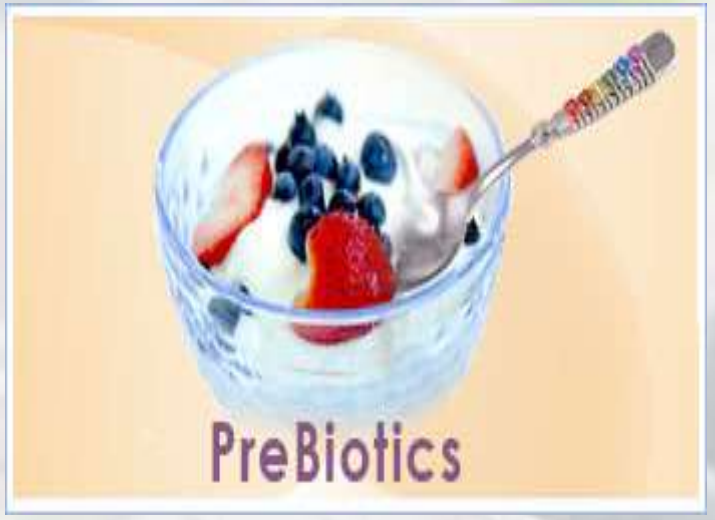
PREBIOTICS & IMMUNITY



Presented By:

Supta Sarkar

HHM-2013-10

M.Sc FN- 2nd Yr.

SEE INSIDE...

- ✓ Introduction
- ✓ Definition
- ✓ Types of prebiotics
- ✓ Mechanism for the effect of prebiotics on immunity
- ✓ Structure & role of different prebiotics
- ✓ Conclusion
- ✓ Reference

Prebiotics

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Introduction

- The concept of prebiotic was introduced by Gibson & Roberfroid, 1995.

- **Prebiotics** is a general term to refer to chemicals that induce the growth and/or activity of commensal microorganisms (e.g., bacteria and fungi) that contribute to the well-being of their host.

- The criteria which must be met in order to be classified a prebiotic, as defined by Gibson & Roberfroid,1995.

-Resistance to hydrolysis or absorption in the upper gastrointestinal tract.

-Fermented by the intestinal microbiota.

-Selectively stimulate the growth and/or activity of beneficial intestinal bacteria, such as Lactobacillius species and Bifidobacterium species.

Definition

- **Gibson & Roberfroid, 1995**:

‘Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth, and/or activity, of one or a limited number of beneficial bacteria in the colon and thus improve host health’.

- **Roberfroid, 2007**:

‘A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health’.

TYPES OF PREBIOTICS

Prebiotic sources:

