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Session 8: Probiotics in the defence and metabolic balance of the organism
Probiotics: beneficial factors of the defence system

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Probiotics, defined as living micro-organisms that provide a health benefit to the host when ingested in adequate amounts, have been used traditionally as food components to help the body to recover from diarrhoea. They are commonly ingested as part of fermented foods, mostly in fresh fermented dairy products. They can interact with the host through different components of the gut defence systems. There is mounting clinical evidence that some probiotics, but not all, help the defence of the host as demonstrated by either a shorter duration of infections or a decrease in the host's susceptibility to pathogens. Different components of the gut barrier can be involved in the strengthening of the body's defences: the gut microbiota, the gut epithelial barrier and the immune system. Many studies have been conducted in normal free-living subjects or in subjects during common infections like the common cold and show that some probiotic-containing foods can improve the functioning of or strengthen the body's defence. Specific probiotic foods can be included in the usual balanced diet of consumers to help them to better cope with the daily challenges of their environment.

Probiotics: Defence: Immune System

Probiotics, defined by FAO/WHO[1] as living micro-organisms that provide a health benefit to the host when ingested in adequate amounts, are components of traditional foods that have been consumed for a long time in various cultures in order to help individuals to cope with infections, particularly of the gastrointestinal tract. Science is providing modern tools that help us to decipher what is legend and what is scientifically relevant. There are data to support the concept that intake of some probiotics and/or foods containing probiotics can provide demonstrated benefits to the defence systems of the host, particularly to the gut defence systems. The gut can be considered to be composed of two main organs. One is the gut microbiota, a prokaryotic organ made up of a huge number of and a great diversity of transient and permanent micro-organisms called commensals. The other one is the gut itself, a eukaryotic organ pertaining to the host, comprising the epithelium and the gut-associated immune system. Gut functions can be classed as three separate functions: digestion and absorption, waste management, and defence.

Each function can be divided into microbial-associated characteristics and gut-associated characteristics[2]. Gut defence is probably the most complex function since it involves an integrated multifaceted system commonly described as involving three components from the gut lumen to the 'milieu interieur': first the microbial barrier, then the gut wall and finally the immune and inflammatory systems[3].

The endogenous gut microbiota is composed of a large variety of phyla, species and strains, with different compositions in the various parts of the gut. The bulk of the gut microbiota is in the colon with 10^{10} micro-organisms/g digestive fluid. Some of these bacteria are within the mucus, and others are 'free' in the lumen. Although most of these bacteria are likely to be neutral as far as the health of the host is concerned, some of them are considered as good bacteria, while some others have a less clear role and may even become deleterious to the host, acting like waiting opportunists. The defence function of the microbiota can be assessed by the measure of colonization

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resistance, which is the capacity of the established microbiota to prevent new exogenous comers from establishing and/or proliferating in the gut.

The gut wall is composed of a monocellular layer of epithelial cells. Most of them are enterocytes, but there are also cells specialized in mucus secretion (Goblet cells) or in secretion of antimicrobial enzymes called defensins (Paneth cells). The defence/barrier function of the gut wall can be assessed by the measure of trans-epithelial permeability and the functioning of tight junctions that maintain intercellular cohesiveness[4].

The immune system is a complex system acting and reacting locally (70% of human immune cells are associated with the gut) and at the systemic level. Recent research has focused on the regulation of the immune system and the interactions within, and components of, inflammatory cascades. Different markers are used to explore the various parts of the immune and inflammatory systems[5], and the integrated functioning can be assessed by the ability of the host to cope with the microbial challenges of the environment and to manage the responses to common or even severe infections.

These three components, namely the microbiota, epithelium and immune systems, can react directly against a pathogen. They are also interacting with each other: the gut microbiota interacts with epithelial cells through Toll-like receptors[6], or the NF-κB cascade[7]; the epithelium is interacting with the immune system and, in fact, some immune cells are part of the epithelium in the Peyer patches; the microbiota interacts with the immune systems and is one trigger for the local inflammation. All these components are quite dynamic with a quick turnover: for example the mucus is renewed within a few hours, and epithelial cells are renewed every 4–6 days[8,9]. The high degree of complexity can also partially explain why the defence capacities do not have a steady status. They fluctuate across the day, the seasons and the lifespan[10]. For example, it is well known that there is a decrease in the defence capacity during malnutrition and with advancing age[11]. The number of elderly consumers is increasing in all countries and the potential health consequences of a normal ageing process altering the normal immune functions will emphasize the important role of immunonutrition in compensating for the physiological risk factor, ageing.

