

ORIGINAL PAPER

Oral delivery of *Lactobacillus casei* Shirota modifies allergen-induced immune responses in allergic rhinitis

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Summary

Background Changes in the composition of the gut microbiota have been implicated in the pathogenesis of allergic disorders, suggesting beneficial interactions between the intestinal immune system and specific bacterial strains. Lactobacilli are naturally present within the complex gastrointestinal microbiota of humans and they are currently present in many probiotic supplements.

Objective We sought to investigate the role that *Lactobacillus casei* Shirota (LcS) may play in modulating seasonal allergic rhinitis (SAR).

Methods The study format was double-blinded, placebo-controlled with 10 SAR sufferers in each group. We have documented and compared changes in immune status arising through the daily ingestion of a milk drink with or without live LcS, over a period of 5 months. Pre-, peak- and post-grass pollen season blood samples were collected for determination of plasma total IgE and grass pollen-specific IgG and IgE levels by an enzyme immunoassay. At the same time, cytokine levels were determined by flow cytometric bead array technology following culture of peripheral blood mononuclear cells for 6 days in the presence or absence of specific grass pollen antigens.

Results Volunteers treated with LcS showed a significant reduction in levels of antigen-induced IL-5, IL-6 and IFN- γ production compared with volunteers supplemented with placebo. Meanwhile, levels of specific IgG increased and IgE decreased in the probiotic group.

Conclusion Changes in antigen-induced production of cytokines were observed in patients treated with probiotics. These data show that probiotic supplementation modulates immune responses in allergic rhinitis and may have the potential to alleviate the severity of symptoms.

Keywords allergic rhinitis, cytokines, IgE, IgG, immunomodulation, *Lactobacillus casei*, probiotics

Submitted 12 October 2007; **revised** 10 March 2008; **accepted** 4 April 2008.

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Introduction

A host-microbe symbiosis is essential for driving the maturation and maintaining a correct homeostasis of the gastrointestinal (GI) immune system through delivery of stimuli by certain microbial antigens [1], and changes in the composition of the commensal microbiota may lead to an increased susceptibility to allergy [2]. Differences in the composition of the gut flora between infants who go on to develop allergy from those who do not are evident very early in life before the development of any clinical signs of atopy [3]. Disturbances in the GI tract have also

been documented in patients with asthma and allergic rhinitis (AR) [4]. These observations make it likely that the intestinal microbiota plays an important or even an essential role in protection from allergy [5]. They also support the concept of an integrated mucosal system in which antigen-specific lymphocytes can home to mucosal effector sites distant from the site of initial antigen exposure to induce systemic sensitization or tolerance. This is one of the rationales for probiotic use.

Specific strains of gut bacteria are known to influence tolerance induction [6] through the recruitment of regulatory T cell activity [7]. It is reasoned that if detrimental changes in our gut microbiota give rise to the dysregulated immunity of allergy, then it must be beneficial to

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re-introduce selected bacterial strains through inclusion in the diet (probiotics). This has been shown to be the case in clinical and other studies where probiotic intervention has succeeded in reversing or modifying some immunological disturbances [8]. Although not proven, the potential of probiotics is recognized in atopic and inflammatory disorders [9].

Allergic responses are linked to T-helper type 2 (Th2) lymphocytes that produce high levels of IL-4, IL-5 and IL-6 that promote IgE synthesis [10]. It has been shown that probiotics have the capacity to create conditions that facilitate a re-direction of the allergen-induced Th2-skewed response to a healthy, regulated balance. Lactic acid bacteria are among the most important probiotic organisms, typically associated with the human GI tract. *Lactobacillus casei* is a species that has been widely studied with respect to its 'health-promoting' properties. It reportedly decreases GI symptoms in children with atopic dermatitis [11], modulates inflammation in Crohn's disease [12] and has strong anti-microbial properties [13]. We hypothesized that inclusion of this probiotic organism in the daily diet of seasonal rhinitis sufferers would modulate their Th2-like immune status and thus reduce the severity of their symptoms. As the symptoms of rhinitis arise through the release of soluble mediators, we measured the levels of Th1- and Th2-associated cytokines and allergen-specific as well as total immunoglobulins. Here we report that differences in IL-5, IL-6 and IFN- γ production between the *Lactobacillus casei* Shirota (LcS)-treated (probiotic) and placebo groups were observed. In addition, we found that a reduction in the levels of pollen-specific IgE was accompanied by an increase in pollen-specific IgG within the probiotic-treated group. We interpret these data as supporting the view that restoration/supplementation of gut microbiota through probiotic administration can be beneficial in seasonal allergic rhinitis (SAR).

