

Probiotics and food-allergic diseases

The definition of probiotics is always evolving, since it includes natural live micro-organisms, cellular subfractions, as well as genetically engineered derivatives or proteins. The scope of probiotic administration is a beneficial change of the intestinal microflora, and improvement of nonimmune or immune resistance in the intestinal tract. Very few controlled human studies have been reported, but many *in vitro* and experimental animal studies point to their safety and potentially useful applications. We shall review the published reports and discuss mainly the prospective uses in the field of allergic diseases, with reference to the implication of the natural (innate) immune system as regulator of the development of abnormal responses to ingested food antigens.

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Introduction

The definition of probiotics is in constant progress, since it includes normal live micro-organisms as commensals of the human digestive tract, and their secreted products, sometimes developing as enterotoxins, and genetically modified strains as well as biotechnology derived products (1). All these different substances, and organisms, are collectively called probiotics when they are thought to exert beneficial effects on the host well-being, mainly operated through the improvement of the gut microflora. Most used are bifidobacteria and lactobacilli, due to their intrinsic properties of resistance in the intestinal transit, the ability to adhere to enterocytes, and largely known safety of human use (2). Nonprobiotics do not survive the long journey from the oropharynx to the distal intestinal tract, so they seem to be unable to modify the existing microbial flora. Conventionally probiotics are considered as dietary supplements, but they have also been used for therapy of diarrhoeic states of undefined origin, and as positive modulators of local (intestinal) mucosal immune responses (3).

Materials and methods

We examined the existing body of published evidence on probiotics administration to humans, the reviews on their *in vitro* effects on immune and non immune functions, and those regarding alterations of the gut microflora in animal studies, as determined by MedLine citations. Our analysis took into account the size and endpoints of human studies, the accuracy of methodology and estimation of

endpoints, the confidence limits of results declared, and for experimental studies the comparative soundness of results in different systems, as well as other indicators of good standardization of methods.

Results

Food allergy and tolerance are influenced by several host factors, including the genetic background, affecting mainly antigen clearance, age of first antigenic contact, due to immaturity of the host immune system, or its weakening associated with aging, individual differences in intestinal antigen uptake, well documented in human and experimental studies, but mainly by the digestive tract microflora (3). The type of gut colonization during the first weeks of life may predispose an individual to atopic disease, due to food or inhalant sensitization (2), and the changes induced by several pathogens in this context may mediate not only resistance to infection, but also the immunological environment for subsequent challenges, including food allergens. Microbial lipoproteins and lipopolysaccharide (LPS), and enterotoxins, are among the most potent modulators of natural and adaptive immune responses (4). Cholera toxin and other heat-labile enterotoxins from *E. coli* and other pathogenic strains (*Staphylococcus*, and others) are potent immunogens and also strong polyclonal activators of the immune system (5). Staphylococcal enterotoxins are able to bind to nonpolymorphic portions of the T cell receptor, delivering an activating signal to the T

lymphocyte, and are known as superantigens. Some of them are endowed with adjuvant activity, which can either enhance a beneficial action of the immune system with regard to other ingested antigens, by means of IgA immune exclusion (1) or down-regulation of potentially harmful responses, or alternatively damage regulatory mechanisms leading to oral tolerance induction. Bacterial LPS interacts with CD14 on macrophages, and lipoproteins with Toll-like receptors (4, 6), inducing cellular activation and transcription of cytokines (IL-12) and inducible nitric oxide synthase with both microbicidal effects and polarization of immune responses towards a type 1 phenotype (i.e. IL-2 and IFN- γ). Antimicrobial peptides are then produced by cells belonging to the innate immune system, including defensins, collectins, pentraxins and complement components (4). Recognition of foreign antigens and pathogens relies upon highly conserved pattern recognition receptors (PRR), sensing biomolecular diversity among species. Another effect of the activation of the innate immune system is the expression of potent costimulatory signals by dendritic and other antigen-presenting cells, for activation of the adaptive (T and B lymphocyte) immune system. Damage to the circuitry of the innate immune system results in disease, as observed in primary immunodeficiencies with genetic inability to express IFN- γ or IL-12 receptors, in cystic fibrosis where altered salinity of the bronchial fluid inactivates antimicrobial peptides, in mutations of mannose-binding lectin, a useful collectin for complement pathway recruitment, and in severe burns.

Bacterial biofilms, such as the intestinal microflora, are a prominent cause of nosocomial infections (7), and they change at sites of inflammation or insertion of inert medical materials as catheters, giving rise to bacterial aggressive species which in over half of the cases are commensal with the human body. Gut commensals achieve their establishment in a competitive ecosystem through molecular sensors allowing use of nutrients. The microflora undergoes several changes during life, and according to the intestinal site.

Probiotics administration dramatically alters the gut microenvironment by promoting a change in the local microflora and in the cytokine secretion. The bacterial motifs engaging PRR on host cells (macrophages, dendritic cells, eosinophils, neutrophils, natural killer and M cells) and the activation of Toll-like receptors induce nitric oxide production and enhance resistance to mucosal infection, promote IgA plasma cell maturation and facilitate oral tolerance induction through epithelial cell presentation of food antigens. The result is down regulation of the inflammatory machinery present at mucosal level, and a stimulation of

monocyte-derived dendritic cells type1 to activate NF- κ B and promote a type 1 response, thus preventing allergic inflammatory events when food antigen is reintroduced. Another possible consequence is the stimulation of type 3 or regulatory T cells to secrete anti-inflammatory cytokines such as IL-10 and TGF- β , which seem to be crucial in inflammatory bowel diseases. In this context, germ-free animals or gene knock-outs (KO) represent further tools to confirm this evidence. Mice which are germ free show defects in oral tolerance induction, and IL-10 KO too.

Not all probiotics have been tested in clinical studies with regard to allergy prevention or treatment, but in the few published studies several differences of activity have surfaced. In particular, fermenting lactobacilli used for yoghurt production appear to be widely diverse in their actions. *L. bulgaricus* seemed to have no effect on immune parameters, whereas it was associated with lower frequency of allergies, but *L. acidophilus* consumption accelerated recovery from food allergy symptoms. These differences have also been observed in infants with eczema and cow's milk allergy using alternative feeding supplemented with *L. rhamnosus*.

Cell wall free homogenates of several lactobacilli, propionibacterium, streptococci and bifidobacteria have also been used in clinical studies and with similar protective effects on food allergy development and Th-2 cytokine orientation as observed in *in vitro* studies.

Safety records for all such micro-organisms, their products and biotechnology by-products (which are at the moment suspended from human use due to new regulations on genetically modified micro-organisms) are so far excellent, but newly isolated probiotics shall pass an extensive battery of safety tests before they can be released for human use.

Discussion

Great care must be taken in transferring data from *in vitro* and experimental animal studies into human use. This general proposition applies also to the use of known probiotics, some of which are already present in human nutrition, such as yoghurt. Their immune-modifying effects and possible antiallergic and anti-cancer actions deserve large clinical studies also to monitor potential adverse effects. Knowledge of their molecular mechanisms of action should also receive major attention. A final word of caution should be reserved for safety aspects, long-term effects, *in vitro* selection of strains, also in spite of the public concerns regarding food, last but not least bovine spongiform encephalomyelitis.

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