UNIVERSITY OF Southampton

INSTITUTE OF DEVELOPMENTAL SCIENCES

Beyond prebiotics:

Microbiota-independent effects of non-digestible oligosaccharides on immunity

Stefania Del Fabbro, PhD student
Faculty of Medicine, Nutrition and Metabolism Group

Outline

1. Non-digestible oligosaccharides (NDOs) and prebiotic mechanisms
2. Microbiota-independent effects of NDOs
3. Potential beneficial role of NDOs in inflammatory bowel diseases
4. Mucosal-associated invariant T cells

Non-digestible oligosaccharides (NDOs)

Dietary fibres that reach the intestine intact

Selectively fermented by health-promoting bacteria

Production of short chain fatty acid (SCFAs) with immunomodulatory properties

Examples of prebiotic NDOs:

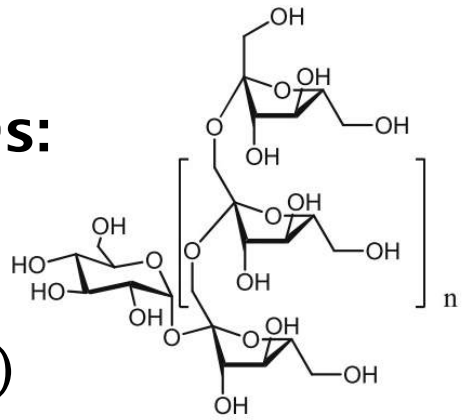
Inulin

Fructooligosaccharides (FOS)

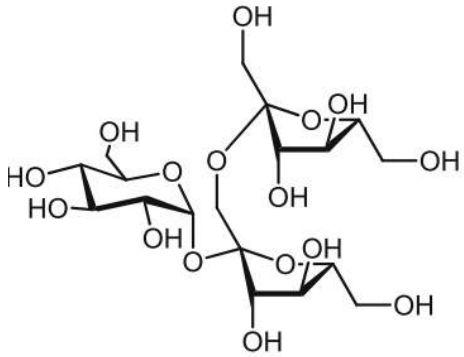
Galactooligosaccharides (GOS)

Mannooligosaccharides (MOS)

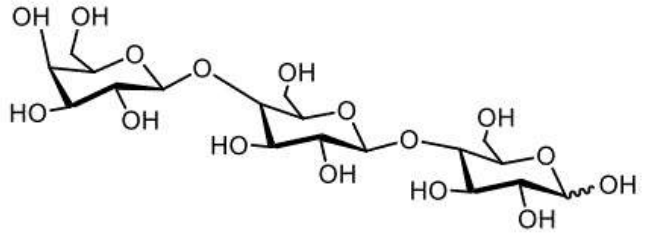
Xylooligosaccharides (XOS)



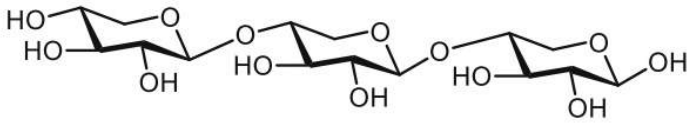
Inulin



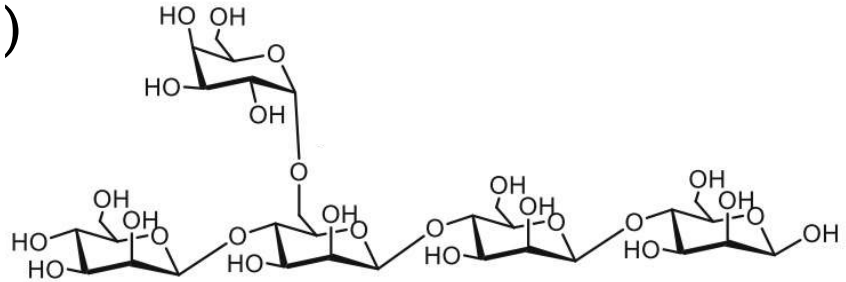
FOS



GOS



XOS

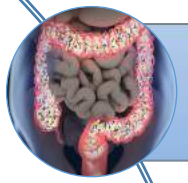


MOS

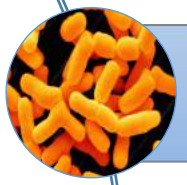
Mano, Appl. Microbiol. Biot. 2017

Effects of NDOs on immunity

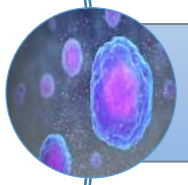
Via modulation of microbiota and their metabolites (SCFAs)



