Extracellular Vesicles: New Bullets to Fight Fungal Infections

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Spotlight

Extracellular vesicles: new bullets to fight fungal infections

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Abstract

While behaving as a commensal yeast in healthy people, *Candida albicans* remains the deadliest fungal pathogen in immunocompromised patients. Halder et al. deciphered unprecedented immunomodulatory properties of monocyte extracellular vesicles in response to Candida infections, paving the way to consider therapeutic innovations for fungal infections but also inflammatory diseases.

Keywords: Extracellular vesicles, TGF-β1, monocytes, Candida

Core text

Candida albicans is a yeast species naturally associated to the human microflora. This fungal agent behaves as a commensal on skin, mucosa, and digestive tract by interacting finely with other microbiome constituents and host immune cells [1]. However, alterations in the host environment (mainly immunosuppression and microbiome disorders) can promote a pathogenic phase and a proliferation of Candida triggering infection termed "candidiasis" [2]. Deep-seated fungal diseases, particularly invasive candidiasis, remain associated to terrible mortality in immunocompromised patients (20-50 %), and active research to better understand fungi-host interactions is still needed to develop innovative therapeutic strategies [3].

Candida yeasts mediate their effects via cell surface interactions and secreted proteins as initial points of contact between Candida and the host. These can be achieved in part through extracellular vesicles (EVs) (large vesicles i.e. microvesicles and small vesicles i.e. exosomes), as a new way to convey fundamental information between cells [4-6]. These microstructures contain proteins, lipids, and genetic information able to modify phenotype and function of the target cells [7]. EV analysis of human pathogenic fungi revealed the presence of proteins with both immunological and pathogenic activities in addition to proteins involved in diverse processes including metabolism, cell wall architecture and signaling, suggesting that they might be active in fungal pathogenesis [8]. In this respect, Candida EVs can elicit a significant biological response in immune cells and can be internalized by macrophages and dendritic cells. These lead to increased nitric oxide production and secretion of IL-12, TNF-α, IL-10, and TGF-β as well MHCII expression in dendritic cells [9]. Although Candida can mediate in part their effects via the release of EVs, a recent manuscript by Halder *et al.* provides evidence that Candida can modulate immune response by generation of human EVs via a direct interaction with immune cells of the host [10].

They first demonstrate that Candida is able to induce vesicle release from human blood monocytes. This can be easily achieved by incubation of isolated human monocytes with complement opsonized Candida leading to a fast and substantial release of EVs. The pathophysiological relevance is provided by live imaging of vesicles released by monocytes in whole blood infected with Candida. Full characterization and quantification of EVs released upon monocyte activation showed that they are double layer vesicles with a mixture of population (diameter ranging from 50 to 200 nm) including large and small EVs. Proteomic analysis revealed that most of the proteins enriched in these vesicles are exosome-related proteins and complement pathway proteins. Importantly, substantial amounts of transforming growth factor-β1 (TGF-β1) were detected in vesicles derived from infected monocytes and this concomitantly with reduced TGF-β1 content within the monocytes. Further isolation of EVs and characterization of TGF-β1-transporting vesicles identified them as exosomes. Thus, not all of the subsets of vesicles transport TGF-β1 suggesting that TGF-β1 might be specifically sorted in exosomes. Interestingly, TGF-β1 is present on the outer membrane of the vesicles. *Ex vivo* tracking of vesicles in Candida-infected whole blood showed a rapid temporal and spatial formation of TGF-β1-transporting vesicles from the intracellular to extracellular spaces of the monocytes leading to increased release of these vesicles in the blood. Furthermore, the pathophysiological relevance has been provided in mice infected with Candida displaying abundant TGF-β1-transporting vesicles in liver and blood.

Then, the authors detailed the mechanism by which Candida activates monocytes to trigger the generation of TGF-β1-transporting vesicles. They have taken advantage that opsonization with the product of component 3 cleavage (C3b), which binds to the complement receptor (CR3) consisting of CD11b (integrin αM) and CD18 (integrin β2) as a key recognition receptor for pathogens, is previously considered to be important in bacteria-induced immune cell vesicle production. They nicely showed that either pharmacological blockade of CR3 on monocytes or knock-out of CR3 receptor subunit CD11b on human acute monocytic leukemia cell line (THP-1) by CRISPR/Cas9 reduces the ability of opsonized Candida to release TGF-β1-transporting vesicles. Interestingly, peripheral blood cells from CR3-deficient mice for CD11 or mouse monocytes generated by isolated bone marrow cells from CD11b-deficient mice infected with Candida are less prone to produce TGF-β1-transporting vesicles. This underscores a central role of CR3 in the production of TGF-β1-transporting vesicles by Candida-infected monocytes. They identified soluble β-glucan of Candida as a ligand of CR3 via its interaction with CD11b on the monocyte triggering the release of TGF-β1-transporting vesicles. One of the key findings is that Candida exploits a conserved physiological regulatory mechanism by the release of an anti-inflammatory cytokine to dampen the immune response against the fungus. Indeed, human complement-opsonized apoptotic bodies from endothelial cells induce production of similar TGF-β1-transporting vesicles in monocytes without infection. Via this pathway, TGF-β1-transporting vesicles inhibit the function of inflammatory cells through the downregulation of LPS-induced IL-6 production by macrophages. Further analysis of the mechanisms by which TGF-β1-transporting vesicles reduce inflammation showed that TGF-β1 binds to monocytes via transforming growth factor beta receptor II (TGF-βRII) in whole blood, and increases the phosphorylation of mothers against decapentaplegic homolog 2/3 (SMAD2/3) and the upregulation of SMAD7. Through this circuitry, TGF-β1-transporting vesicles reduce the early phase of Candida infection associated with increased IL-1β production via upregulation of the inhibitor of nuclear factor kappa B, IκBα. Thus, in the early systemic infection, Candida uses these vesicles to reduce host immune response and therefore favors its survival. Moreover, TGF-β1 is expressed in the liver of Candida-infected mice and is localized to the endothelium. Candida infection in mice increases the release of TGF-β1-transporting vesicles from blood cells. Although it would be interesting to determine the cellular origin of these vesicles, they can interact with TGF-βRII expressed on surface of endothelial cells to upregulate several anti-inflammatory genes including TGF-β1 amplifying the TGF-β1 signal to further exert their anti-inflammatory properties.

These findings highlight a novel function of EVs as a biological vector of TGF-β1 used by Candida to reduce the early induction of pro-inflammatory cytokines in the host. The yeast utilizes this pathway not only to escape from the immune response but also to reduce systemic infection. Hence, the relative contribution of the two components (the host TGF-β1-transporting vesicles and Candida released

vesicles) in the course of the infection should be considered, for a better understanding of how the fungus modulates immune response. They also advance for a possible therapeutic use of soluble β-glucan as an immune suppressor in inflammatory diseases.

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