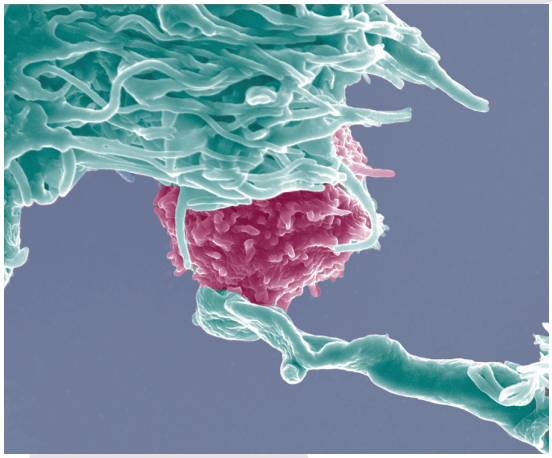
Confluence
DISCOVERY TECHNOLOGIES
An Aclaris Therapeutics Company

TECHNICAL BRIEF
Immune Cell Biology



T cells are the "quarterbacks" of the immune system and their regulation is vital to infectious disease, autoimmunity and cancer. As important are the cells of the innate immune system, responsible for presenting antigen to T cells, or responding to various Toll-like receptors (TLRs). It is the interaction of these cells with each other, and with malignant cells, which defines the success of the immune response against tumors. Similarly, it is the interaction of these cells with "self" epithelial and endothelial tissues, that defines the balance between tolerance and autoimmune inflammation. Studying these cells in vitro and in vivo provides specific insight into their biology, and allows predictions of therapeutic potential for small molecules that impact their differentiation and activation. The Confluence Discovery Technology (Confluence) team has a wide range of experience in developing and validating these functional assays.

We have expertise in the analysis of:

- the earliest signal transduction events post antigen and cytokine receptor stimulation (0 – 20 minutes)
- cytokine production and cytolytic activity (4 – 24 hours)
- maturation/function of antigen-presenting cells (1 – 5 days)
- differentiation into effector T cell subsets (e.g., TH17 cells, cytolytic T cells, 3 – 7 days)

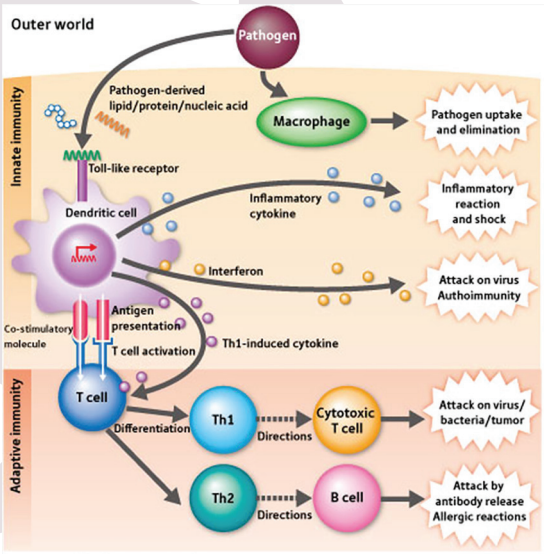
While initial assays are typically carried out in vitro with purified cell populations or mixture of cells derived from lymphoid organs of peripheral blood, we have also developed in vivo assays that will allow rapid analysis of dosed compounds. Such assays are the first window into the therapeutic potential of an oral drug and allow precise correlations of PK-PD relationships. We recommend building such an assay specific to your immunological target prior to embarking on longer-term, disease model efficacy studies.

Applications

Adoptive transfer of CD4+ helper T cell subsets derived from T cell receptor transgenic mice and subsequent immunization with cognate antigen.

Adoptive transfer of CD8+ cytotoxic T cells derived from T cell receptor transgenic mice and subsequent analysis of antigen-specific cell killing.

Injection of various TLR ligands (e.g., LPS, imiquimod) and analysis of antigen presenting cell cytokine production.

In vitro differentiation and activation of T helper cell subsets

For all the assays described above, in addition to MSD- based analysis of secreted factors, MSD-based analysis of phospho-specific signaling events and measurement of specific cell killing – cells can be analyzed by FACS for intracellular and membrane- expressed proteins.