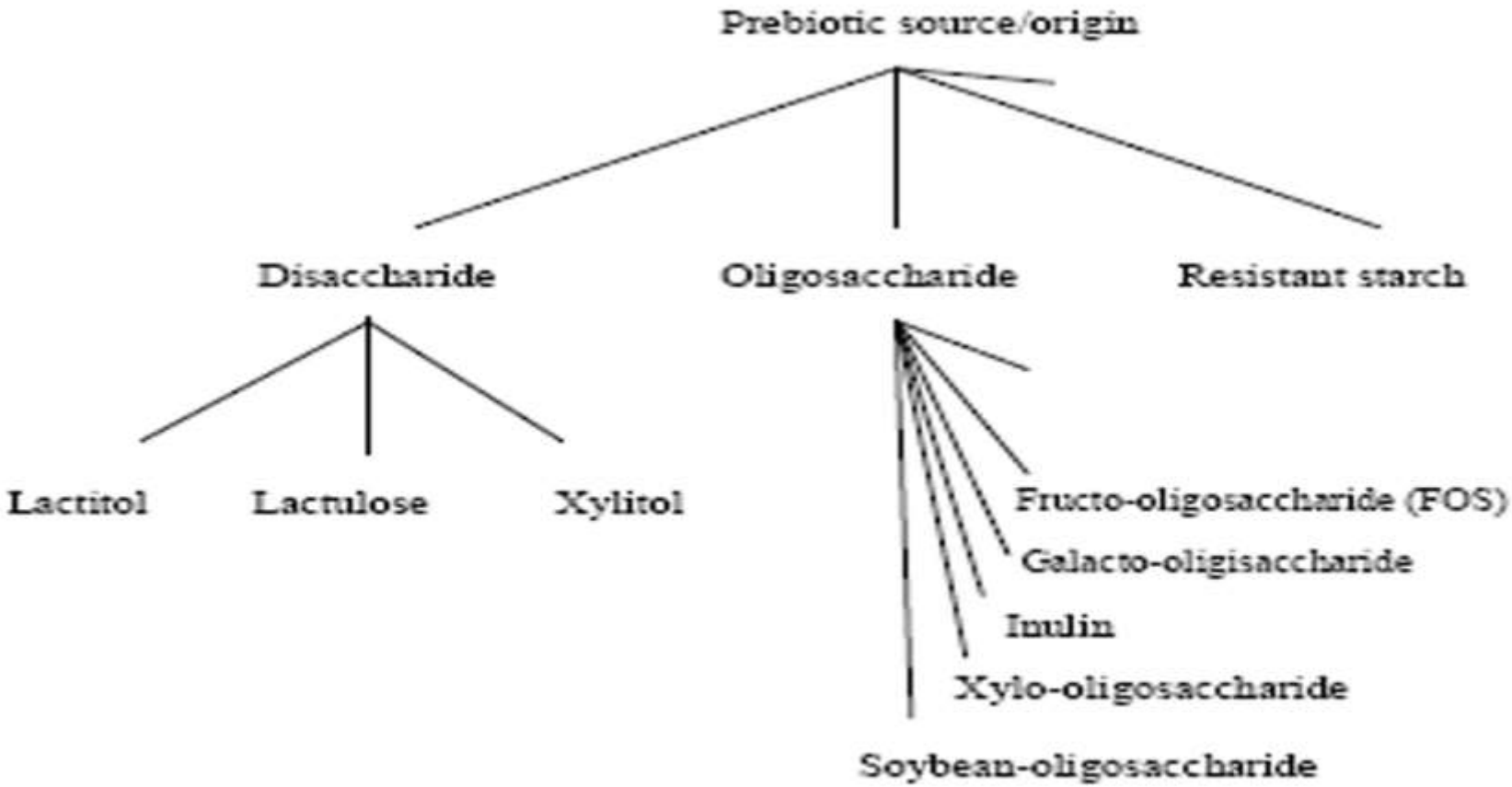


FIGURE 4. Prebiotic origin. Adapted from Magalhães *et al.* (23)./ *Origen de los probióticos. Adaptado de Magalhães et al. (23).*

- Only two particular prebiotics then fully met the refined definition of prebiotics: Inulin(IN) and Trans-galactooligosaccharide (Roberfroid, 2007).

- Other authorities also classify resistant starch, fructooligosaccharide (FOS) and lactulose as prebiotics. Mannan Oligosaccharides (MOS) have been termed as prebiotics but would more correctly be termed **immunosaccharides** (Roberfroid, 2007).

- Carbohydrates including gluco-oligosaccharides, isomalto-oligosaccharides, nigero-oligosaccharides, oat b-glucans, raffinose, soyabean oligosaccharides (SOS), and xylo-oligosaccharides are considered as **candidate prebiotics**.

Mechanism for the effect of prebiotics on immunity

- The underlying mechanisms of how prebiotics modulate the immune system are not known at present.

- However, experimental data suggests that these compounds exert effects in the GALT and also point to a few different mechanisms that might explain these effects:

1. Selective changes in bacterial composition and bacterial products which modulate cytokine and antibody production;
2. Production of SCFAs and their interactions with leukocytes
3. Modulated mucin production
4. Interaction with carbohydrate receptors of pathogens inhibiting their enhancement to epithelial cells as well as receptors on immune cells.

A. SELECTIVE CHANGES IN BACTERIAL COMPOSITION AND BACTERIAL PRODUCTS:

- It is well known that prebiotics increase the no. of beneficial bacterial (i.e bifidobacteria and lactobacilli).
- Probiotics when administered orally are known to increase the secretion of IgA in the small intestine and the feces and to stimulate PP B lymphocyte IgA production.
- They are also known to exert effects on systemic immune functions and various immune parameters in the lungs, spleen and peritoneal cells.