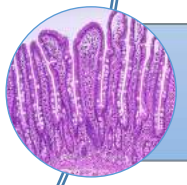
Growth of bacteria with immunomodulatory activity; maintenance of gut homeostasis



Inhibition of pathogens → competition for nutrients; inhibition of adhesion to gut cells; production of antimicrobial compounds



Production of SCFAs, which affect cytokine expression and immune cell functions

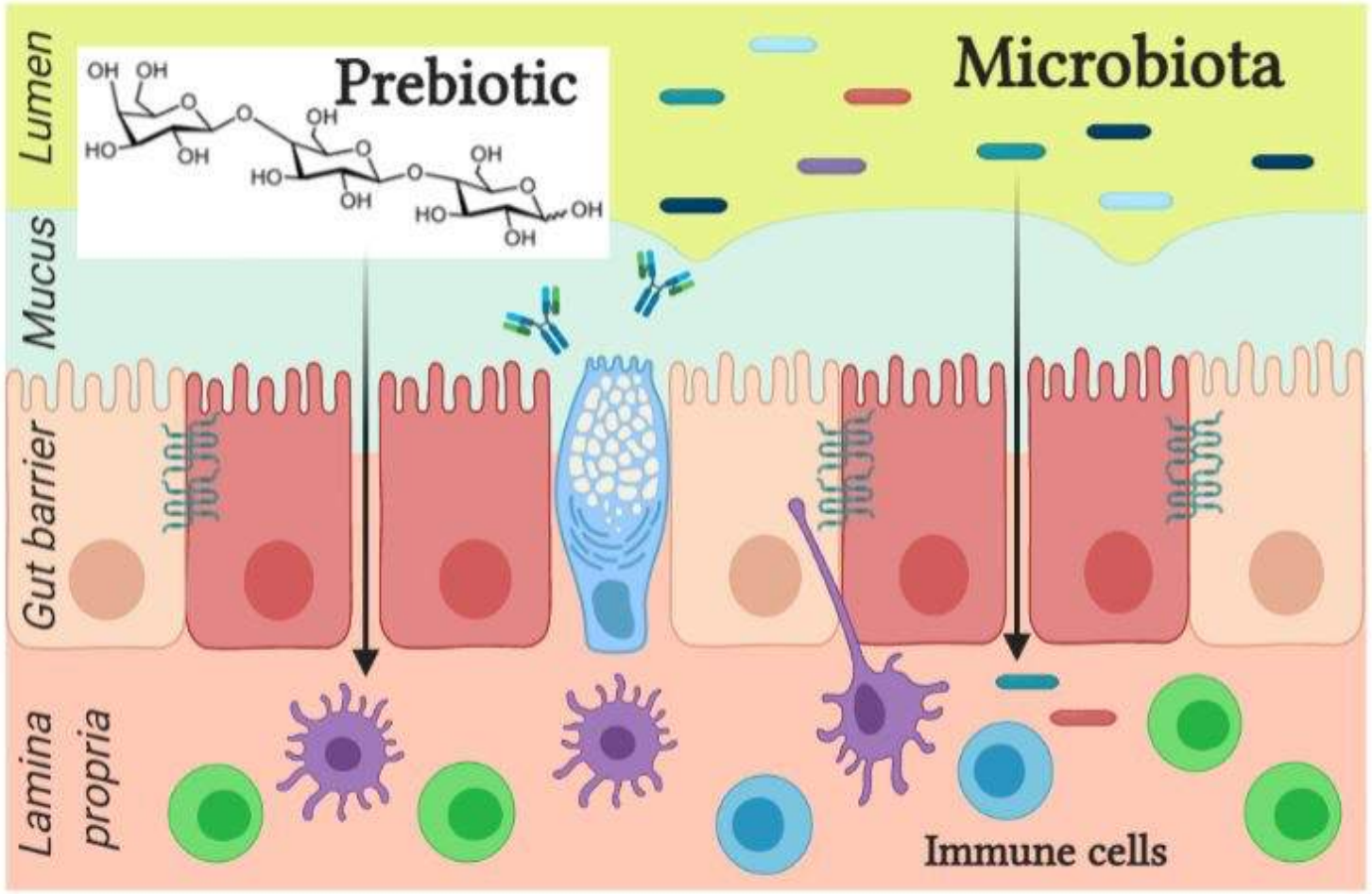


Maintenance of epithelial barrier integrity

Enhancement of antibody response to seasonal influenza; prevention of allergy and infections in early life

