

EDITORIAL

Clinical & Experimental Allergy

Probiotic-derived factors: efficient treatment for allergic asthma?

This editorial discusses the findings of the article in this issue by H. Harb et al. [10] pp. 353–364.

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Amongst the multiple factors responsible for the development of allergic disease, environmental microbial exposures greatly influence the occurrence of this group of inflammatory conditions. As originally formulated by Strachan, the hygiene hypothesis suggested that the susceptibility to allergen sensitizations is correlated with a reduced overall exposure to microbial stimulation during pregnancy and the neonatal period as well as with the composition of the gut microbiota [1, 2]. Great progress has highlighted the importance of immune regulatory mechanisms triggered through the innate immune activation by microbia in the intestinal tract to finely tune Th1-Th2 balance and tolerance. The potential of probiotics, live microorganisms which confer health benefits when administered in adequate amounts, stimulated great interest in prevention or treatment of the Th2-biased allergic responses [3]. Notably, their mucosal immunomodulatory properties were shown to promote the production of pro-Th1 (IL-12) or pro-Treg (IL-10) cytokines following immune cell stimulation including dendritic cells [4]. Although anti-allergic effects of probiotic treatments were evidenced in different animal models, contrasting results were obtained from clinical studies which evaluated the influence of perinatal/postnatal probiotic supplementations in the initiation as well as the progression of allergic diseases [5–9]. These inconsistent data, which could result from the use of species-

specific probiotic strains and/or suboptimized doses, formulations or treatments regimens, has so far prevented authorities recommending that pregnant women and their infants, in countries with high allergic disease prevalence should take probiotics.

Whereas the great majority of the studies reporting the beneficial effects of probiotics on allergic inflammation were performed with living bacteria, the contribution of probiotic-derived factors from cell-free culture supernatants has remained poorly characterized.

In this issue of *Clinical & Experimental Allergy*, Harb et al. evaluated not only the immunomodulatory properties of *L. rhamnosus* GG (LGG)-derived supernatants on cultured mouse macrophages but also assessed, following neonatal supplementation, the anti-allergic effects of these conditioned media on the allergic inflammation in a mouse model of ovalbumin sensitization [10].

The investigators found that LGG-derived supernatants induced TNF α and IL-10 in a mouse macrophage cell line, although to a lesser extent than seen with intact LGG. Although such strong IL-10 induction was previously observed with supernatants from cultured *B. breve* [11], it is the first report which evidenced such cytokine stimulation using LGG-derived conditioned media. It is however quite difficult to compare the strength of the immunomodulatory properties between bacteria and conditioned supernatants, notably because the quantification of the stimulators in these materials is problematic. Nevertheless, the strong IL-10 production triggered by LGG-derived supernatant highlighted that such probiotic-derived compounds could display efficient adjuvant capacity for the prevention of Th2-biased allergic responses.

Interestingly, the authors also assessed the impact of the bacterial growth phase on such immunostimulation. Compared with supernatants from mid-exponential and

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stationary growth phases, the one from late exponential bacterial growth phase displayed the most prominent activity, suggesting that the release of the stimulating factor(s) from LGG cultures was dependent on the growth phase of the probiotic. These data were consistent with a previous report showing that exponential-phase probiotic cultures displayed more immunostimulatory properties compared with stationary phase cells [12]. These finding must be associated with the observation that growth phase-associated changes occur in the proteome and transcriptome of LGG [13]. Gene deletion analysis would certainly bring precious informations about the regulation of the expression/secretion of the stimulating factors from LGG supernatant.

The demonstration that oral/intragastric probiotic administrations were shown to be efficient only in the initial stage of the colonization process (time of opportunity) led the authors to initiate LGG-derived supernatants neonatal supplementations in 2-day-old newborn mice. These animals were subsequently sensitized with unadjuvanted ovalbumin and subsequently challenged with the same aerosolized allergen. Although such treatment influenced neither the sensitization to the allergen nor the airway hyper-reactivity, the oral neonatal supplementation with processed LGG supernatants reduced the allergic airway inflammation *in vivo* as judged by the reduction of eosinophils and goblet cells numbers through airway production of IL-10 in comparison with untreated control mice.

