ImmunoTools *special* Award 2023

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Bacterial extracellular vesicles in the regulation of the immune system: a new frontier in the host-microbes interaction

The human body is constantly challenged by numerous microbes. In particular, bacteria largely colonize some mucosal surface (e.g. gastrointestinal and respiratory tract), and communicate with each other and with the host cells, resulting in a beneficial (mutualism/commensalism) or detrimental (pathogen) relation for one or both the organisms.

Regardless of their pathogenic role, bacteria can produce specific extracellular vesicles (bEVs), enclosed entities of 20-300nm, that carry proteins, lipids, and nucleic acids. bEVs structure and content vary depending on the yielder bacterial species. Both Gram-positive and -negative bacteria can produce vesicles, with dissimilar characteristics due to their structural differences. This diverse assortment of components, combined with the ability of bEVs to disseminate within the body, led bEVs to interact in multiple ways with host cells. Depending on the packaged cargo, bEVs have a broad spectrum of action and are involved in pathogenesis, antibiotic resistance, nutrient uptake, and nucleic acid transfer.

The immune system can perceive structure and molecular compounds produced by bacteria, triggering responses to defend the host against microbial infection. Among the cells of the immune system, Myeloid-derived suppressor cells (MDSC) are pathologically expanded myeloid cells that acquire immunosuppressive properties under the influence of host- and microbes-derived factors. MDSC are subdivided into two main subtypes, based on their morphology, and cell surface markers: polymorphonuclear (PMN) MDSC and monocytic (M) MDSC. Their main biological function is immune suppression of T and NK cells, modulation of cytokines produced by macrophages, and the impairment of DC differentiation. Several mechanisms are involved in these functions such as the production of arginase-1,

inducible nitric oxide synthase (iNOS), indoleamine dioxygenase (IDO), cyclooxygenase (COX), and reactive oxygen species (ROS). MDSC emerged as one of the players in the pathogenesis of several infectious agents associated with chronic or acute inflammation, however, the mechanisms regulating MDSC expansion and suppressive functions were not clearly depicted.

In this project, we will investigate the ability of Gram-positive and negative derived-EVs to modulate the immune response activity triggering the MDSCs differentiation. For this purpose, we will isolate bEVs from different opportunistic bacteria (e.g *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*) using ultracentrifugation. Primary monocytes or neutrophils will be collected from healthy donors and stimulated with bEVs. After 4 days we will evaluate the differentiation toward an immunosuppressive cell phenotype by the evaluation of cellular markers (CD14, CD11b, CD66b, CD33, HLA-DR) and the cytokines production (TGF-β, IL-10, TNF-α). We will also assess the immunosuppressive activity by evaluating the proliferation ability and cytokine production (IFN-γ) of CD4+ and CD8+ T lymphocytes, as well as their levels of apoptosis (Annexin V).

Results obtained with the generous help of ImmunoTools will allow to explore new mechanisms at the base of the host-microbes interaction mediated by bEVs. Since opportunistic bacteria in immunocompromised patients could be very hurdle to contrast, better knowledge of the bEVs communication pathways could allow the development of new defense strategies against these pathogens.

Key Reference

Toyofuku, Masanori et al. “Composition and functions of bacterial membrane vesicles.” Nature reviews. Microbiology vol. 21,7 (2023): 415-430. doi:10.1038/s41579-023-00875-5

ImmunoTools *special* AWARD for **Andrea Sabatini** includes 10 reagents

FITC - conjugated anti-human CD11b

PE - conjugated anti-human CD8, CD14, IFNgamma

PerCP - conjugated anti-human HLA-DR

APC - conjugated anti-human CD3, CD66b, CD80, CD86, AnnexinV

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