [1]. While these cytokines are key inflammatory mediators in many processes, we still do not fully understand the molecular mechanisms driving their production, processing, or release. A recent paper by Yang et al. [2] implicates pannexin 1 (PANX1) as a novel intermediary of the TNF-alpha/IL-1beta inflammatory pathway in the human umbilical vein endothelial cell (HUVEC) line (Fig. 1A).

Yang and colleagues found that TNF-alpha increased *PANX1* transcription. Prior to this study, NF-kB was not a known transcriptional regulator of PANX1, and knowledge of *PANX1* transcriptional regulation was limited [3]. Sparked by their report, a preliminary bioinformatics analysis (using open source tools [4]) revealed (at least) two putative NF-kB binding sites upstream of *PANX1*. Additionally, our PANX1 interactome [5] included key NF-kB signaling proteins, such as toll-like receptor 3. These findings support the authors' conclusions and indicate further mechanistic insight into the novel TNF-alpha/NF-kB/PANX1/IL-1beta signaling axis is now warranted.

Prolonged exposure (24 h) to TNF-alpha increased surface expression of PANX1, which facilitated entry of Ca^{2+}, thereby enhancing IL-1beta expression and release in a feed-forward manner. Using several orthogonal approaches the authors ruled out contribution of purinergic signaling (via PANX1-mediated ATP release), and postulated Ca^{2+} influx occurred directly via PANX1 channels. This model diverges from purinergic signaling acting as a key component in regulation of IL-1beta [6]. These seemingly contradictory findings could be reconciled by recognizing that P2X7 receptors and PANX1 regulate two distinct parts of the IL-1beta life-cycle. Here the amounts of ATP released via PANX1 were deemed insufficient to activate P2X7 receptor/inflammasome regulation of IL-1beta processing and release. Instead, PANX1 increased IL-1beta transcription, and thereby also IL-1beta release. Indirectly these findings infer that the levels of TNF-alpha used to stimulate the cells were insufficient to activate the inflammasome. To better understand how PANX1 regulation of IL-1beta might crosstalk with inflammasome-mediated regulation of IL-1beta in endothelial cells, it would be important to identify the types of purinergic receptors present in these cells (and key inflammasome accessory proteins) and to expose the cells to a wider range of stimuli, including confirming those that effectively activate the inflammasome. Overall these data raise several questions surrounding possible mechanistic differences between cell types (e.g. immune *v.* non-immune cells) or cell states (e.g. healthy *v.*

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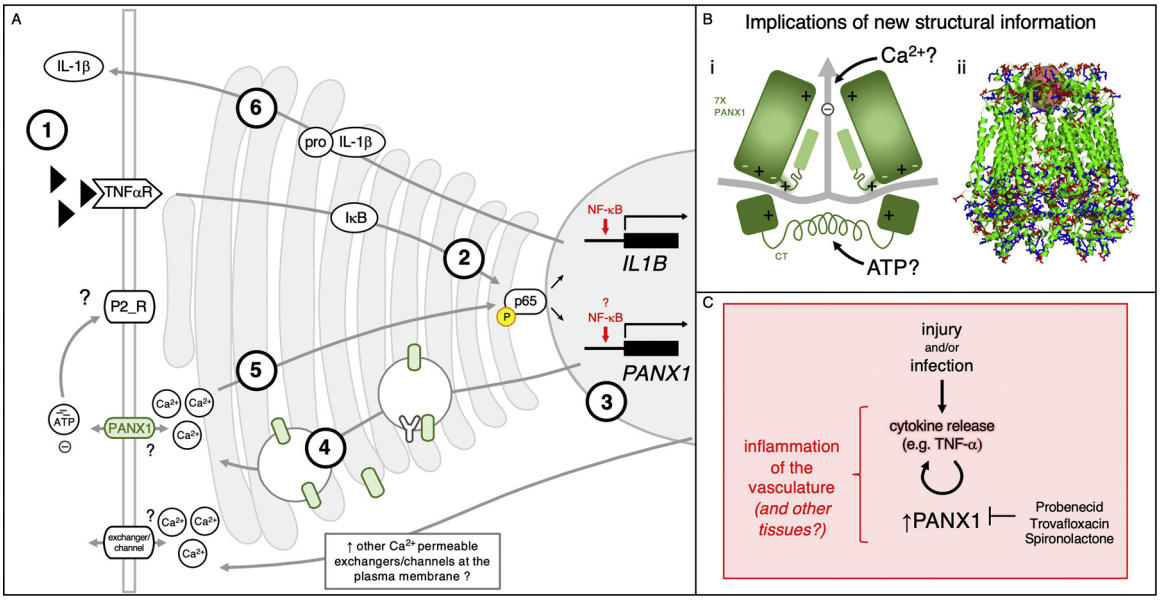

