Tuberculosis's cargoman

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Tuberculosis's cargoman: bacteria load RNA into host extracellular vesicles

Mirit Biton†, Paula Abou Karam† & Neta Regev-Rudzki

Tuberculosis remains one of the deadliest infectious diseases worldwide. *Mycobacterium tuberculosis* (*M.tb*) has developed various mechanisms to manipulate the human host, in particular by disrupting the host phagosome and the immune response. It is becoming evident that secreted extracellular vesicles (EVs) are involved in the dynamic crosstalk between *M.tb* and the host cells. These vesicles shuttle different cargo components, such as RNA, lipids, and proteins, between cells. In this issue of *EMBO Reports*, Cheng and Schorey [1] describe a previously unknown EV-mediated process, regulating *M.tb* RNA loading into EVs and their internalization by naïve macrophages. They identify the mycobacterial Sec2 secretion system as involved in RNA loading into EVs and show that secreted vesicles contain bacterial RNA that not only promotes IFN-β production upon entry into target cells, but also leads to *M.tb* clearance via the activation of the host's RIG-I/MAVS signaling pathway. Importantly, combined treatment with secreted EVs and antibiotics decreases bacterial load in a mouse model, improving lung pathology compared to treatment with antibiotics alone.

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See also: **Y Cheng & JS Schorey** (March 2019)

Mycobacterium tuberculosis (*M.tb*) is a leading cause of human mortality in the developing world. As an airborne pathogen, *M.tb* mainly infects alveolar macrophages, although various phagocytic cells recruited to the infected lung also ingest bacteria and likely play an important role in the outcome of tuberculosis (TB) [2]. The ability to infect macrophages is crucial to bacterial spread and dissemination. Therefore, *M.tb* has developed a number of strategies to enter phagocytic cells, employing numerous receptors expressed by phagocytic cells that bind mycobacteria [2]. The immune host cells, on their part, have developed several defense "tools" against invading pathogens. A recently discovered mechanism of host–pathogen communication functions via extracellular vesicles (EVs) [3,4].

Extracellular vesicles are membrane-bound vesicles that play important roles under both physiological and pathological conditions, by regulating various cellular functions in recipient cells (reviewed in [4,5]). It has been demonstrated that EVs contain multiple types of bioactive cargo (genetic material and proteins), including different types of RNA [5]. These RNA can be transferred to recipient cells, remain active, and modify the cell fate by regulating gene expression and, in some instances, the production of novel proteins (in the case of mRNA transfer) [6].

Extracellular vesicles are also released from *M.tb*-infected macrophages, but their role in regulating an immune response during *M.tb* infection is still incompletely understood. *In vitro* infection studies have shown that they contain mycobacterial components, including proteins and RNA [6,7]. *In vivo* experiments strengthen these findings, as TB proteins and RNA transcripts were also identified in EVs isolated from the serum of TB patients [6,7]. These EVs were further shown to stimulate the production of pro-inflammatory cytokines and chemokines [6], such as TNF-α, IL-1β, and CCL2, and to increase the rate of apoptosis in host cells, as well as the recruitment of macrophages and neutrophils to the lung [8]. Other functions include the induction of migration of T cells *in vitro*, the stimulation of antigen-specific $CD4^+$ and $CD8^+$ T cells *in vivo*, and the activation and maturation of bone marrow-derived dendritic cells [3]. A previous study by Singh and peers impressively showed that host mRNA transcripts enriched in EVs from *M.tb*-infected macrophage can undergo translation in recipient cells [6]. While it is clear from these studies that EVs derived from *M.tb*-infected macrophages affect the host immune system, which host or mycobacterial components affect the host immune response remained undefined.

Cheng and Schorey now expand on their previous study [6] to reveal an entire sequence of EV-mediated events that leads to an immunological response in target cells [1]. So far, it was unclear which *M.tb* and host components are required for transport of *M.tb* RNA into EVs. Remarkably, using ΔsecA2 *M.tb*, the authors demonstrate that the bacterial Sec2 secretion system is required for the release of RNA from *M.tb* for subsequent packaging into host EVs (see Fig 1). Regarding the effect of EV-loaded *M.tb* RNA on target cells (i.e., macrophages), the authors were able to demonstrate the activation of an entire signal transduction pathway, starting from RNA engagement of the RIG-I/MAVS RNA-sensor, which resulted in the phosphorylation of TBK1 and activation of IRF3, leading to the production of IFN-β [1]. Interestingly, *M.tb* RNA binding to RIG-I/MAVS also restricts *M.tb* growth in the target macrophage. Specifically, cultured macrophages that were exposed to EVs from *M.tb*-infected macrophages were more efficient in controlling *M.tb* infection, likely, in part, by increasing the co-localization of *M.tb* with the phagosome's NADPH oxidase subunits [1]. The recruitment of the NADPH oxidase and the subsequent production of reactive oxygen species lead to the activation of the LC3 pathway and promote phagosome maturation.

