A three-dimensional collagen construct to model lipopolysaccharide-induced activation of BV2 microglia

ABSTRACT

Background: We report a novel method of culturing microglia in three dimension (3D) using collagen as a substrate. By culturing microglia within a matrix, we aim to emulate the physical state of microglia embedded within parenchyma. Methods: BV2 microglia cell suspensions were prepared with type I collagen and cast into culture plates. To characterise the BV2 microglia cultured in 3D, the cultures were evaluated for their viability, cell morphology and response to lipopolysaccharide (LPS) activation. Conventional monolayer cultures (grown on uncoated and collagen-coated polystyrene) were set up concurrently for comparison. Results: BV2 microglia in 3D collagen matrices were viable at 48 hrs of culture and exhibit a ramified morphology with multiplanar cytoplasmic projections. Following stimulation with 1 μg/ml LPS, microglia cultured in 3D collagen gels increase their expression of nitric oxide (NO) and CD40, indicating their capacity to become activated within the matrix. Up to 97.8% of BV2 microglia grown in 3D cultures gained CD40 positivity in response to LPS, compared to approximately 60% of cells grown in a monolayer ($P < .05$). BV2 microglia in 3D collagen gels also showed increased mRNA and protein expression of inflammatory cytokines IL-6, TNF-α and the chemoattractant MCP-1 following LPS stimulation. Conclusions: In summary, BV2 microglia cultured in 3D collagen hydrogels exhibit multiplanar cytoplasmic projections and undergo a characteristic and robust activation response to LPS. This culture system is accessible to a wide range of analyses and provides a useful new in vitro tool for research into microglial activation.

Keyword: Microglia; Lipopolysaccharide; Collagen matrix; Three-dimensional cultures