Demonstration of the impact of some probiotics on the defence capacity of the host

A meta-analysis of nine randomized controlled clinical trials in children using Lactobacilli as probiotics (*Lactobacillus rhamnosus* or *L. reuteri*) reported a significant reduction in duration of diarrhoea of 0·7 d in probiotic groups as compared to controls[12]. Agarwal & Bhasin[13] reported a significant 25% reduction of diarrhoea duration in a group of 175 children admitted into care centres for diarrhoea in India; in this study the controls received either a local fermented dahi (without *Lactobacillus casei*) or a UHT fermented milk, and the test group received a specific probiotic (*L. casei* DN-114001) in fermented milk. Canani *et al.*[14] compared the effect of intake of five different

probiotics or probiotic combinations during acute diarrhoea in children, and reported a significant benefit for *L. rhamnosus* and for a combination of *Lactobacillus bulgaricus*, *L. acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*. This study confirmed that not all probiotics behave similarly.

Szajewska *et al.*[15] reported a significant effect on gut barrier defence challenged by antibiotics in a meta-analysis of six randomized clinical trials on 766 children. The significant reduction of the relative risk of antibiotic-associated diarrhoea in children was different for different probiotics: RR 0·2 for the *Saccharomyces boulardii*, 0·3 for the *L. rhamnosus* and 0·5 for the combination of *Bifidobacterium lactis* and *S. thermophilus*. In adults, Cremonini *et al.*[16] reported a significant reduction (RR 0·396) in antibiotic-associated diarrhoea with *S. boulardii* and *L. species* in a meta-analysis of seven randomized controlled trials including 881 subjects. In a recent a randomized controlled trial on 135 hospitalized elderly subjects receiving antibiotic therapy, Hickson *et al.*[17] reported a significant reduction of antibiotic-associated diarrhoea from 34 in the control group to 12 in the probiotic group (*L. casei* DN 114001) and, more strikingly, an absence of *Clostridium difficile* toxin in the stools of the probiotic group, while there were 17 positive samples in the control group. McFarland[18] confirmed, in a meta-analysis of six randomized controlled trials, a significant risk reduction (RR 0·59) of *C. difficile*-associated diarrhoea with the probiotic *S. boulardii*.

The beneficial impact of probiotics on defence capacities of the host go beyond the gut. A randomized clinical trial[19] compared the morbidity of a cohort of free-living elderly during winter time and reported that the number of illnesses was not different between the control and probiotic groups, but that the duration was significantly shorter: 8·3 d in the control group and 7·0 d in the probiotic group (*P*<0·01). This is a 15% reduction of disease state. This beneficial effect has been confirmed more recently in a similar randomized controlled study[20] conducted on 1000 free-living elderly receiving either a milk fermented by the specific probiotic or a non-fermented control. Results were also similar: the same number of infections in both groups, but a significant reduction of duration (6·5 *v*. 8 d) of infections in the probiotic group. It is not easy to quantify the health consequences of such reduction. One way to assess it is to calculate the benefit of 1 d of hospitalization in an elderly ward. It becomes clear that for the community the benefit is going beyond the statistical value.

Thus, there are convincing reports on the efficacy of some probiotics in improving the defence capacities of the host. It is of interest to explore mechanisms by which probiotics have these actions.

Mechanisms of action of some probiotics on the gut microbiota

Effect on the composition of the gut microbiota

The intake of either yoghurt or a specific probiotic fermented milk (*L. casei* DN 114001) was able to increase

significantly the number of young children with an adequate number ($>10^6$/g) of lactobacilli in their stools[21]. The rate of acquisition of lactobacilli was higher for the probiotic group and a higher colonization was achieved in that group. Both effects vanished in the weeks following the end of intake.

Effect on the function of the gut microbiota

One of the main defensive functions of the gut microbiota is colonization resistance that prevents transient micro-organisms from becoming permanent components of a given microbiota. The first demonstration of this probiotic effect was by Nissle[22] in 1916 when he was able to clear healthy typhoid carriers of their *Salmonella* infection. The efficacy of some probiotics in defence against antibiotic-associated diarrhoea is a clear demonstration of the benefit of these probiotics to help restore the function of colonization resistance that has been weakened or disrupted by antibiotics. Similarly, the prevention of emergence of opportunistic micro-organisms such as *C. difficile* is another indication[17] of the beneficial effect of some probiotics on the defensive function.

