M1 and M2 Macrophages – Technology Overview

Differentiated M1 and M2 Macrophages derived from CD14⁺ Monocytes

Product overview

AvantiCell Science (ACS) derives polarised M1 and M2 Macrophages (Mϕ) from CD14⁺ monocytes obtained from human blood samples with full ethical permission.

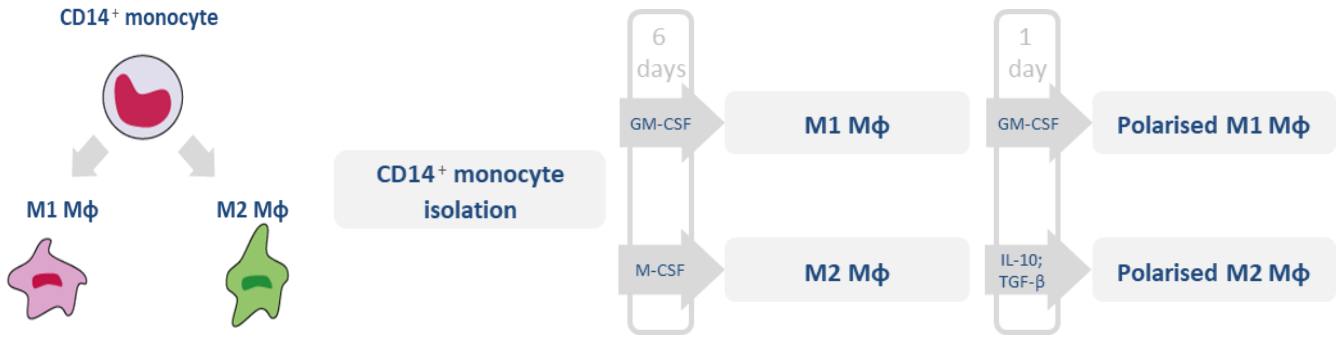
- M1 macrophages are generated via GM-CSF treatment for 7 days
- M2 macrophages are generated via M-CSF for 6 days, and IL-10/TGF-β for 1 day

Monocyte derived macrophages (moMϕ) are characterised by fluorescence-activated flow cytometry, cytokine induction and cell health indices.

Cells are banked using ACS Cryotix™ in-plate cryopreservation technology.

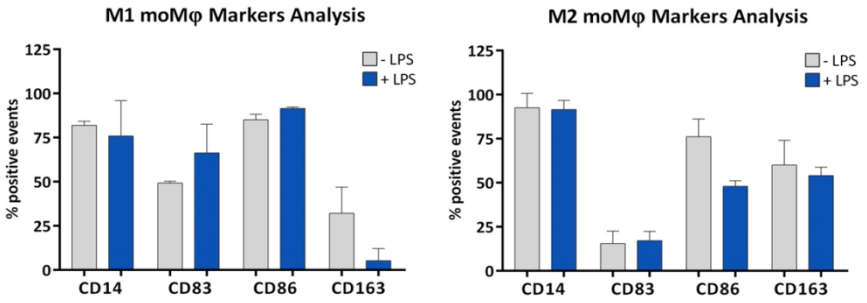
Macrophages (Mϕ) are phagocytic leukocytes which can be differentiated from blood monocytes and which detect, engulf and terminate pathogens, dead cells and cellular debris. Monocytes leave the circulation and migrate to tissues in response to threat detection, where they circulate as differentiated patrolling Mϕ. The Mϕ population is widely distributed and heterogeneous between tissues, which is reflected in pathogen recognition and inflammatory response. Mϕ classification, is reflective of polarisation status. ACS derives human polarised M1 and M2 Mϕ from isolated CD14⁺ monocytes (moMϕ).

The inflammatory M1 Mϕ, are classically activated by lipopolysaccharide (LPS) or Interferon-ɣ (IFN-ɣ). Tissue-resident M2 Mϕ are alternatively stimulated by exposure to cytokines such as M-CSF, IL-4, IL-10 or IL-13, and are associated with wound healing and tissue repair.