- Intestinal epithelial cells are involved in both innate and adaptive immune responses and act by transducing signals from luminal pathogens to adjacent immune cells of the intestinal immune system, via specific germinal-encoded pattern-recognition receptors, such as **Toll-like receptors (TLRs)** and **cytoplasmic receptors**.

- TLRs are able to discriminate between the normal commensal biota and pathogens and induce the transcriptional activation of a no. of genes mediating immune and inflammatory responses.

- **Pathogen-associated molecular pattern (PAMPs)** [eg., endotoxin(lipopolysaccharide), lipoproteins, lipopeptides and imidazoquinolines] present on diverse microbes are initially recognized by TLRs and their interaction result in the activation of intracellular signaling pathways, nuclear translocation of transcription factor NF-κB and the transcription of pro-inflammatory cytokines.

- The changes that occur in the composition of the intestinal microbiota due to prebiotic fermentation could potentially reduce the presence of PAMPs and thereby exert a positive effects on the immune system.

- Prebiotics also promote an increase in the bacterial cell-wall components that are recognized by TLRs and in DNA derived from luminal bacteria that, in turn, stimulate the intestinal immune system.

- Cytoplasmic components and cell-free extracts of probiotics have also been demonstrated to produce some of the same immune effects (eg, IgA production by PP and macrophage stimulation) as live bacteria.

B. PRODUCTION OF SCFAs:

- The major end products of carbohydrate fermentation are SCFAs, of which acetate, propionate and butyrate are quantitively the most important in the human colon.
- The production of SCFAs in the colon averages 400 mmol/day, with a range of 150-600 mmol/day.
- All SCFAs are rapidly absorbed from the large intestine and stimulate salt and water absorption: principally, the gut epithelium, liver and muscle metabolize them, with virtually none appearing in the urine and only small amounts appearing in the feces.

- The three major SCFAs are trophic when infused into the colon and these trophic properties have important physiological implications in addition to maintaining the mucosal defense barrier against invading organisms.

- Butyrate is known to suppress lymphocyte proliferation, inhibit cytokine production of Th1-lymphocytes and upregulates IL-10 production.

- It also suppresses expression of the transcription factor NF-κB and upregulates TLR expression.

- Butyrate is also believed to protect against colon cancer as it inhibits DNA synthesis and induces cell differentiation.

- It has been demonstrated in a rat model that supplementing total parenteral nutrition with a SCFA mixture results in increased NK cell activity(Pratt et al.,1996).

- Pharmacological doses of acetate administered intravenously to both healthy individuals and cancer patients also increased NK cell activity and peripheral blood antibody production.

- In addition, it has been shown that serum glutamine levels are raised following lactulose administration and suggested that increased SCFA levels were responsible for this (glutamine is a preferred substrate for lymphatic tissue).

- Therefore, SCFA production in the large intestine could potentially reduce the requirement of epithelial cells for glutamine, making it available to the cells of the immune system.

C. MUCIN PRODUCTION:

- The first line of defense of the mucosa against luminal contents is the mucous layer, which is mainly composed of high-molecular-weight glycoprotein (mucins) that are secreted by goblet cells.

- The effect of prebiotics on mucin production has been reported in a study, where it was shown that inulin administration resulted in increased mucin production in rats.

- Greater mucin production was found to be associated with a lower incidence of bacterial translocation across the mucosa following dietary fibre supplementation.

- It has been shown in a perfused rat colon model that the production of acetate and butyrate from the fermentation of dietary fibre stimulates mucin secretion, but fibers do not have the same effect on their own.

- Pathogens and beneficial commensal bacteria are able to modulate mucin synthesis by regulating some of the mucin genes.

- Currently there are 16 identified mucin genes, but further work is needed to fully explain the function of each of them and to identify new genes.

D. CARBOHYDRATE RECEPTORS:

- Studies suggest that some prebiotics are directly involved in protecting the gut from infection and inflammation by inhibiting the attachment of pathogenic bacteria or their toxins to the colonic epithelium.

- This attachment is necessary before pathogens can colonize and cause disease and it is mediated by glycoconjugates on glycoproteins and lipids present on the microvillus membrane.

