Inflammation

IMMUNE CELL ACTIVATION

Background

Human PBMCs will be collected from blood samples of healthy donors and M2 cells will be isolated by beads separation protocol. Isolated cells will be then exposed either to the CLIENT's proprietary molecules for defined timings or to control Antibody, along with a positive (i.e. LPS) or negative (to be defined by CLIENT) control.

Stimulated cells will be then processed for the following activation parameters:

Molecular Biology

- Quantitative gene expression of target inflammatory genes (max 10 genes)

Biochemical Characterization

- Inflammatory cytokine production (i.e. IL-1a, IL-1β, IL-2, IL-4, IL-6, IL-10, VEGF)
- Total ROS production (i.e. DCF-DA fluorescent assay)
- Phagocytic activity (i.e. Vybrant Assay)
- Mitochondrial damage (i.e HCS Mitochondrial Health assay)
- Cell viability and toxicity: (i.e. MTT assay)

Pathology Model

Mast cell activation will be monitored by quantitative analysis of allergen-induced histamine release. In particular, the following parameters will be measured:

Antigen induced release of β−hexosaminidase

Mast cells play essential roles in provoking the pathogenesis of allergic reactions via the regranulation process, measuring the degree of degranulation reflects the level of mast cell activation. βHexosaminidase released by these cells during this process has been reported to be a suitable marker for determining the degree of degranulation (Guo et al 2009).

Antigen-induced release of Tryptase

Degranulation, the secretion of cytoplasmic granules, is a key step in the inflammatory response of leukocytes (e.g. basophils, neutrophils, eosinophils, and mast cells). In addition to histamine, these secretory granules contain many proinflammatory mediators including heparin, cytokines, chemokines, and many proteases. Tryptase, a tetrameric serine proteinase, has emerged as the major component of mast cell granules, comprising up to 20% of the total protein of mast cells derived from lung, colon and skin tissue (he et al 1998, 2004). Because it is stored almost exclusively in mast cells, tryptase is a popular indicator of mast cell activation and a target for therapeutic intervention in allergic diseases.