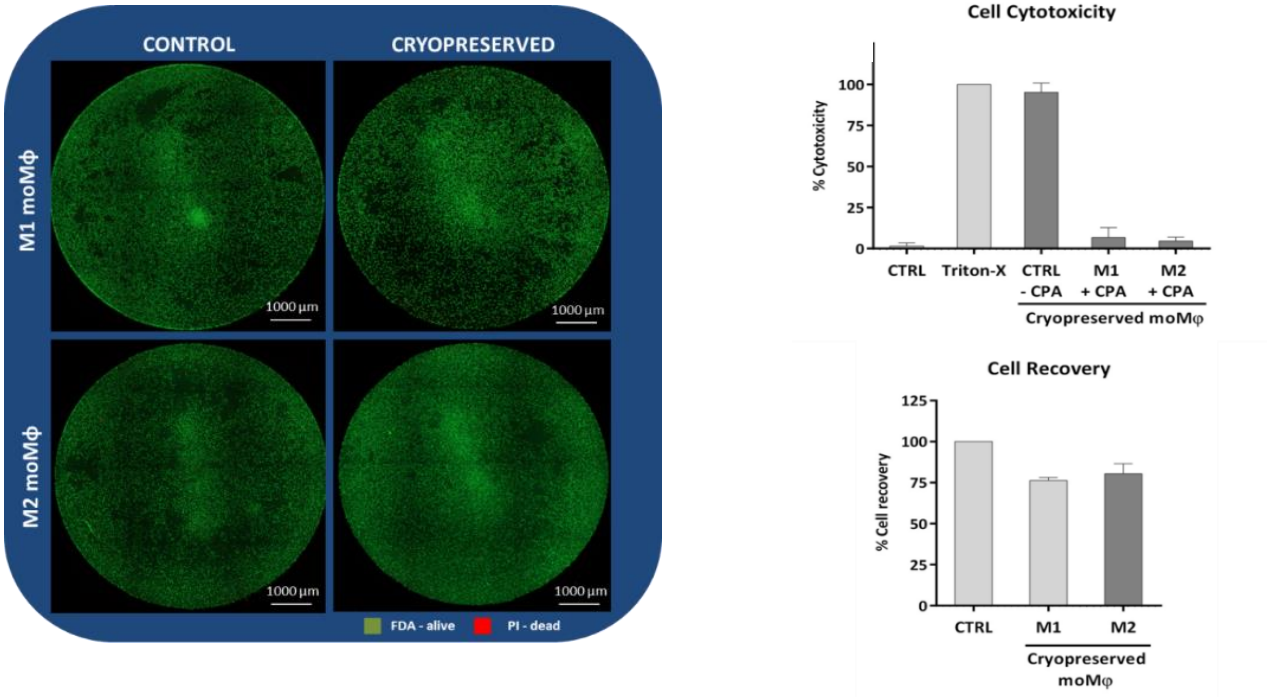
Polarised M1 and M2 macrophages are generated from CD14⁺ monocytes treated with GM-CSF (M1) and M-CSF, IL-10 and TGF-β (M2).

ACS M1 and M2 moMϕ are characterised for cell surface expression markers via flow cytometry, and for cytokine induction via ELISA. M1 moMϕ express high levels of CD14, CD83 and CD86, while expression of CD163 is low. M2 moMϕ express high levels of CD163, with low CD83 expression.



Surface marker expression of M1 and M2 moMϕ.

Differentiated ACS M1 and M2 moMϕ have been cryopreserved *in situ* in 96-well plates using proprietary ACS Cryotix™ cryopreservation technology. High cell recovery and viability is observed upon plate thawing, as revealed by live/dead staining with Fluorescein Diacetate (FDA) and Propidium Iodide (PI), respectively. Cytotoxicity assays confirmed that neither the cryoprotectant nor freezing procedure were toxic to cells and cell counts confirm >80% M1 and M2 moMϕ recovery from frozen across the cryopreserved plate.



