Neuroimmune activation and off-target toxicity testing of cell therapies using a novel brain-on-a-chip system

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INTRODUCTION

Adoptive cellular therapies such as chimeric antigen receptor T (CAR-T) cell therapy have shown promise in cancer treatment. However, concerns arise in how the safety of these therapies can be systematically approached. Animal models are not sufficient for testing due to their limitations and have limited translatability. However, humanized-cell models, including co-culturing human immune cells with human cells or co-culture with human-embryonic stem cell-derived immune cells on a microscale, can provide critical insights into the interactions between immune cells and human tissues. However, these models remain limited by the inability to recapitulate the complexity of human immune systems.

METHODS

Culture settings

Hepatic cells and HMC3 cells (BioreclamationIVT) were maintained in EMEM supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1% Glutamax. Jurkat cells (HL-60; Sigma-Aldrich) were maintained in RPMI-1640 medium supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1% Glutamax. Conditioned medium was collected from activated cells on day 14 after 24 hours. HepaRG™ cells and NoSpin HepaRG™ cells (TRL/Lonza), iCell HepG2 cells and HMC3 cells (ATCC) were maintained in EMEM supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1% Glutamax. NoSpin HepaRG™ cells were maintained in culture over a 5-day period (n=6 for all 3D conditions and n=3 for 2D). Primary human T cells (Astarte) were maintained in culture over a 5-day period (n=6 for all 3D conditions and n=3 for 2D). Conditioned medium from activated cells was added to the PerfusionPal system or to a 96-well plate. Human cortical cultures show increased inflammatory gene and protein expression in response to pro-inflammatory stimuli.

Human cortical cultures formed viable, 3D tissues in SeedEZ™ and PerfusionPal™

Culturing in PerfusionPal potentiates hepatic CYP activity for drug toxicity testing

Culturing human hepatocytes in the PerfusionPal system consistently recapitulates the capacity of primary human hepatocytes to metabolize a broad range of drugs. Hepatocytes, rat cortical cells, and HMC3 microglia release CXCL10 in response to CXCL10. Expression of microglia markers responds to LPS and interferon gamma. Contraction of these cells to LPS and interferon gamma was confirmed by Western Blot analysis using Neurolucida software controlling a Nikon Eclipse 80i upright microscope.

PerfusionPal supports simultaneous, long-term culture of a rat cortical model and a human liver model

Human cortical cultures may produce more robust cortical cultures

CONCLUSIONS/FUTURE DIRECTIONS

• The unique human cortical cell culture model shows promise in terms of cellular viability and response of individual cellular components to inflammatory stimuli.
• Secreted factors from activated T cells administered to the neural cultures elicit a measurable inflammatory response.
• A more detailed analysis of cytokines and chemokine release will be carried out.
• T cells and human cortical cell cultures will be grown simultaneously in PerfusionPal to model T cell-brain interactions.
• PSC-derived microglia are being generated in-house to produce a more physiologically relevant response.

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