Shokryazdan, Med. Microbiol. Immunol. 2017; Wilson, J. Gastroenterol. Hepatol. 2017; Liu, PLoS One 2016; Fernández, AIMS Microbiology 2015; Akatsu, Geriatr. Gerontol. Int. 2016; Arslanoglu, J. Nutr. 2008

Beyond microbiota-dependent effects



NDOs may modulate immunity in a **non-prebiotic manner**, especially in individuals with **increased gut permeability**

1. In what conditions are prebiotics in direct contact with immune cells?

2. Can prebiotics directly affect immunity in a microbiota-independent way?

Only few studies focus on direct effects of oligosaccharides

Del Fabbro, Proc. Nutr. Soc. 2020

Evidence for intestinal transportation of prebiotics

Table 1. *In vitro* studies providing evidence for intestinal transportation of prebiotics

Reference	Treatment	*In vitro* model	Study design	Findings
(33)	Neutral and acidic HMO fractions (5 mg/ml)	Caco-2 cells	Caco-2 cells grown on filter inserts in minimal essential medium. 200 µl of transport buffer with neutral and acidic HMO fractions applied. HPLC-MS analysis of HMO in basolateral compartment	Neutral HMO use transcellular and paracellular pathways to cross Caco-2 monolayer; acidic components use only paracellular pathways
(34)	scGOS/lcFOS	Caco-2 cells	Transfer of scGOS/lcFOS via Caco-2 monolayer measured by HPAEC-PAD. Sample preparation as in Ref. (33)	Transfer of scGOS/lcFOS detected with the rate of transfer of 4–14%, depending on molecular size and structure

HMO, human milk oligosaccharides; Caco-2 cells, human epithelial colorectal adenocarcinoma cells; HPLC-MS, high-performance liquid chromatography MS; scGOS/lcFOS, short-chain galactooligosaccharides/long-chain fructooligosaccharides; HPAEC-PAD, high-pH anion-exchange chromatography with pulsed amperometric detection.

- **Prebiotics transported through gut *in vitro***
- **4-14% rate of transfer**

Del Fabbro, Proc. Nutr. Soc. 2020; Gnoth, J. Biol. Chem. 2001; Eiwegger, Pediatr. Allergy Immunol. 2010

Evidence for intestinal transportation of prebiotics

Table 2. Human studies supporting evidence for intestinal transportation of prebiotics

Reference	Treatment	Population	Study design	Findings
(35)	Infant formula with FOS (3 g/l)	Term infants (*n* 84) aged 1 to 8 (±3) days	Controlled, randomised and blinded clinical study to determine the safety of use of FOS and ability to detect oligosaccharides in urine and plasma of infants randomised to receive FOS-enriched formula, control formula or breast-feeding for 16 weeks. Anthropometric measures, urine, stool and plasma samples taken	No adverse effects with FOS supplementation. Prebiotic effect of FOS on lactobacilli. FOS with DP = 4 in plasma and urine of infants fed with FOS-enriched formula
(36)	HMO; fortified human milk; infant formula with FOS; infant formula with GOS or *B. animalis*	Mother–preterm infant dyads (*n* 4)	Clinical study where preterm infants received human milk with Similac® Human Milk Fortifier or unsupplemented human milk followed by human milk with fortifier Prolact + 4® or formula milk Similac® Special Care® 24 High Protein either with GOS or with *B. animalis*. Samples of milk, urine and stool collected for analysis by nanoflow LC-TOFMS	HMO and oligosaccharides with 3 < DP < 9 identified and quantified in urine and stool of infants

FOS, fructooligosaccharides; DP, degree of polymerisation; HMO, human milk oligosaccharides; GOS, galactooligosaccharides; *B. animalis*, *Bifidobacterium animalis*; LC-TOFMS, liquid chromatography time-of-flight MS.