- Certain prebiotic oligosachharides contain structures, similar to those found on the microvillus membrane, that interfere with the bacterial receptors by binding to them and thus preventing bacterial attachment to the same sugar on microvillus glycoconjugates.

- For example, α-linked TOS, present in human milk, are known to have anti-adhesive properties and be capable of toxin neutralization.

- Recently, a novel TOS mixture, which contains an oligosachharide alpha anomeric configuration, was shown to significatntly decrease the attachment of enteropathogenic *Escherichia coli* (EPEC) and *salmonella enterica* serovar Thyphimurium in vitro.

- In addition, immune cells also express specific carbohydrate receptors which mediate various cellular reactions when activated. For example, C-type receptors expressed on phagocytic cells.

- It is hypothesised that carbohydrate moieties on the prebiotic may interact with receptors on immune cells.

- Although a specific fructose receptor has not yet been identified, receptors for b-glucan and mannose have been identified on immune cells, and in vitro, fructose has been shown to alter non-opsonic phagocytosis, suggesting that a receptor for fructose on immune cells may exist.

- In addition, some oligosaccharides, for example OF, can bind to receptors on pathogenic bacteria and prevent them from attaching to this same sugar on the epithelial membrane, thus preventing adherence.

Structure & role of different prebiotics

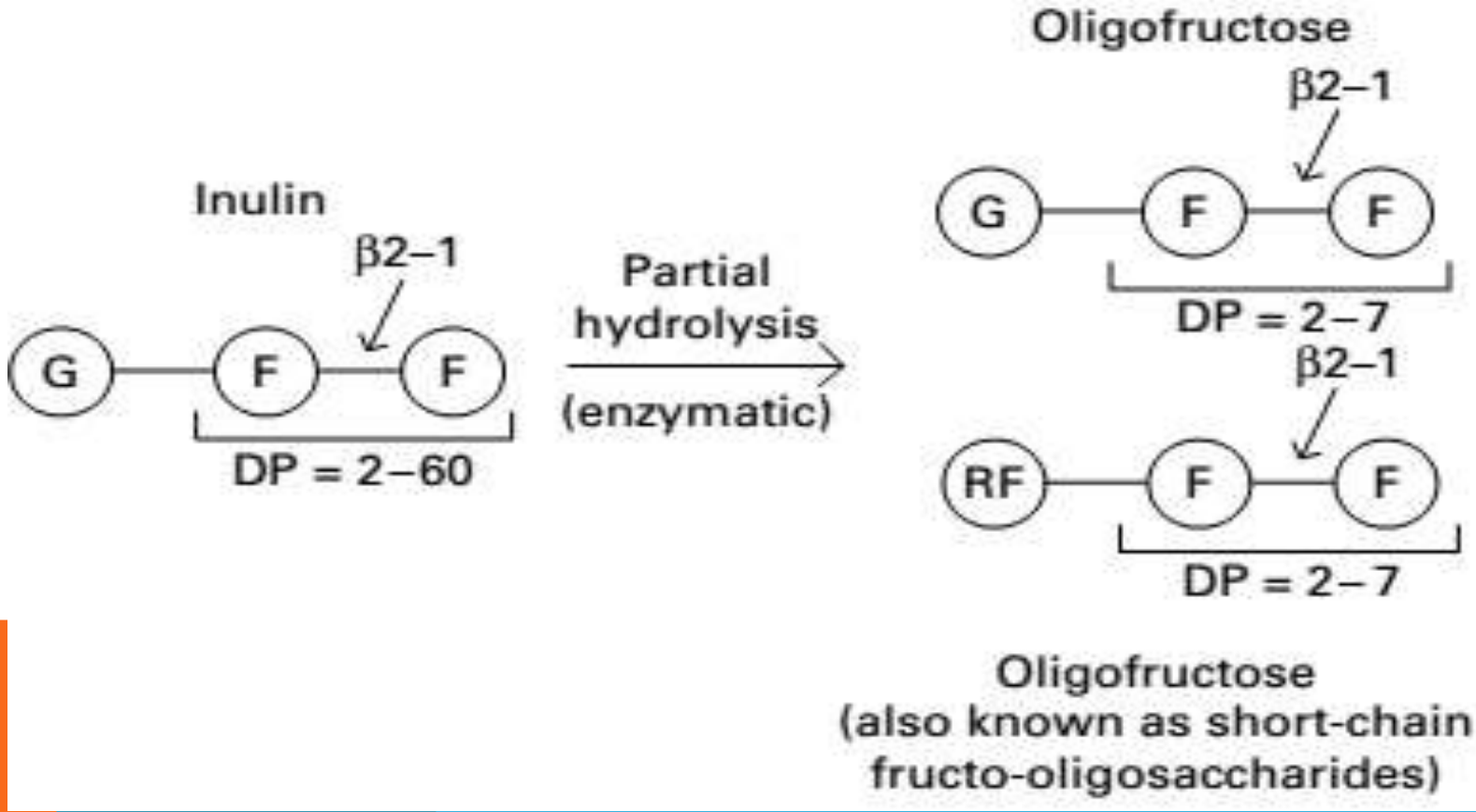
- β2-1 Fructans, which include inulin (IN) and fructo-oligosaccharides (FOS), fulfil the criteria for prebiotics. (Gibson, et al.,2004).