The authors explained the absence of modulation of the allergen-specific antibody response by the LGG-derived compounds by the interruption of the treatment during the allergen sensitization, as a previous study observed a reduction of OVA-specific IgE when LGG cells were orally administrated before and during the sensitization [14]. Once again, the lack of effects on allergen-specific antibodies as well as hyper-reactivity could result also from the suboptimization of the prophylactic treatment. The lack of IgE modulation by LGG supernatants despite the triggered *in vivo* IL-10 production could be consistent with data showing that the IL-10 level and total IgE was not correlated in children with IgE-dependent food allergy [15]. Moreover, CD4⁺ naïve T cells from BALB/c mice, the mice strain used in this study, are capable of producing significant amounts of IL-4 [16] and it was previously reported that IL-10, together with IL-4, is capable of enhancing allergen-specific IgE synthesis in unprimed B cells exposed to allergen [17]. The IL-5/IFN γ cytokine balance was surprisingly not modified in the airways despite the production of IL-10. Unfortunately, the effect of LGG-derived supernatant on the recall cytokine secretion by lymph nodes or splenocytes was not characterized in that study. Nevertheless, the demonstration that rather

the circulating but not the local lung IL-5 is required for the development of antigen-induced airway eosinophilia [18] suggests that LGG-derived media likely modulate the systemic IL-5 production. The failure of LGG-derived supernatant treatments to reduce the specific IgE response and the airway hyper-reactivity indicated that such conditioned media did not influence also the production of IL-4 and IL-13, those cytokines promoting the IgE production and AHR development respectively. Complementary studies must be performed to more deeply clarify the effects of LGG-derived supernatants on the Th2 cytokine profile.

This study raises at least two key questions: what is (are) the immunostimulatory compound(s) present in the LGG-derived supernatant as well as the mechanism(s) related to (their) anti-inflammatory effects? The authors demonstrated that the bioactive compound(s) present in LGG-derived supernatants was(were) protease-sensitive and could stimulate, similarly to the intact bacteria, TLR2 and NOD2 signalling pathways *in vitro*. It would be very interesting to evaluate the anti-allergic effects of LGG-derived supernatants in NOD2 or TLR2 deficient mice. The TLR2- and NOD2-dependence of the LGG-induced anti-inflammatory effect, mainly orchestrated by dendritic cells were previously described including in animal model of colitis [19]. Whereas lipoteichoic acid as well as peptidoglycans from probiotics can activate TLR2, NOD2 is activated by the minimal peptidoglycan structure muranyl dipeptide (MDP) [4]. These innate immune activations by these pathogen-associated molecular patterns (PAMPs) result from the release of cell wall fragments through the activity of bacterial peptidoglycan hydrolases [20]. Consequently, the presence of peptidoglycan fragments in the LGG conditioned media more likely explain the TLR2-, NOD2-dependence of the immunomodulatory effects. Intriguing results arise with the protease sensitivity of LGG-derived compounds displaying such activity as cell wall containing peptides with D-amino acids often escape digestion by the proteases. The peptide interbridge, cross-linking the parallel glycan strands from the peptidoglycan, could represent the protease-sensitive element of the LGG-derived components. It must be pointed out that the main secretory proteins from LGG are Msp1 (p40) and Msp2 (p75), which share homology with cell wall hydrolases and such biological activity was at least reported for Msp1 [21]. The proteolytic degradation of such enzymes could affect the shape processing of the peptidoglycan fragments released by LGG and then impair the innate immune activation.

In the context of allergic asthma, the relevance of ovalbumin is questionable as this protein is not an airborne allergen. Complementary studies, using house dust mite or pollen allergens must be conducted to confirm the promising results obtained in this study. Whereas the present clinical applications of probiotics lie essentially

in the prevention of allergic diseases through oral perinatal/neonatal supplementation with living bacteria, the results from this study suggest that LGG-derived supernatant could be considered as promising mucosal adjuvants to elicit anti-allergic responses. Moreover, it is not excluded that the intranasal administration of these immunostimulatory compounds could prove more efficient for the treatment/prevention of allergic asthma.

Lactobacillus species are generally not considered pathogenic. Notably, a range of products based on LGG are commercialized for more than 20 years by the Finnish company Valio. However, ingestion of probiotics has been linked to rare case of infections and endocarditis highlighting that the analysis of risk/benefit of probiotics usage should be likely considered on a strain-by-strain basis [22]. Consequently, the use of conditioned media or the isolated functional molecule(s)

from probiotic-derived supernatants could represent an advantage not only in terms of safety but also manufacturing.

Insights gained through these findings will thus open the route towards the design of a new family of therapeutic tools for the treatments of allergic diseases.

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