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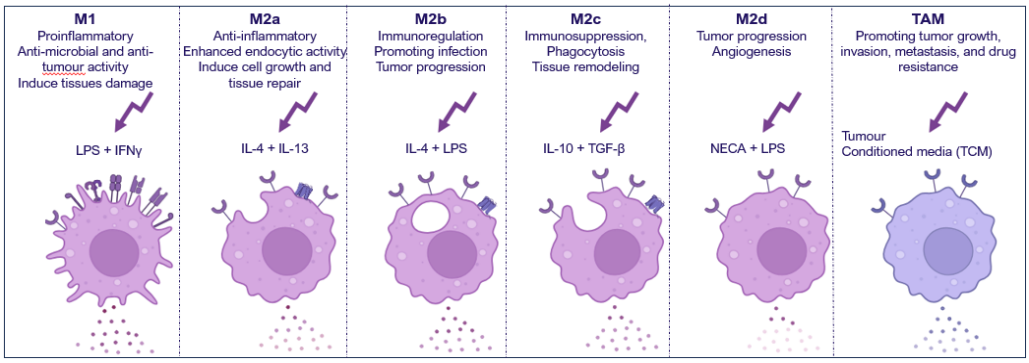
NEXUS BIOQUEST

Macrophage Polarisation Assays

Macrophages can differentiate into a range of activated phenotypes driven by external signals under various disease conditions. Therapeutic driven modulation of macrophage phenotype has the potential to modulate many different disease types where macrophage play a key role in either driving or regulating disease. We present here a range of macrophage polarisation conditions that model macrophage phenotypes found in different disease states. These assays can be used to understand how a therapetic may modulate macrophage phenotype and function.

Methodology

Monocyte derived macrophages (moM) are differentiated from monocytes isolated from healthy blood donors followed by polarisation into a range of phenotypes M1/ M2a-d and TAM phenotypes using the stimuli described below. Readouts include flow cytometric analysis of surface markers associated with different subsets, production of cytokines and chemokines and ability to phagocytose. Therapeutics can be added at the monocyte to macrophage differentiation phase or polarisation phase to prevent or drive polarisation into a distinct macrophage phenotype.



