ImmunoTools *special* Award 2023

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The role of bacterial membrane vesicles on the polarization of human macrophages and dendritic cells.

Overview and aim:

Bacterial membrane vesicles (BMVs) are bilayered micro- to nanoscale structures that are released by both Gram-positive (Gm +ve) and Gram-negative (Gm -ve) bacteria via a variety of mechanisms. As a miniature form of a cell, these vesicles contain a variety of biomolecules, including proteins, lipids, nucleic acids, and several chemical compounds. These biomolecules can be transferred to other bacterial cells or even host cells, enabling them to play a role in many different biological processes, including virulence, the development of biofilms, and intercellular communication [1]–[4]. During an inflammatory process driven by bacterial infections, BMVs come in contact with host cells, but the results have not been investigated so far.
Our project's primary goal is to give more insights regarding the relationship between BMVs and immune cells, specifically human macrophages and dendritic cells.

Project Description:

As previously stated, BMVs are secreted through different mechanisms depending on the surrounding environment and the type of bacterium (Gm +ve or Gm -ve) [1]. Therefore, we will choose a bacterium from each class, *Staphylococcus aureus* (Gm +ve) and *Klebsiella pneumonia* (Gm -ve) since they are two of the most prevalent pathogens responsible for many healthcare-associated inflammations [5].

Using a density gradient centrifugation technique, we will initially separate monocytes from human buffy coats to isolate them from the red blood cells and plasma. After centrifugation, the mononuclear cells will be collected from the interface between the plasma and the density gradient medium. These mononuclear cells will be washed and resuspended in a culture medium.

Cytokines from ImmunoTools such as recombinant human granulocyte-macrophage colony-stimulating factor (rh GM-CSF), recombinant human macrophage colony-stimulating factor (rh M-CSF), and interleukin-4 (IL-4) are going to help us to differentiate monocytes into macrophages or dendritic cells. GM-CSF will be used to differentiate monocytes into classically activated macrophages (M1 macrophages), M-CSF + IL-4 will be used to differentiate monocytes into alternatively activated macrophages (M2 macrophages), and GM-CSF + IL-4 will be used to differentiate monocytes into dendritic cells.

Once differentiated in vitro, cells can be exposed to bacterial microvesicles (BMVs) isolated from clinical and reference strains, and the phenotypic differentiation and inflammatory imbalance can be evaluated with the help of ImmunoTools antibodies and cytokines using two methods:

1. Fluorescence-activated cell sorting analysis:
   We will use antibodies for the characterization exploiting (i) anti-human antibody against inducible nitric oxide synthase (iNOS), an enzyme that catalyzes the production of nitric oxide in response to an inflammatory stimulus, (ii) anti-human antibody against CD206, a mannose receptor required for phagocytosis and antigen presentation, and (iii) anti-human antibody against CD11b, a protein responsible for cell migration, adhesion, and phagocytosis.
2. Enzyme-linked immunosorbent assay (ELISA):
   In this method, we will analyze the conditioned media for the presence of pro- and anti-inflammatory cytokines using antibody pair for interleukin-10 (IL-10), interleukin-1beta (IL-1beta), and tumor necrosis factor-alpha (TNF-alpha).

Finally, by using ImmunoTools reagents, we will be able to broaden our understanding of whether BMVs have the ability to affect the inflammatory responses of immune cells and their implications on the immune system, which will be reflected in inflammation processes during infections.

References:

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[3] A. Hendrix *et al.*, “Extracellular vesicle analysis,” *Nature Reviews Methods Primers*, vol. 3, no. 1, p. 56, Jul. 2023, doi: 10.1038/s43586-023-00240-z.

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[5] A. V. Mironova, A. V. Karimova, M. I. Bogachev, A. R. Kayumov, and E. Y. Trizna, “Alterations in Antibiotic Susceptibility of Staphylococcus aureus and Klebsiella pneumoniae in Dual Species Biofilms,” *Int J Mol Sci*, vol. 24, no. 10, p. 8475, May 2023, doi: 10.3390/ijms24108475.

ImmunoTools *special* AWARD for **Mahmoud Elsayed Mosaad Shalata**
includes 10 reagents

FITC - conjugated anti-mouse CD40L, CD80

PE - conjugated anti-mouse CD62L

recombinant murine cytokines: rm G-CSF, rm GM-CSF, rm IFN-gamma, rm IL-4

FITC - conjugated anti-human CD14

PE - conjugated anti-human CD16

recombinant human cytokines: rh IFN-gamma

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