Mechanisms of action of some probiotics on the gut wall

Some probiotics can induce a change in the composition of gut mucus and therefore reduce the binding capacity of some micro-organisms. This has been shown in a cell model where a co-incubation of colonocytes with a specific Lactobacillus (*L. casei* DN 114001) changed significantly the ratio of sialic acid and galactose of the secreted mucus and inhibited the adhesion of a fluorescently labelled *rotavirus*[23]. Some probiotics can also strengthen the gut barrier, reducing its permeability, as demonstrated by the change in trans-epithelial electrical resistance and the restoration of tight junctions as expressed by the amount of zona occludens proteins[24]. This was clearly demonstrated when the gut cell barrier was challenged by an entero-pathogenic *Escherichia coli*[24]. The permeability of the gut barrier can be modulated by different probiotics[25,26]. Part of this activity is provided by soluble factors released from probiotics as reported by Putaala *et al.*[27]. A last main defence tool of the gut wall is the secretion of defensins by Paneth cells. There are *ex vivo* data reporting a stimulation of production of some defensins by different probiotics[28,29], and a human trial confirmed that intake of a probiotic was able to increase the amount of β defensin 2 in the faeces[30].

Mechanisms of action of some probiotics on the immune and inflammatory systems

The complexity of the systems as well as the number of biological markers and models that are used to assess the functions of the immune system generate a body of data that are impossible to summarize in a few lines, but these findings have been reviewed in some detail elsewhere[31]. However, it is useful to illustrate the different potential effects of different strains even within the same *Lactobacillus* species. Maassen *et al.*[32] measured the impact on the cytokine profile of different lactobacilli given orally to conventional mice, and they reported different pro- and anti-inflammatory cytokine secretion profiles (interferon-γ, IL-10 or IL-2) following exposure to *L. murines* or *L. casei* or *L. gasseri* or *L. plantarum*, or *L. fermentum* or *L. brevis* or *L. reuteri*.

The consumption of probiotic fermented milks can also modulate some cellular markers of the immune system. Using an academic examination stress as a triggering factor, Marcos *et al.*[33] reported a significant change in the blood concentration of lymphocytes and CD56 cells in the probiotic group as compared to the control one. Pujol *et al.*[34] reported a significantly smaller decrease of blood natural killer cells induced by an intense cycling test in the probiotic group as compared to the control group after 1 month of daily intake of a large amount of fermented milk. Some probiotics may also change the functionality of circulating immune blood cells, such as monocytes. Parra *et al.*[35] reported a significant improvement of the oxidative burst capacity of monocytes in a randomized controlled trial enrolling 45 healthy adults for an 8-week consumption of a fermented milk or a non-fermented control. Modulation of the antibody response after a vaccination is recommended to explore the impact of food and nutrition on the immune system. A recent randomized controlled trial reported a significant improvement of the antibody response after the seasonal influenza vaccination in a group of institutionalized elderly consuming a specific probiotic 1 month before and several weeks after the vaccination[36]. The kinetics of the antibody responses were similar in both the control and the probiotic group, but the level was significantly higher in the probiotic group. Finally, some probiotics may have an impact on the regulation of the inflammatory response, at least at the mucosal level. An *ex vivo* study reported the effect of different micro-organisms on the inflammatory response, expressed by the cellular secretion of TNFα, of gut samples collected from patients with Crohn's disease undergoing surgery[37]. The probiotic *L. casei* tested was able to reduce dramatically the inflammatory status of the inflamed cells, while some other lactobacilli, including some from the human gut microbiota, were unable to change significantly the inflammatory status. This interaction of prokaryotic and eukaryotic cells is intriguing, but the discovery of a family of receptors (Toll-like receptors) at the surface of the gut epithelial cells and triggered by different constituents of the microbiota is opening a new door for understanding another part of the cross-talk between these two communities[6].

Conclusions

Intake of some probiotics as components of fermented milks results in a significant improvement of the host's defences and in the capacity to cope with different challenges from the environment, either infection or stresses that weaken part of the defence systems. The effect of probiotics can be explained either by a direct effect on the

gut microbiota, its composition or its defensive function (colonization resistance) or by an effect on the gut mucosa (e.g. mucus, permeability and defensins) or by an effect on the immune and inflammatory systems. However, probiotics, like other exogenous micro-organisms, are transient in the gut, and a regular intake is necessary to provide a sustainable benefit.

Acknowledgement

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