- Transport of prebiotics across the gut epithelium **in infants**

- **Lack of literature on healthy adults and those with increased gut permeability**

Del Fabbro, Proc. Nutr. Soc. 2020; Prieto, FFIJ. 2005; De Leoz, Anal. Bioanal. Chem. 2013

2. Can prebiotics directly affect immunity in a microbiota-independent way?

- **13** studies reviewed
- **HMO, FOS, inulin, GOS**
- All prebiotics **directly modulated cytokine production** (IL-6, IL-8, IL-10, IL-12, MCP-1, MIP-3α and TNF-α) **and immune cell maturation** (lymphocytes, DCs) ***in vitro***, with mechanisms involving toll-like receptor ligation
- One *in vivo* study in germ-free mice reinforced *in vitro* evidence

HMOs

- Clear anti-inflammatory properties *in vitro*, which might explain protective effects against allergy/infection *in vivo*

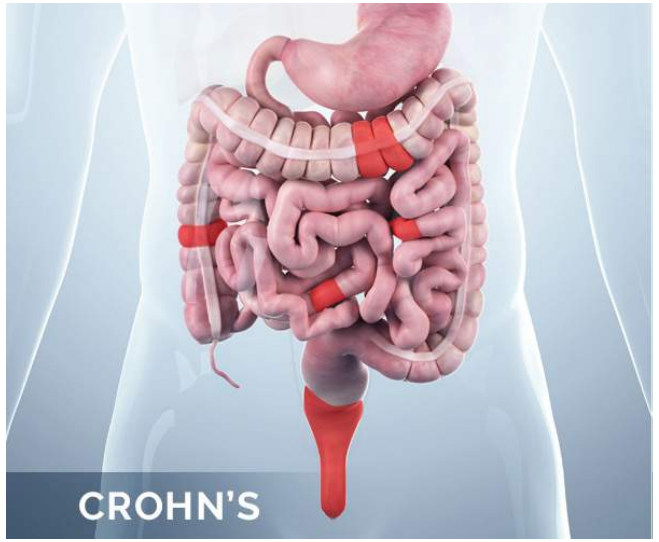
FOS, inulin and GOS

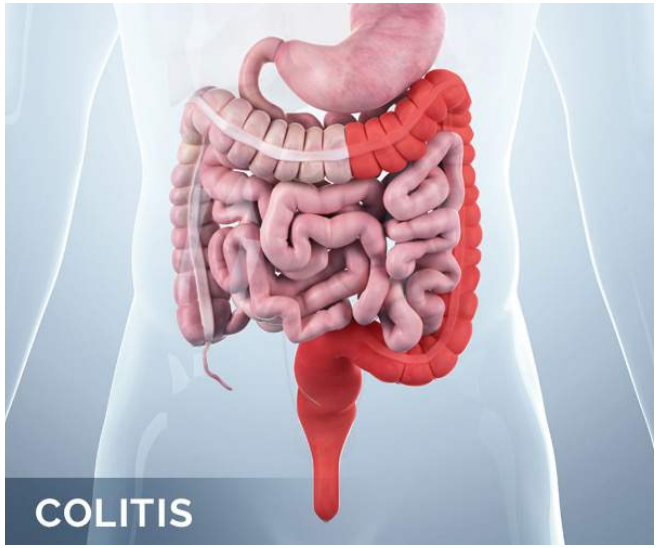
- Various outcomes, including anti-inflammatory / pro-inflammatory effects
- Different doses/types; cell culture models; chain lengths;

Del Fabbro, Proc. Nutr. Soc. 2020

Inflammatory bowel diseases (IBD) and prebiotics

- IBDs are chronic and relapsing conditions affecting the GI tract
- Associated with inflammation, dysbiosis and increased gut permeability
- No cure, only maintenance treatments (e.g. immunosuppressants, anti-inflammatory drugs and antibiotics)
- **Increasing interest in prebiotics as a preventive/support therapy in IBDs**





Ng et al., The Lancet 2017; Maloy and Powrie, Nature 2011; Calder et al., Brit. J. Nutr. 2009