- According to Roberfroid, 2007, only two particular prebiotics then fully met the refined definition of prebiotics: Inulin(IN) and Trans-galactooligosaccharide (TOS).

INULIN:

- Inulin is the most common type of FOS.
- IN is a linear carbohydrate molecule which contains β-(2→1) fructosyl–fructose linkages with a terminal glucose.
- IN may contain between two and sixty fructose residues with an average of twelve. (fig.1)
- Partial enzymatic hydrolysis of IN yields a FOS known as oligofructose(OF), which can have a terminal glucose or fructose residue (fig.1)
- In OF there can be two to eight (average five) fructose residues with a terminal glucose residue or a chain of three to eight (average five) fructose residues.

FIG.1:



Oligofructose
(also known as short-chain
fructo-oligosaccharides)

Dietary Source of Inulin (IN):

- IN is found naturally in a variety of plant foods such as bananas, barley, chicory, garlic, artichoke, leeks, onions and wheat.

- Oligosaccharides, including some believed to be prebiotics, are present in human breast milk.

- The presence of oligosaccharides in large amounts in breast milk suggests that these compounds may play an important role in early infant development, perhaps of the gut, its microbiotia and the immune system.

Role of Inulin on immunity:

- The effect of β2-1 fructans upon macrophage number and function has been studied, with the results suggesting that macrophage functions are enhanced by the addition of β2-1 fructans to the diet.

- Peritoneal macrophage phagocytic activity was increased in rodents given IN or OF for varying periods of time (Kelly-Quaglian *et al.*,2003).

- In rats, OF-enriched IN (100g/kg) increased caecal secretory IgA concentrations(Roller *et al*., 2004).

- The number of T cells in the MLN of rats was increased upon 10% OF or IN supplementation and also the blood IL-2 and IL-4 concentrations were increased upon the supplementation for 4 months (Trushina *et al.*, 2005).

- A study shows the percentage of blood B cells (defined as CD19+) was increased in young male adults after consumption of a bread containing IN (Seidel *et al*., 2007).

REFERENCE	Prebiotics dose used	Animal studied	Immune effect
Stillie et al, 2005	Inulin (4·8 % w/w diet)	Rats (21 d old, male & female); diabetes resistant or diabetes prone	**In diabetes-resistant rats:** ↑Small intestine length ↓ Number of splenocytes ↑CD8+ Lymphocytes in PP ↑ Proliferation of splenocytes and MLN cells to mitogens ↓Production of IL-4 and ↑ production of IL-10 by stimulated splenocytes **In diabetes-prone rats:** ↓Number of splenocytes ↑IgA+ cells in jejunal lamina propria ↑B lymphocytes in PP ↓Production of IL-4 and ↑production of IL-10 by stimulated splenocyte

FRUCTOOLIGOSACCHARIDES (FOS):

STRUCTURE:

- FOS consists of several β(1-2) or β(1-6) linked fructose units which may be linked to glucose residues.