Materials and methods

Subjects

The number of individuals selected for recruitment was based on guidance from the current literature, which led us to believe that this number should give us a significant and measurable difference in the selected immune parameters. Before recruitment, a power calculation was performed based on the assumption that the data would be parametric and that the participants would be fairly uniformly distributed between the groups.

Twenty AR sufferers with a history of seasonal allergic rhino-conjunctivitis during March–June and with detectable levels of pollen-specific IgE antibodies in the blood before the start of the pollen season were recruited into the study from our volunteer database and advertisements in

local newspapers. All were otherwise healthy. They were aged 18–45 years, did not normally consume fermented dairy products, were not pregnant, did not smoke or suffer from asthma and were unlikely to holiday abroad during the study period. The last two exclusion criteria had the greatest impact on sample size. Recruited volunteers were allocated a code number and placed into one of two age- and sex-matched groups (10 in each). However, due to non-compliance, one of the volunteers had to be excluded, leaving nine in one group and 10 in the other. Ethical approval for the study was granted by the IFR Human Research Governance and Norwich Research Ethics Committees, and all participants gave their informed consent.

Probiotic or placebo drinks

All dairy milk drinks were supplied by Yakult Europe B.V. with HACCP certification for the safety of materials, production process and microbiological analysis (issued by TNO, Holland). The probiotic and placebo drinks were identical in packaging, appearance and composition, except for the presence of 6.5×10^9 LcS per 65 mL probiotic drink bottle.

Grass pollen antigens

Early (GX1) and late (GX2) pollen antigens were purchased as six grass mixes of each, from Pharmacia Diagnostics (Uppsala, Sweden). Each allergen was reconstituted at 10 mg/mL in sterile serum-free RPMI 1640. The proteins were allowed to dissolve for at least 2 h at room temperature and then microfuged to remove any insoluble material. Solubility was 20%, giving a final concentration of 2 mg/mL.

Study design

The study took place during 2005–2006 at the Institute of Food Research in Norwich (UK) and data were analysed in 2007. Pre-season (April) fasting, heparinized peripheral blood samples were taken for processing as described below. Over a period of 2 weeks, each volunteer was then given 2 weeks' supply of either probiotic or placebo dairy drink for their daily consumption. Compliance with the study was checked through fortnightly recovery of any surplus drinks, when a fresh consignment was delivered to each volunteer. A second fasting blood sample was taken from each volunteer during the peak grass pollen period (June) and a final one was taken 4 weeks after the end of the grass pollen season (late August). All blood samples were processed identically.

Plasma immunoglobulin G and immunoglobulin E responses

Heparinized peripheral blood was centrifuged at 800 g for 10 min at room temperature, and the plasma was removed

for storage at -80°C until analysis. Total and specific IgE levels were measured by a cellulose-disc-based enzyme immunoassay with the Ridascreen system (Darmstadt, R-Biopharm AG, Germany). Specific IgE was determined for grass pollen mixtures (GX1 and GX2 antigens described earlier).

Culture of mononuclear cells

Mononuclear cells (MNCs) were derived from heparinized peripheral blood by Histopaque 1077 (Sigma Aldrich, Dorset, UK) gradient centrifugation, according to the manufacturer's instructions. MNC were cultured in duplicate (2×10^6 cells/mL) in RPMI 1640 tissue culture medium (TCM) supplemented with 5% autologous plasma, glutamine, penicillin and streptomycin in the presence or absence of either early (GX1) or late (GX2) pollen antigens, each at a final concentration of $2 \mu\text{g/mL}$. On the 6th day, cells were harvested and their supernatants were frozen at -80°C for cytokine analysis at a later date.