Department of Biomolecular Sciences, Faculty of Biochemistry, Weizmann Institute of Science, Rehovot, Israel. E-mails: neta.regev-rudzki@weizmann.ac.il
†These authors contributed equally to this work

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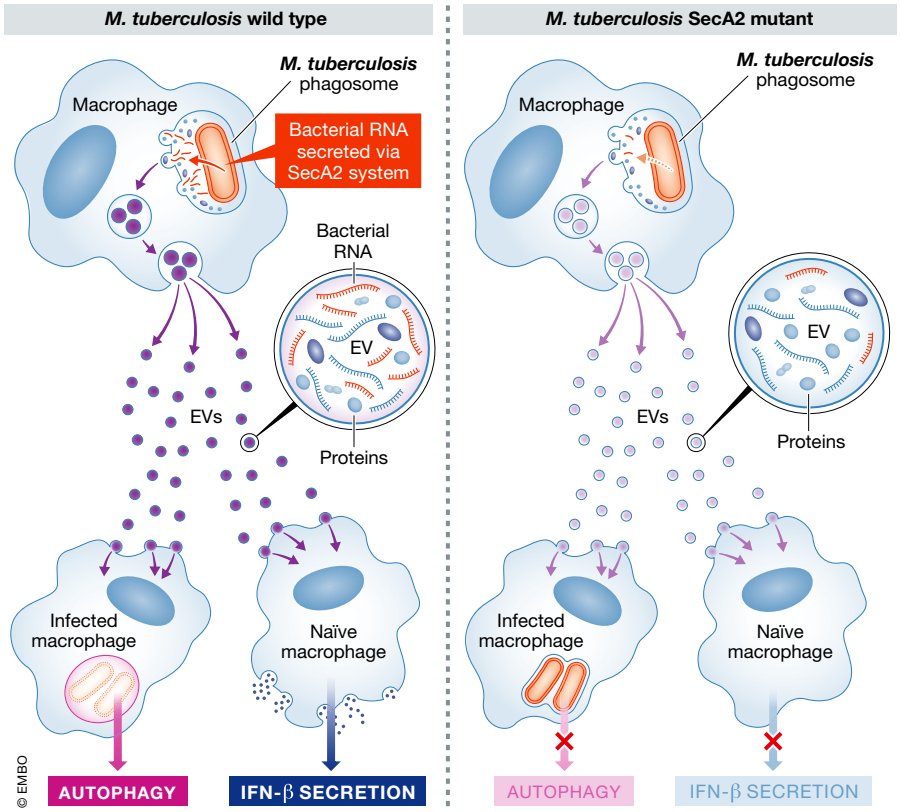

Figure 1. The SecA2 secretion system is required for *M.tb* RNA incorporation into EVs and immune activation.
Macrophages infected with wild-type *M.tb* expressing a SecA2 secretion system release EVs loaded with *M.tb* RNA molecules (red). These EVs are then internalized by naïve macrophages, leading to the production of type 1 IFNs, promoting *M.tb* killing within the phagosome. Macrophages infected with SecA2 mutant *M.tb* fail to release *M.tb* RNA within the vesicles. Consequently, internalization of these EVs devoid M.tb RNA does not promote IFN-β production or restrict bacterial growth.

These results suggest that EVs secreted from *M.tb*-infected macrophages can play a therapeutic role in fighting *M.tb*. Indeed, Cheng and Schorey went on to demonstrate that the combined treatment of EVs and the antibiotic moxifloxacin has a considerable therapeutic effect *in vivo*. Mice treated with EVs from *M.tb*-infected macrophages displayed smaller granuloma-like lesions in the lung and lower bacterial burden in the lung and spleen. In MAVS$^{-/-}$ mice, this phenomenon was abolished, suggesting that this process is MAVS-dependent [1]. Thus, the authors not only delineated the process of packaging the bacterial RNA into EVs and deciphered the mechanism by which it effects host macrophage response, but also present how this knowledge can be translated into clinical benefit.

A major question that remains open is who benefits from packaging mycobacterial RNA into host EVs. If internalization of EVs derived from *M.tb*-infected macrophages by naïve cells results in a lower bacterial load, why would *M.tb* bacteria promote "its own killing"? Either the host exploits bacterial mechanisms to its own benefit, or there is a hidden motive of *M.tb* that has yet to be revealed. One potential clue may come from the *M.tb* RNA-induced production of type I IFN by EVs, as published data suggest that IFN-β production is beneficial to *M.tb* replication *in vivo* [9]. Therefore, it is likely that the balance of these positive and negative effects of *M.tb* RNA will dictate who benefits from its release.

While the biological rationale for this EV-mediated bacterial activity remains to be clarified, this study clearly demonstrates the promise of studying pathogenic EVs to develop effective therapies. One of the major advantages in EV treatment is the ability to target specific subsets of cells, since the internalization of EVs is likely to be mediated by specific receptors [5]. An increasing number of studies have demonstrated the effect EV cargo has on the pathogenesis of infectious diseases [4]. Although it is evident that EVs play a major role in the modulation of infectious diseases, we are still missing applicable *in vivo* models, as we lack the tools to specifically block EV biogenesis. Therefore, it is unclear how blocking EV secretion by bacteria will affect disease progression. Additionally, there remain significant gaps in our understanding of the mechanism involved in selective packaging of cargo components into EVs. Finally, recent studies in mammalian systems have indicated that there are several EV populations, which differ in size, surface markers, cargo components, density, and more [10]. Major efforts in the EV field are directed toward characterizing these populations and their contribution to the infection process.

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Conflict of interest

The authors declare that they have no conflict of interest.

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