DIETARY SOURCE:

- They can be found naturally in some cereal crops as well as fruits and vegetables such as bananas, onions, chicory root, garlic, asparagus, onion and leeks.

Role of FOS on immunity:

- They stimulate the growth of bifido bacteria and to inhibit growth and multiplication of potentially pathogenic bacteria such as enterobacteria, clostridia and salmonella.

- Fermentation of FOS leads to the production of short chain fatty acids, which is substrate for energy metabolism in the colonic mucosa stimulating epithelial cell growth.

- Short chain FOS can enhance IgA content and thus play a major role in immunity.

- A **study** conducted by Bourgot *et al*.,2014 says that maternal scFOS supplementation modified the intestinal immune functions in piglets in association with increased colostral immunity.

- Thirty-four sows received a standard or a scFOS supplemented diet (10 g scFOS/d) for the last 4 weeks of gestation and the 4 weeks of lactation.

- Colostrum and milk immunoglobulins (Ig) and TGFβ1 concentrations were evaluated on the day of delivery and at d 6 and d 21 postpartum.

- Colostral IgA ($P<0.05$) significantly increased because of scFOS and TGFβ1 concentrations tended to improve ($P<0.1$).

- Such results underline the key role of maternal nutrition in supporting the postnatal development of mucosal immunity.

Another study shows dietary supplement of FOS to the Caspian roach (*Rutilus rutilus*) fry increase the serum Ig levels, lysozyme activity and alternative complement activity (ACH50) and thus improving the innate immune response (Soleimani et al.,2012).

PreBiotics

TRANS-GALACTOOLIGOSACCHARIDES (TOS):

STRUCTURE:

- **Galacto-oligosaccharides** (GOS), also known as oligogalactosyllactose, oligogalactose, oligolactose or **transgalactooligosaccharides (TOS),** belong to the group of prebiotics.

- GOS/TOS generally comprise a chain of galactose units that arise through consecutive transgalactosylation reactions, with a terminal glucose unit. However, where a terminal galactose unit is indicated, hydrolysis of GOS formed at an earlier stage in the process has occurred.

- The degree of polymerization of GOS/TOS can vary quite markedly, ranging from 2 to 8 monomeric units.

Role of TOS/GOS on immunity:

- Galactooligosaccharides, GOS can positively influence the immune system—indirectly through the production of antimicrobial substances as the result of galactooligosaccharide fermentation, that can reduce the proliferation of pathogenic bacteria, and directly by interaction with immune cells.

- In infants the usage of GOS has been shown to have a potential role in allergy prevention and reduction of infectious diseases.

- Several studies have shown that GOS and FOS fermentation can lead to the production of **butyrate**, the principal fuel for colonic epithelial cells. This SCFA also stimulates apoptosis (Rowland 1998) and may be a protective factor in carcinogenesis (Scheppach and Weiler 2004).

- **Propionate** is also produced from GOS, and has been shown to be anti-inflammatory with respect to colon cancer cells (Nurmi et al. 2005).

Role of TOS/GOS on Mucosal Immune System:

- The intestinal mucosa contains large amounts of sIgA which has a protective role against adherence and invasion by harmful bacteria and viruses.
- Levels of sIgA in faeces have been found to correlate with an increased ability to neutralize and clear viruses.
- Infants have an immature immune system, and because of the lack of transfer of immunoglobulins from breast milk, formula-fed babies have low levels of sIgA, which can lead to increased risk of gastrointestinal infection.
- Thus, the addition of prebiotics such as GOS alone or in combination with probiotic bifidobacteria or lactobacilli with immunomodulatory abilities that are able to utilize the substrate, has potential to increase production of sIgA in the body.

ROLE OF OTHER PREBIOTICS ON IMMUNITY:

OLIGOFRUCTOSE:

- The studies conducted with recognized prebiotic fibres (oligofructose) have shown increased lymphocyte and/or leucocyte numbers in GALT (Gaskins *et al*., 1996; Pierre *et al*., 1997; Field *et al*.,1999) and peripheral blood (Kaufhold *et al*., 2000).

PECTIN:

- More $CD4^+$ T cells have been found in mesenteric lymph nodes in rats fed a diet containing 5% pectin, compared to less fermentable cellulose (Lim *et al.,* 1997).