Cytokine analysis

Frozen culture supernatants were thawed rapidly (at 37°C) for cytokine analysis with multiplexed bead-array technology. A Th1–Th2 10-plex human cytokine kit (BMS 716FF) was purchased from Bender MedSystems (Vienna, Austria) and used according to their instructions. The following cytokines were measured: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN- γ and TNF- α . Data were acquired on a Beckman Coulter FC500MPL cytometer and analysed with software provided by Bender MedSystems.

Statistical analysis

ANOVA models were used to study the effect of the probiotic drink and the seasonal period on cytokine and Ig production. For each subject, the modelled quantity was the logarithm of the ratio between the Ig or cytokine concentration in peak- or post- and pre-rhinitis season, i.e.

$$R = \text{LnCpeak}(\text{or Cpost}) - \text{Ln Cpre}$$

IgS were measured in blood plasma while cytokines were analysed in MNC supernatants collected after their 6-day culture in the presence (challenged) or absence (unchallenged) of early (GX1) or late (GX2) pollen antigens. The ANOVA models included the three factors: treatment (probiotic vs. placebo drink), seasonal period (peak- or post-rhinitis period) and challenging antigen (none, GX1 and GX2), and the interactions among them. The *P*-values associated with the model terms indicated the significance of the effect of treatment, seasonal period and challenging antigens on cytokine and Ig ratios. When the effect of the antigens was significant, a Tukey test was carried out to compare the means of this factor.

In addition, a canonical discriminant analysis was performed to differentiate between the four groups of measurements (placebo/peak-season, placebo/post-season, probiotic/pre-season and probiotic/post-season) with those Ig and cytokine ratios that showed some variation through consumption of probiotic and/or period of the AR season. These were IL-1 β , IL-5, IL-6, IFN- γ , TNF- α , IgE and IgG. This analysis was carried out independently for datasets of unchallenged cultures and those challenged with GX1 or GX2 pollen antigens.

Results

There were no statistically significant differences in cytokine profiles or Ig secretion between the two study groups at the start of the study.

Profile of allergen-specific cytokine production is modified in Lactobacillus casei Shirota-treated volunteers

Table 1 shows the measurements obtained according to the experimental design while Table 2 shows the *P*-values derived from statistical analysis of the ANOVA model terms that included the three factors (treatment, period and antigens) and their interactions. There were notable inter-group differences in IL-5, IL-6 and IFN- γ secretion (Table 2). IL-5 levels were always significantly higher in the placebo group, irrespective of seasonal period or antigenic challenge. Unlike IL-5, there was no difference in the spontaneous production of IL-6 in unchallenged cultures, but significant increases were seen in culture supernatants collected from the placebo but not the probiotic group, following *in vitro* MNC stimulation with GX1 or GX2 allergens (Table 2). As these effects occurred irrespective of the nature of the challenging allergens or the seasonal period, they could be attributed directly to the treatment given (i.e. probiotic). There were no seasonal differences in the amounts of IFN- γ produced by subjects ingesting probiotic or placebo drinks, but moderate treatment-related differences were apparent following *in vitro* allergenic challenge. These were more marked in response to GX2 late pollen allergens, with less produced by the probiotic than the placebo group. The cytokines IL-2, IL-4, IL-8 and IL-12p70 were unaffected by treatment, challenge or seasonal period. Group-associated and seasonal differences in IL-10 production did not reach significance due to a high degree of variability, but we observed a consistent and unexpected trend that suggested a decline from base line in IL-10 ratios within the probiotic group (data not shown). We have elected not to dismiss this observation as it could be potentially interesting, practically relevant and may attain significance in a larger sample size. There were no measurable differences in IL-1 β and TNF- α production between groups, but there was an inverse relationship between them that appeared to be

Table 1. Cytokine and Ig ratios derived with respect to pre-season values ($\pm$ SD)

