Development of *ex vivo* models for traslational research in organic and fuctional intestinal disorders

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Background: the irritable bowel syndrome (IBS) is a functional bowel disorder characterized by abdominal pain and changes in bowel habits; inflammatory bowel diseases (IBDs) are organic intestinal disorders characterized by recurrent inflammatory episodes and ulcerations of the bowel wall. Notwithstanding profound differences due to the extent of the inflammatory processes occurring in IBD, both disorders share some pathophysiological features including increased trans-epithelial permeability and altered neuro-immune interactions. Rodent models of IBDs show intestinal wall damage and an aberrant immune response, although to achieve an enhanced pathophysiological resemblance with humans, they must lack important immune components (e.g. $IL10^{-/-}$ mice, adoptive transfer). As for IBS, its functional nature makes the reliability of current animal models questionable. In this scenario, *ex vivo* models can turn out to be a valuable tool for proof of concepts tests and for the detection of novel biomarkers interspecies in order to increase the translational value.
Recent clinical trials showed the efficacy of mesenchymal stromal cells (MSC) in the treatment of IBD and its complications. Interestingly, MSC immunomodulatory properties were ascribed to their MSC supernatants alone. MSC mediators, rather than contact-mediated T-cell regulation, may indeed exert a therapeutic function. Finally, Zaniboni and colleagues showed that pericytes isolated from porcine vascular aortic tissue (pAVPC) exhibited all MSC properties. **Aims**: characterize a neuronal primary culture obtained from porcine myenteric plexus compared to an akin preparation obtained from guinea pigs; analyze the effect of pAVPC supernatants on neuronal cultures treated with lipopolysaccharide (LPS) in terms of morphological changes, neuronal differentiation and immune response. **Methods:** 5 Pigs between 40 days and 3 months of age and 9 guinea pigs of 3 months of age were used in the experiments. Neuronal ganglia were placed in culture after isolation and digestion of the longitudinal muscle layer from segments of proximal ileum and let settle for 48 h. Later, both neuronal culture from pigs and guinea pigs were treated for 24 h with increasing LPS concentrations (0,1-1-10 µg/ml) and/or pAVPC conditioned and unconditioned medium; Immunofluorescence analysis toward HuD (1:100), GFAP (1:200), GAP43 and Tubulin (1:200) was applied to detect respectively neurons, glial cells, growth cone associated protein and the cytoskeletal structure. TUNEL analysis was performed to detect apoptosis in both cellular cultures. SYBR green qRT-PCR was applied to quantify al the markers above but Tubulin with the following primers: HuD F -5' -CCTCGCGAATCCTGGTTGAT-3'R - 5' -TCGGTTCTGTAGCACCACTG-3'; GFAP F - 5' -GCCTGCCAAGTGTAGACAGA- 3' R - 5' -TAATGACCTCCCCATCACGC- 3'; GAP43 F .- 5' - AGCCAAGGAGGAGCCTAAAC - 3' R - 5' - TCAGGCATGTTCTTGGTCAG - 3'. **Results and conclusions:** LPS treatment showed a decrease of neurons and a proportional increase of glial cells at LPS 0.1 µg/ml and LPS 1 µg/ml in guinea pig and pig neuronal cultures respectively. A protective effect of pAVPC conditioned medium (CM) on apoptosis induced by LPS 1 µg/ml (+21,1%) was shown by TUNEL; preliminary qRT-PCR analysis showed a ˜6 folds increased expression of GFAP in guinea pig but not in pig neurons treated with CM and LPS 1 µg/ml. Unlikely, the same treatment produced a ˜ 4 folds increase of GAP43 expression in pig but not in guinea pig neurons. Taken together these data show a morpho-functional consistency of guinea pig and pig myenteric neuronal cultures and protective effect of pAVPC mediators on neuronal survival to bacterial products. However, further data are needed to confirm or deny a global consistency of biomolecular features of differentiation and immune response in these two models. **GRANTS:** The study was supported in part by a grant from the Fondazione del Monte Bologna-Ravenna. ID ROL:FdM/3208.