Effects of prebiotics on IBDs: animal models and human clinical trials

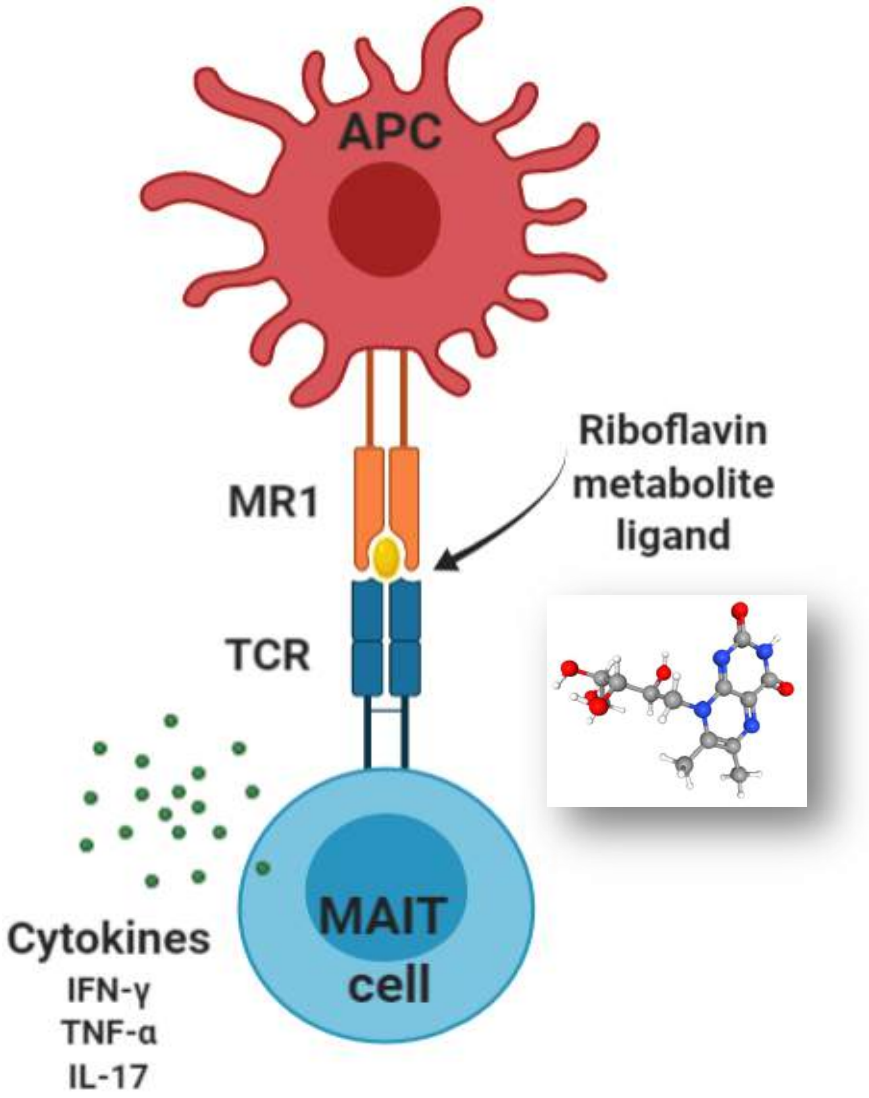
- Prebiotics promising in IBDs for role in restoring gut microbiota homeostasis and affecting cytokine production and immune cell maturation

- Various prebiotics (lactulose, inulin, goat milk oligosaccharides, GOS and FOS) have different potential in attenuating inflammation
- More studies using the experimental colitis model are needed

- Human clinical trials available for FOS and inulin, but not for GOS or other NDOs
- Inulin appears promising in reducing IBD symptoms and inflammation. Only few studies with FOS and no studies with GOS
- More research using standardised methods needs to be conducted

