Letter to the Editor

Th17 skewing in the GALT of a Crohn disease patient upon Lactobacillus rhamnosus GG consumption

Dear Sir,

We wish to report on a recent observation we made and that might represent a useful hint for the employment of the so-called probiotics in the management of some pathological immune reactions of the gut.

Probiotics have been defined as “live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002) [1]. Currently, there is a certain consensus that probiotic bacteria can help in the relief of symptoms associated to several human diseases, including some immunopathologies such as atopic diseases and inflammatory bowel diseases (IBD), chronic infections [2], neoplasms and they have even been proposed to play a role in the complex immune network underlying atherogenesis [3,4]. The beneficial effects of probiotics, which are usually administered per os, have been ascribed to different factors: help in the absorption of otherwise indigestible nutrients, especially complex carbohydrates; release of anti-inflammatory soluble factors; production of antimicrobial substances; competition for adhesion, sites and nutrients with pathogenic bacteria; stimulation of the immune system [5]. In particular, this last mechanism, i.e. the immunomodulatory activity of probiotics, has been widely investigated and different commensal bacteria, which are usually administered as probiotics, have been shown to possess relevant properties, such as the ability to finely polarize both the innate and the adaptive immune response [5]. These commensal bacteria should mainly exert direct effects on antigen presenting cells such as dendritic cells (DCs), monocytes, macrophages, and, to a minor extent, B-cells, without necessarily being the source of antigen. In vitro, different strains of commensal bacteria possess the ability to induce a discrete production of cytokines by myeloid DCs, which in turn would differently polarize the subsequent adaptive immune response toward specific T helper (Th) cell subsets (Th1, Th2, Th17) or even T regulatory cells [6–8]. These observations, especially the ability to polarize T cell response, support probiotics as interesting candidates in the treatment of immune-mediated pathologies, such as allergies and Crohn disease (CD), characterized by a disruption of the Th balance and an excess of, respectively, Th2 or Th1 immune response.

Despite these interesting observations, the usefulness of probiotics administration in these illnesses is often argued on the basis of a lack of evidence that, upon their consumption, commensal bacteria passing through the gut, apparently without colonizing it, could establish close interactions with local antigen presenting cells or other immune cells. As a matter of fact, a main obstacle to these contacts is supposed to be represented by the mucus layer in the intestinal lumen.

We had the opportunity to analyze biopsies of gut-associated lymphoid tissues (GALT) performed during routine colonoscopy in patients (pts) followed up for either previously resected colon cancer or CD; some of these pts received Lactobacillus rhamnosus GG (LGG) as oral administration of $6 \times 10^9$ CFU three times per day. LGG (ATCC 53103) is a well-documented probiotic strain described to be endowed with the capability of ameliorating the symptoms of inflamed gut mucosa [9]. In a pt with CD and in five pts with normal intestinal mucosa (the latter in follow up for colon cancer) we managed to analyze GALT biopsies before and after 20 days of treatment with LGG (at the dosage indicated above), which was a suitable opportunity to evaluate the immune effects of a probiotic treatment on gut mucosa-associated immune system. All biopsies were carried out in the distal region of ileum (terminal 10–15 cm). GALT biopsies were dissociated to single cell suspensions and isolated cells were stimulated with a polyclonal stimulus (PMA/ionomycin). Then, cytokine production by GALT-associated T lymphocytes were detected by flow cytometry with the aim of assessing differences of T cell polarization before and after treatment with LGG probiotic.

In CD pt, after treatment with LGG, we observed a striking skewing of the polarization of GALT-associated T lymphocyte from Th1 to Th17 (Fig. 1). In human, the generation of Th17 polarization requires the presence of IL-23 and IL1ß, both produced by accessory cells such as macrophages and DCs. In agreement with our observation, we found that LGG strain administered to these pts was able, in vitro, to induce in monocyte-derived DCs an extraordinary production of IL-23 if compared to other Lactobacilli usually employed as probiotics, i.e. Lactobacillus reuteri (ATCC PTA 5289) and Lactobacillus acidophilus (NCFM) (Fig. 2); conversely, IL1ß was released at comparable levels by all the bacterial strains tested. The strain-specific release of a large amount of IL-23 by DCs co-cultured with LGG is a strong suggestion for considering the switch of T cell polarization toward Th17, observed in the CD patient, as an in vivo effect of LGG administration. However, we did not observe any change of GALT-associated T cell polarization in all the five pts displaying a normal non-inflamed gut mucosa.

We propose that while the use of probiotics in healthy subjects might be of limited efficacy, their employment in the presence of a deeply altered gut mucosa surface, as occurring in IBD, might affect the locoregional immune system. This effect would take place because the alterations of the normal mucosal structure causes gut barrier failure, making the encounter between probiotics and gut-associated accessory immune cells more likely to occur. In this context, since unstirred mucus layer is a major component of the physiological gut barrier, it is conceivable that we could expect an immunological relevant effect of probiotic administration only when a profound alteration of these structures has occurred.

In conclusion, these preliminary observations call for further studies aimed to confirm our current proposal that the administration of probiotics might affect gut-associated immune system.

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mainly in pts with IBD rather than in subjects with normal non-inflamed mucosa.

We must underline that putative immunological outcomes observed upon administration of probiotics are not necessarily related to clinical benefits and, also in this case, further controlled studies are warranted. These further investigations should aim to establish not only the clinical outcome but also the kind of bacteria to employ in the different inflammatory diseases, which often display divergent immunopathogenesis; since it has been clearly shown that different bacteria species, and even strains of the same species, are able to induce the production of a distinct cytokine milieu [6–8], each clinical condition should require a peculiar probiotic treatment.

Meanwhile, our current observation just represents one of the few indications that consumption of commensal bacteria might have immunological effects in humans.

Conflict of interests

None of the authors have any conflicting financial interest related to this study.

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References

Irene Bonaccorsi  
Laboratory of Immunology and Biotherapy, Dept. Human Pathology, University of Messina, Italy

Carmelo Buda  
Surgical Endoscopy Unit, University Hospital—Policlinico G. Martino, Messina, Italy

Stefania Campana  
Claudia De Pasquale  
Daniela Oliveri  
Laboratory of Immunology and Biotherapy, Dept. Human Pathology, University of Messina, Italy

Antonio Cascio  
Infectious Disease Unit, Dept. Human Pathology, University of Messina, Italy

Guido Ferlazzo*  
Laboratory of Immunology and Biotherapy, Dept. Human Pathology, University of Messina and Cell Therapy Program, University Hospital—Policlinico G. Martino, Messina, Italy

* Corresponding author at: Laboratory of Immunology and Biotherapy, University of Messina, Pad. H—A.O.U. Policlinico, Via Consolare Valeria, 1, 98125 Messina, Italy.  
Fax: +39 090 221 2043.  
E-mail address: guidoferlazzo@unime.it (G. Ferlazzo)

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