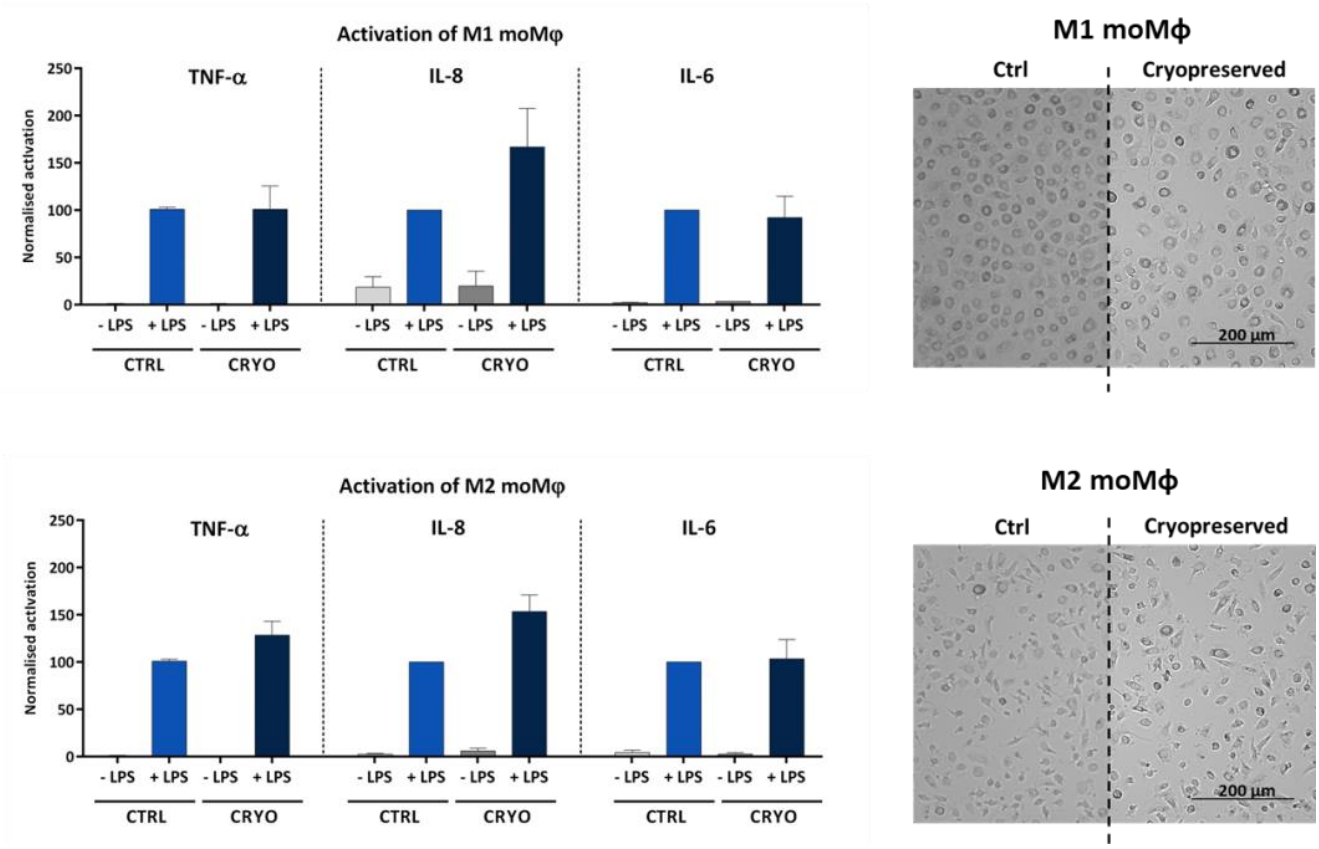
High cell viability is observed in control and cryopreserved M1 and M2 moMϕ (left). Cytotoxicity of cryopreserved cultures is low when the appropriate cryoprotective agent (CPA) is utilised. Triton-X and CPA-free wells served as controls (upper right). Post-thaw cell recovery (expressed as a percentage of non-frozen control) is >80% (lower right).

Cytokine induction in ACS M1 and M2 moMϕ is observed upon stimulation with LPS. Upon treatment, moMϕ show increased release of several cytokines, including TNFα, IL-8 and IL-6. TNFα is a pyrogenic cytokine which stimulates acute

phase immune responses and is known to play a pathological role in many inflammatory diseases. IL-8 is a known chemotactic factor that attracts neutrophils, basophils, and T cells to sites of inflammation, while IL-6 is a pleiotropic cytokine which has both pro-inflammatory and anti-inflammatory functions, from inflammation to wound repair.

Cytokines, which mediate intercellular communication, are essential for macrophage function and are, for example, released by cells upon encounter with viral or bacterial threats in health and disease. Cytokines can be pro-inflammatory or anti-inflammatory and bind to cell surface receptors to initiate signalling cascades which ultimately influence cell behaviour and overall immune response.

Cytokine induction in M1 and M2 moMϕ, which were thawed and recovered from in-plate cryopreservation, was observed via ELISA. Sensitivity to LPS and subsequent secretion of TNFα, IL-8 and IL-6 was found to be retained.



LPS stimulated TNFα, IL-8 and IL-6 cytokine release was retained in cryopreserved M1 and M2 Mϕ.

ACS Cryotix™ in-plate cryopreserved moMϕ can be utilised as a test platform for many cell-based assays, including macrophage induction or testing of pro-inflammatory or anti-inflammatory materials.