Mucosal-associated invariant T (MAIT) cells

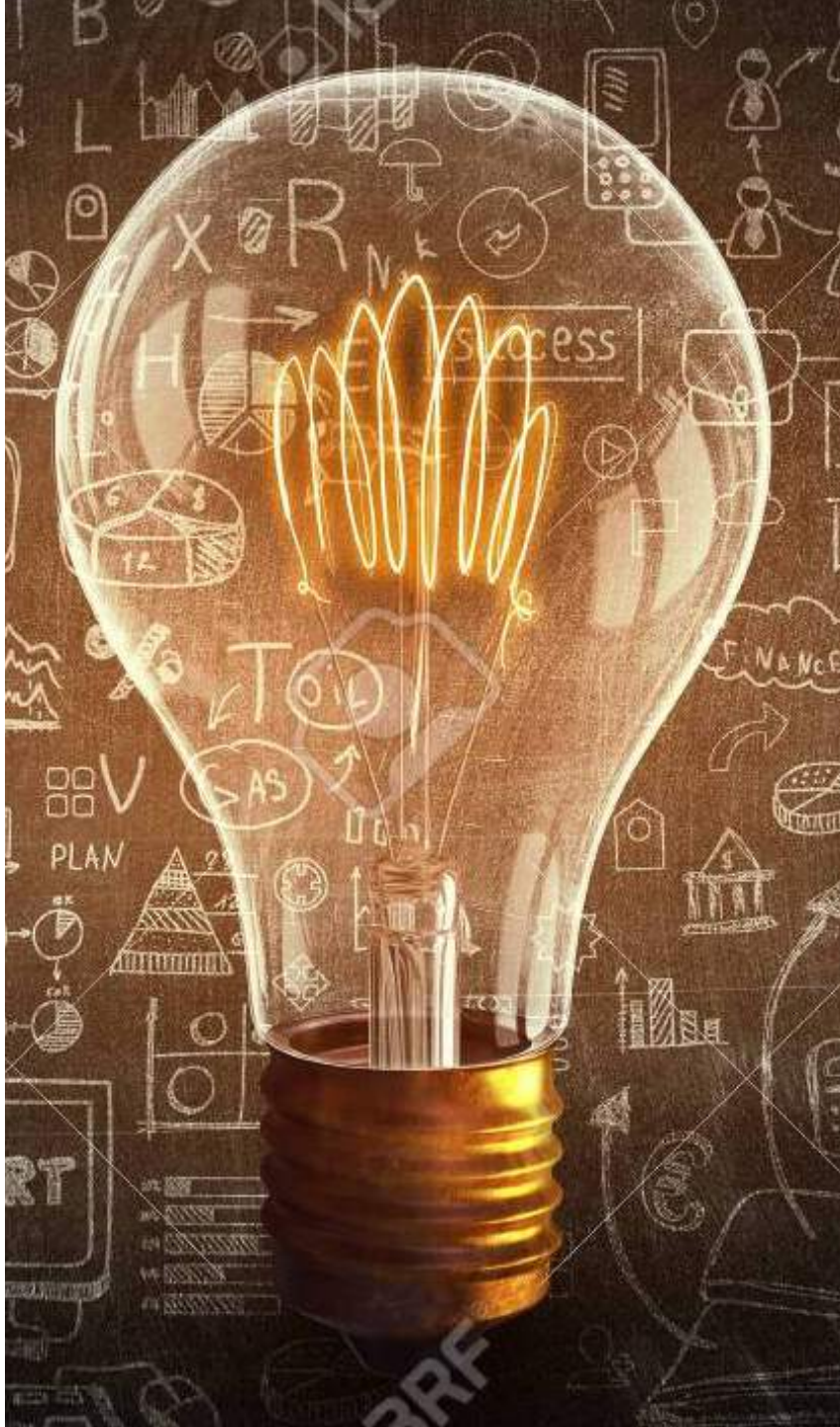
- **T cells** with a key role in **immune surveillance**
- Found in **high numbers** within the **gut**
- **Alterations** in MAIT cell frequencies and activation status found in **IBD patients**
- **Intermediates** produced by **gut bacteria** during **riboflavin** synthesis are **MAIT cell ligands**
- Unknown metabolites synthesised by **probiotic strains** activate or modulate MAIT cell function
- **MAIT cells: an important gut-immune link**



Haga, J. Gastroenterol. Hepatol. 2016; Serriari, Clin. Exp. Immunol. 2014; Johansson, Front. Immunol. 2016

Future applications and proposed areas of development

- From the literature **different types and structures of prebiotics** seem to have **different effects on immunity** (*anti-inflammatory v. pro-inflammatory*)
- There is a **lack of studies on transportation of NDOs across the intestinal barrier** in healthy and diseased adults. Current research focuses on infants
- There are convincing preliminary data to support **NDOs as immunomodulators in the management of IBD**, but their mechanisms of action are still unclear and larger standardised studies are needed



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Thank you!

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