Fig. 1. PANX1 involvement in TNF-alpha mediated regulation of IL-1beta in endothelial cells: summary of new findings, open questions and clinical implications. A) PANX1 modulates inflammation in endothelial cells by regulating intracellular Ca^{2+}. During an inflammatory response, TNF-alpha acts as a *priming signal* (1) and through NF-kB signaling (2) induces IL-1beta and PANX1 biosynthesis and processing (3). Exposure to TNF-alpha for 24 h increases PANX1 channels at the plasma membrane (4), where it regulates Ca^{2+} entry through an ATP-P2 purinergic receptor independent pathway, further enhancing IL-1beta processing (5) and release (6). B) (i) Cartoon of PANX1 structure based on new structural data [10] with electrostatic surface potential roughly represented as darker green denoting approximate areas of relatively positive charge density and lighter green denoting approximate areas of relatively negative charge density; (ii) In addition to positive charges lining the proposed 7 side "tunnels" there are also acidic (negatively charged) residues that could facilitate Ca^{2+} permeability (red and blue stick cartoons indicate the location of acidic and basic amino acid residues lining the PANX1 tunnels. The sphere represents carbenoxolone binding site in the ribbon cartoon). The various conditions underlying permeability to smaller anions (e.g. Cl^-) and cations (e.g. Ca^{2+}) and larger anions (e.g. ATP) and cations (e.g. spermidine) remains to be determined. **C**) This work underscores the clinical potential for targeting PANX1 in the context of inflammatory diseases (e.g. injury and infection) involving the vasculature, and potentially other tissues and cell types. The figure was created using Microsoft PowerPoint (v16). The PANX1 structure cartoon was created using BIOVIA Discovery Studio.

apoptotic cells), potential contributions of alternative purinergic molecules (e.g. ADP, adenosine and UTP also may be released via PANX1), and the role of putative high-concentration extracellular ATP microdomains.

The permeability of PANX1 channels also remains a topic for further study. In addition to its role as a nucleotide and metabolite channel, previous work suggested PANX1 channels are permeable to ions of various size and charge [7]. This includes small anions like Cl^-, but also cations like Ca^{2+}, as suggested by Yang et al. Recent structural reports detailing PANX1 subunit and channel composition imply PANX1 channels might more favourably pass negatively charged molecules, such as Cl^- and ATP, due to the presence of strategically placed positive residues (Fig. 1B, part i), but our modelling also revealed the purported tunnels are additionally lined with a set of negatively charged amino acids (aspartate and glutamate; Fig. 1B, part ii) which could facilitate cation or Ca^{2+} ion transport. This raises the possibility that the various possible open configurations associated with different stimuli (described in detail in [7]) might preferentially support alternate permeability to anions or cations. Another possibility could be that PANX1 indirectly regulates Ca^{2+} influx, for example by modulating the activity of a Ca^{2+} transporter, exchanger, or channel. For instance, PANX1 interacts with N-methyl-D-aspartate (NMDA) receptors [8], which are present and functional on peripheral endothelial cells (e.g. [9]) suggesting PANX1-NMDAR crosstalk could be involved in the current context.

PANX1-mediated inflammatory signaling (such as release of ATP and other nucleotides, inflammasome activation and cytokine release) has been implicated in several peripheral and nervous system pathologies. Given the ubiquitous expression of PANX1, TNF receptors, and IL-1beta, PANX1 upregulation of *IL1B* transcription could be implicated in many of these pathologies, and therefore could represent a potential therapeutic target (Fig. 1C). To bring this knowledge into the clinic, it will be important to determine whether approved PANX1 blockers affect this novel mechanism.

In summary, these exciting new findings provide a new perspective on the role of PANX1 in inflammation, connecting it to TNF-alpha and NF-kB. This work has laid the groundwork for future studies that will undoubtedly help us unravel the complexities of inflammatory disease pathogenesis and treatment in endothelial cells as well as other cells expressing PANX1.

Author contributions

L.A.S. was responsible for conceptualization and prepared the initial outline. L.A.S., J.C.S.A., L.E.W.S., R.C.C., E.V.D.S., I.P., P.P.N.R. and B.A.M. wrote and revised the manuscript. J.C.S.A. L.A.S. and P.P.N.R. prepared the figure. J.C.S.A. and L.E.W.S. made equal contributions.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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