ImmunoTools *special* Award 2014

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Mechanisms involved in the development of inflammation

Neutrophils are myeloid cells which represent the first line of defense against bacterial and fungal infections. Neutrophils kill microbes by means of destructive molecules such as proteases and highly reactive oxygen species, and can also produce a variety of proteins, including cytokines, chemotactic molecules, and other mediators that are involved in their effector functions. Beyond their microbicidal effectiveness, they can also mediate extensive damage to adjacent healthy tissues. Infections are the major triggers of neutrophil recruitment, although many different sterile stimuli, including mechanical trauma, ischemia, toxins, minerals, crystals and chemicals also lead to neutrophil accumulation in the tissues.

Neutrophils contribute to clear infections not only by phagocytosis, but also by means of neutrophil extracellular traps (NETs), a meshwork of chromatin fibers that are decorated with granule-derived antimicrobial peptides and enzymes, such as myeloperoxidase (MPO), elastase and cathepsin G. NETs represent an important strategy to immobilize and kill invading microorganisms.

Our laboratory is focused on the study of factors that regulate the development of inflammation. We have different research projects carried out by PhD students that involve neutrophils and dendritic cells. We have recently reported that human neutrophils produce and release IL-1 beta and that both serine-proteases and caspase-1 contribute to IL-1β processing. We are currently investigating the mechanisms involved in the release of this cytokine. To this aim, we will isolate human neutrophils from healthy donors and after determining by immunostaining with anti-CD14 that the presence of monocytes in neutrophils samples is negligible, we will stimulate the cells in the presence or absence of inhibitors of different metabolic processes and evaluate IL-1β and IL-8 release to culture supernatants. We will also determine intracellular IL-1 beta, IL-18 (another caspase-1-dependent maturation cytokine) and GM-CSF, and different neutrophil activation markers such as CD11b,

CD18 and CD62L expression. We will also evaluate neutrophil viability by FITC-Annexin V and 7-AAD staining and flow cytometry.

To complement these studies, we plan to carry out in vivo experiments in mice. We will study the mechanisms involved in neutrophil IL-1 beta secretion by mouse neutrophils. In this part of the project, we will employ antibodies specific for Gr-1 and Ly6G for the identification of neutrophils.

Another project of our lab focuses on studying neutrophil extracellular traps (NETs) generation. In this case, after isolation, healthy human neutrophils will be stimulated to release NETs by treatment with different agonists in the presence or absence of different cytokines. We will confirm NET formation by immunostaining with anti-MPO and anti-dsDNA by confocal microscopy.

If I am awarded with the ImmunoTools special Award 2014, the antibodies specific for CD18, CD11b, CD62L, CD14, IL-1beta, IL-18, MPO, dsDNA, GM-CSF, mouse Gr-1, mouse CD62L, the recombinant human cytokines CXCL8, IL-17A, GM-CSF, IL-1 beta, TNF alfa, IFN gamma and TRAIL, the ELISA kits for detection of IL-8 and TNF alpha, and annexin V, would be invaluable tools to materialize our projects.

ImmunoTools *special* AWARD for **Analía Trevani** includes 20 reagents

FITC - conjugated anti-human CD11b, CD14, CD62L, Annexin V,

PE - conjugated anti-human CD18,

human IL-8 ELISA-set for 96 wells, human TNFa ELISA-set for 96 wells (each 3 reagents),

recombinant human cytokines: rh IL-8/ CXCL8, rh IL-17A, rh GM-CSF, rh IL-1beta, rh TNF alpha, rh IFN-gamma, rhTRAIL,

FITC - conjugated anti-mouse Gr1,

PE - conjugated anti-mouse CD62L

DETAILS more AWARDS