LACTULOSE:

- Studies have documented that feeding lactulose is associated with increases in IgA secretion or IgA+ cells in GALT (Kudohet al.1998; Kudohet al.1999), a decrease in the CD4+/CD8+ ratio in the spleen (Kudoh et al. 1998), and an increase in the phagocytic function of intraperitoneal macrophages (Nagendra & VenkatRao, 1994).

- Immunological effects have been observed after adding prebiotics such as FOS and lactulose to the diet, including increases in mucosal immunoglobulin production, mesenteric lymph nodes, Peyer's patches, and altered cytokine formation and lymphocyte numbers in the spleen and intestinal mucosa (Schley and Field, 2002).

The few studies that have examined the effects of prebiotic fibres on the immune system are reviewed in the Table:

REFERENCE	SUBJECTS	EXPERIMENTAL FIBRE DOSE	CONTROL DIET/ FIBRE DOSE	IMMUNE EFFECT
Field et al., 1999	Adult mongrel dogs	Fermentable fibre mixture (beet pulp, Oligofructose, gum arabic), 8·7 g/kg	Cellulose, 8·3 g/kg	• ↑ CD8+ cells in Intra Epithelial Lymphocytes(IEL), PP and LP • ↑ CD4+ cells in MLN and peripheral blood. • ↑ T cell mitogen responses in MLN and IEL
Lim et al.1997	Sprague-Dawley rats	Pectin, konjak mannin, or chitosan, 5 % w/w	cellulose, 5 % w/w	• ↓serum and MLN IgE (all fibres) • ↑serum IgA and IgG (pectin) • ↑MLN IgA and IgG (pectin, chitosan) • ↑CD4+ T cells in MLN (pectin) • ↑INF-γ in MLN (pectin)

REFERENCE	SUBJECTS	EXPERIMENTAL FIBRE DOSE	CONTROL DIET/ FIBRE DOSE	IMMUNE EFFECT
Pierre et al.1997	Min mice	Oligofructose (from sucrose), wheat bran, or resistant starch, 5·8 % w/w	Cellulose, 2 % w/w	• ↑ number of PP in small intestine (short-chain FOS)
Yamada et al., 1999	Sprague-Dawley rats	PHGG, guar gum, HM pectin, or glucomannan, 5 % w/w	cellulose, 5 % w/w	• ↑IgA in spleen and MLN (all fibres). • ↑ IgG in spleen (glucomannan, pectin) and MLN (all fibres). • ↑ serum IgA (guar gum, glucomannan, pectin) and IgM (glucomannan).
Yun et al., 1997	C57BL/6 mice (immunosuppressed	Oat b-glucan, 3 mg every 48 hr	Diet not specified	• ↑non-specific and antigen-specific IgG in serum. • ↑ IFN-γ-and IL-4-secreting cells in spleen and MLN.

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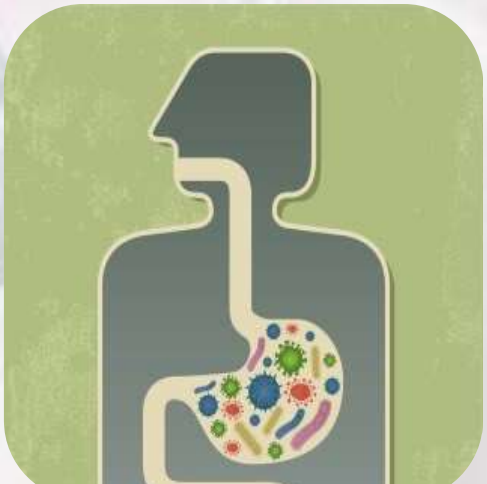
Conclusion

- Chemicals or food ingredients, selectively fermentable.
- supports the beneficial bacteria.
- Plays major role in immunity
- Mainly 2 types of prebiotic fulfill all the criteria.
- ***Prebiotics*** *can be* combined with ***probiotics*** to form ***Synbiotics*** which can be given as nutritional supplements to get **synergic effect**.



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SO ... DO YOU HAVE ANY
QUESTIONS FOR ME?