Proceedings of the Nutrition Society (2023), **82 (OCE2)**, E78 doi:10.1017/S0029665123000873

46th Annual Scientific Meeting of the Nutrition Society of Australia, 29 November – 2 December 2022, Sustainable nutrition for a healthy life

Bacterial metabolites of dietary fibre fermentation, propionate and butyrate, reduce type 2 cytokine responses by peripheral blood mononuclear cells from subjects with asthma

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Asthma is a chronic inflammatory disease of the airways, typically driven by type 2 (T2) and/or eosinophilic airway inflammation. In asthma, epithelial insults such as respiratory virus infection or exposure to aeroallergens induce the release of epithelial-derived cytokines, interleukin (IL)-25 and -33.[(1)] These cytokines act on immune cells, including T-helper 2 (T_H2) cells and T2 innate lymphoid cells (ILC2s), to induce T2 cytokine release (IL-4, -5, and -13). Short-chain fatty acids (SCFAs) are hypothesised to reduce T2 inflammation, suggesting a therapeutic effect.[(2)] This was an *in vitro* study examining the effect of SCFA treatment on T2 responses by cells derived from adults with doctor-diagnosed asthma ($n = 18$). Peripheral blood mononuclear cells (PBMCs) were isolated via density centrifugation from whole blood samples and seeded in 24-well plates at a density of 2×10^6 cells/mL. PBMCs were treated with 1 mM of sodium acetate, propionate or butyrate for 3 hours prior to culture with 25 ng/mL interleukin (IL)-2, 10 ng/mL IL-25, and 10 ng/mL IL-33, with or without 1 multiplicity of infection (MOI) rhinovirus A1 (RVA1; RVA1/Newcastle/2018). Cells were incubated for a total of 5 d at 35°C, 5% CO_2. Concentrations of IL-5 and IL-13 were measured in cell culture supernatants via ELISA. Gene expression of T2 transcription factor, *GATA3*, and receptors for epithelial-derived cytokines, *ST2* and *IL17RB,* were measured via RT-qPCR, analysed using fold change ($2^{-\Delta\Delta Ct}$). IL-2, IL-25 and IL-33 induced significant IL-5 (1997.1 (1364.9, 2183) pg/mL, median (interquartile range, IQR); $p < 0.001$) and IL-13 (1146 (702.6, 2076) pg/mL; $p < 0.001$) production by PBMCs isolated from subjects with asthma. The addition of RVA1 did not significantly ($p > 0.05$) change IL-5 and -13 production compared to IL-2, -25, and -33 alone. Acetate treatment had no significant effect on induced IL-5 and -13 compared to untreated cells. Propionate and butyrate treatment significantly lowered IL-5 (propionate, $p = 0.004$; butyrate, $p < 0.001$) and -13 (propionate, $p = 0.001$; butyrate, $p = 0.008$) production compared to untreated cells. The mRNA expression of *GATA3* was not significantly changed with IL-2, -25, and -33 or SCFA treatment. IL-2, -25, and -33 upregulated *IL17RB* and *ST2* mRNA expression by 30- and 6-fold, compared to unstimulated cells. However, propionate impaired up-regulation of *IL17RB* ($p = 0.020$), while butyrate led to lower *IL17RB* ($p < 0.001$) and *ST2* ($p = 0.012$) expression compared to untreated cells. Treatment with bacterial metabolites, propionate and butyrate, reduced T2 inflammatory cytokine production by immune cells following exposure to epithelial-derived cytokines. This observation appears to be driven by SCFA-mediated downregulation of the receptors, IL17RB (IL-25 receptor) and ST2 (IL-33 receptor). This study highlights new mechanisms, and suggests strategies that increase circulating SCFAs, including soluble fibre supplementation, may attenuate T2 inflammation in asthma.

References

1. Lambrecht BN & Hammad H (2015) *Nat Immunol* **16**, 45–56.
2. Williams LM, Scott HA & Wood LG (2019) *J Nutr Intermed Metab* **18**, 100108.