ImmunoTools *special* Award 2014

Thomas Bertalot, PhD

University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Università di Padova,
Via Marzolo 5, 35131 Padova, Italy

Enteric Nervous System microenviroment and inflammation

The enteric nervous system (ENS) is the most complex division of the peripheral nervous systems (PNS) in vertebrates. It controls key aspects of gut functionality including the motility, secretion of gastric acid, water and electrolytes and the regulation of blood flow [*Furness, 2012*]. It is composed of several neurons and glial cells, organized into interconnected ganglia, embedded in the wall of the gastrointestinal tract. Several clinical and experimental evidences suggest that homeostatic control of gut function results from an adaptive changes involving cells, extracellular matrix and soluble factors. Intestinal agangliosis or Hirschsprung's disease demonstrate that altered bioavailabilities of neurotrophic factors drive to pathological conditions. In this study, in order to better characterize the immunoprotective response of neurons or glia under LPS-based simulated *in vitro* inflammation conditions, ENS cells, isolated from the myenteric plexus of the Sprague Dawley rats [*Schaefer et al., 1997*], were cultured in medium deprived (BM) or supplemented (SM) with bFGF, EGF and GDNF (ImmunoTools). Particular attention was given to the regulation mechanisms mediated by TLR4 and Wnt signalling. At time of isolation, immunophenotypical characterization by flow cytometry showed the expression of stem cell, progenitors, neuronal and glial markers. Culturing in SM and BM showed a specific modulation of neuronal and glial differentiation and a greater responsiveness mediated by CD349 (SM) and TLR4 (BM) was observed. Moreover, a neuronal subpopulation co-expressed the receptors TLR4 and CD349 suggesting that this cell population may be involved in the maintenance of homeostasis and in the regulation of inflammatory processes. Furthermore, only SM cultures formed neurosphere-like structures. Wnt3a stimulation activated the canonical Wnt pathway through CD349 and qPCR analysis demonstrated anti-inflammatory activity. In addition, a cross-talk between LPS/TLR4 and Wnt pathway was demonstrated by western blotting. Differentiation processes

are also influenced by the extracellular matrix (ECM). In this study, the modulatory effect induced by ECM was evaluated assessing an *in vitro* model: ENS-derived cells cultured on a decellularized ECM of adult rat jejunum. Acellular matrixes (AMs) were provided using a modified enzyme detergent decellularization protocol [*Meezan et al., 1975*]. Histological study, SEM and quantification of residual DNA verified the complete decellularization. Immunofluorescence and western blotting demonstrated that the structural proteins such as collagen I, III , IV and laminin were preserved. After culturing ENSc on AMs for 7 and 14 days, the ECM demonstrated to influence the ENSc spatial organization, exerting a synergic effect with the factors present in the culture medium. In fact, only the AM cultures with SM, showed ganglion-like structures partially interconnected and positive for βIII tubulin. ENSc cultured on acellular matrix may represent a useful *in vitro* model for toxicological and pharmacological studies as well as a possible tissue scaffold in regenerative medicine. Future prospective will focusing on the characterization of the interaction between immune cells and ENSc with particular attention to CD349/TLR4 positive neurons.

References

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