Phenotypic Readout

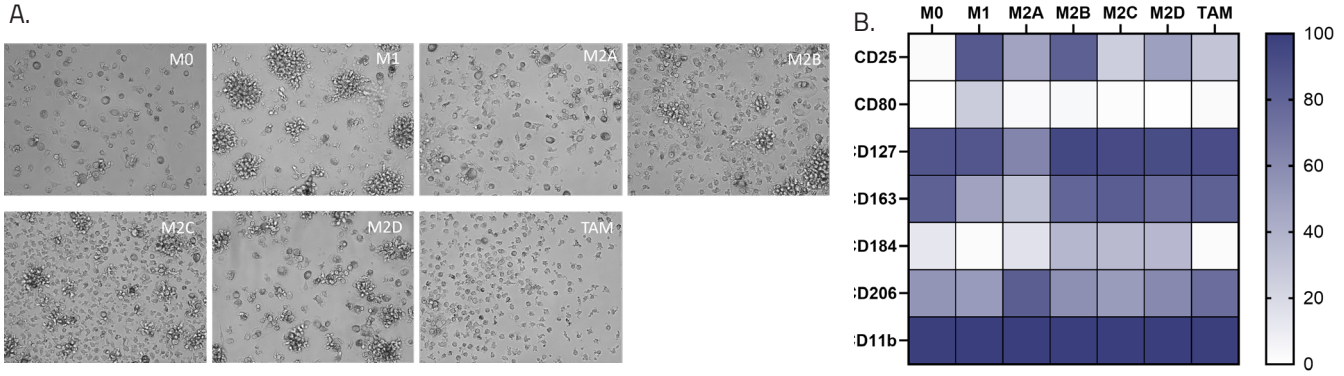
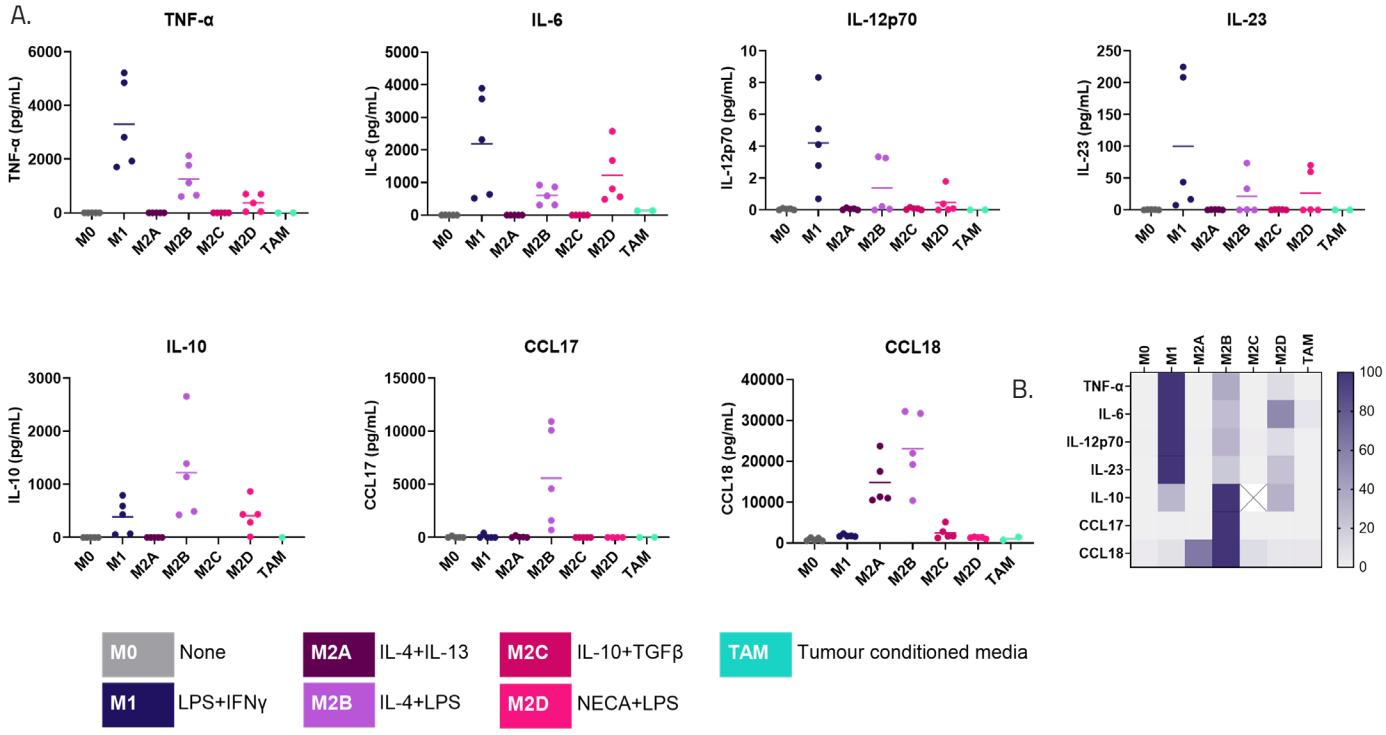


Figure 1. Vitro differentiation and polarization of macrophages under varying culture conditions modulates the phenotypic characteristics of the cells. Differences in morphology are observed by light microscopy (A) alongside distinct profiles in surface marker expression as determined by flow cytometry summarised in (B). Proinflammatory signals generate an M1 phenotype with increased expression of CD25 and CD80. In contrast, alternatively activated M2 macrophages exhibit a spectrum of anti-inflammatory phenotypes including an increase in CD206 (M2a) and CD184. CD163 remains high on most M2 and TAM phenotypes but is downregulated by M1 macrophage.

Cytokine Readout

Figure 2. Effector cytokines and chemokines released by macrophage are important immune mediators and profiling of these factors provides an insight into macrophage functionality and polarisation and how this may have been modulated by a therapeutic. Multiplex analysis of cytokine release by polarised macrophage subsets using MSD (A) shows that each subset has a distinct cytokine and chemokine signature (B). M1 macrophages secrete pro-inflammatory cytokines TNF-a, IL-6, IL-12p70 and IL-23; in contrast M2b macrophages secrete IL-10, CCL18 and CCL17. M2a macrophage only secrete CCL18.



Related Assays

- M1/M2a-d phenotypic and functional (flow cytometry/cytokine/ phagocytosis) characterisation
- Medium throughput 384well M1/M2a screening assays
- M2 co-culture assays: maintenance of epithelial barrier tight junction/ function; fibroblast (FMT) and epithlial (EMT)
- M1/M2 co-culture assays: macrophage modulation of effector T cell function (proliferation and cytokines)

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