Immunomodulatory effects of dietary supplements on IPEC-J2 cells

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	pigs
Type of cells/tissues/organs	IPEC-J2, porcine small intestinal cells

DESCRIPTION

Method keywords

immune response

cell culture

ELISA

MTT

citotoxicity

lps challenge

Scientific area keywords

gut health

immunomodulation

postbiotic

gut barrier

intestine

Method description

Prebiotics are used by the gut microbiota creating metabolites that exert a positive effect on the host (postbiotics). Prebiotics are available on the market for human and animal consumption and may induce differential effects on the host's health and metabolism. They can be tested by in vitro methods to evaluate their capacity to exert a beneficial effect on gut inflammation and barrier by using in vitro assays and cell cultures. IPEC-J2 cells are intestinal epithelial cells non-transformed obtained from the jejunal segment from a newborn and un-suckled piglet. Cells are grown in flat bottom flasks/plates at 37°C and 5% CO2 humidified atmosphere in complete DMEM/F-12 supplemented with 1% penicilin-streptomicyn, 5% fetal bovine serum, Lglutamine, EGF, Isulin, transferrin and selenium. Screening of cytotoxicity of the products of interest by MTT test in a concentration series of the product of interest to establish the dose. Incubation for 24 hours with the chosen concentration of product and parallel LPS challenge. Collection of supernatants and cells for the measurement of the immune mediators released in the medium by the cells, such as cytokines. Collection of cells for RNA extraction and evaluation of gene expression changes induced in the cells by qPCR methods. In parallel, cells can be cultured in snapwell for studying barrier integrity properties by measuring permeability changes and transepithelial resistance in Ussing chambers.

Lab equipment

CO2 incubator ; Water bath ; Hood (sterile) ; Centrifuge ; Cell counter ; Pipet controller ; Pipettes ; Spectrophotometer ; qPCR ; Ussing chambers.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

1. Fast, easy to set up, reproducible, high-throughput screening capacity. 2. Limited ethical and economic constraints. 3. A valuable tool for initial investigation on gut health studies prior to in vivo tests.

Challenges

The gut is a complex tissue where several cell-types constitute the intestinal barrier, this model however only includes intestinal epithelial cells, and thus, it is a muchsimplified system.

Modifications

The battery of analysis after incubation with the substrates of interest can vary according to the interest of the research. The products of interest have been described as pure prebiotics solutions, however, there are also probiotics and the combination of them as synbiotics that can also be tested. It might be of interest to do an in vitro batch fermentation before incubation with cells to study the postbiotic juice (product of the fermentation of prebiotics/synbiotics in the gut) to screen and evaluate the immunomodulatory effects on intestinal cells. For evaluation of the immune effects of postbiotic juice, before incubation with cells, it should be sterilefiltered.

Future & Other applications

The system could be improved by co-culture in trans-well of intestinal epithelial cells in combination with immune cells.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Uerlings, J., Schroyen, M., Bautil, A., Courtin, C., Richel, A., Sureda, E. A., ... Everaert, N. (2019). In vitro prebiotic potential of agricultural by-products on intestinal

fermentation, gut barrier and inflammatory status of piglets. British Journal of Nutrition, 1–37. https://doi.org/DOI:10.1017/S0007114519002873.

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