Allergenic challenge	Period	Treatment	IL-1 β	IL-5	IL-6	IFN- γ	TNF- α	IgE	IgG
None	Peak	Placebo	1.19 $\pm$ 1.25	1.00 $\pm$ 1.99	1.79 $\pm$ 4.06	1.34 $\pm$ 2.61	1.58 $\pm$ 1.86	0.25 $\pm$ 0.42*	N/T
		Probiotic	1.13 $\pm$ 1.35	1.31 $\pm$ 1.67	1.27 $\pm$ 2.69	2.01 $\pm$ 1.01	1.65 $\pm$ 1.62	0.24 $\pm$ 0.57*	N/T
None	Post	Placebo	1.25 $\pm$ 0.25	3.32 $\pm$ 1.79	3.00 $\pm$ 4.95	2.61 $\pm$ 2.51	1.40 $\pm$ 1.77	0.41 $\pm$ 0.46*	N/T
		Probiotic	1.35 $\pm$ 0.19	1.68 $\pm$ 1.93	1.16 $\pm$ 3.32	1.17 $\pm$ 3.00	1.23 $\pm$ 1.73	0.43 $\pm$ 0.64*	N/T
GX1	Peak	Placebo	1.94 $\pm$ 1.46	2.18 $\pm$ 1.55	16.44 $\pm$ 3.32	2.61 $\pm$ 3.00	1.51 $\pm$ 1.63	1.46 $\pm$ 1.60	1.08 $\pm$ 1.49
		Probiotic	1.75 $\pm$ 1.72	1.43 $\pm$ 1.75	2.64 $\pm$ 2.08	1.63 $\pm$ 2.29	1.51 $\pm$ 1.77	1.17 $\pm$ 1.75	1.17 $\pm$ 1.39
GX1	Post	Placebo	15.03 $\pm$ 2.25	2.41 $\pm$ 1.73	14.59 $\pm$ 4.22	6.42 $\pm$ 3.86	1.06 $\pm$ 1.63	1.65 $\pm$ 1.80	0.92 $\pm$ 1.34
		Probiotic	10.81 $\pm$ 2.03	1.36 $\pm$ 1.92	2.46 $\pm$ 1.95	1.63 $\pm$ 4.26	0.76 $\pm$ 1.45	1.26 $\pm$ 1.75	1.38 $\pm$ 1.63
GX2	Peak	Placebo	2.46 $\pm$ 1.51	2.14 $\pm$ 1.73	25.53 $\pm$ 3.74	2.61 $\pm$ 2.89	1.31 $\pm$ 1.67	1.60 $\pm$ 1.82	1.25 $\pm$ 1.60
		Probiotic	2.64 $\pm$ 2.98	1.35 $\pm$ 2.64	3.53 $\pm$ 1.90	1.70 $\pm$ 2.44	1.11 $\pm$ 1.68	1.51 $\pm$ 1.60	1.35 $\pm$ 0.49
GX2	Post	Placebo	23.34 $\pm$ 2.08	1.95 $\pm$ 1.57	11.82 $\pm$ 3.67	5.58 $\pm$ 2.80	1.12 $\pm$ 1.62	1.77 $\pm$ 1.99	1.08 $\pm$ 1.45
		Probiotic	15.33 $\pm$ 1.77	1.19 $\pm$ 2.44	2.12 $\pm$ 1.86	1.20 $\pm$ 1.72	0.73 $\pm$ 1.38	1.35 $\pm$ 1.39	1.34 $\pm$ 2.05

Cytokines were measured in culture supernatants while allergen-specific IgE and IgG were measured in plasma.

*Total IgE; N/T, not tested.

Table 2. Significant changes in cytokine and Ig ratios attributed to treatment (placebo/probiotic), season (peak-/post-rhinitis period) or allergen (GX1/GX2 compared with unchallenged cultures) shown as *P*-values associated to the terms of the ANOVA model developed

Allergenic challenge	Changes due to	IL-1 β	IL-5	IL-6	IFN- γ	TNF- α	IgE	IgG
None	Treatment	-	< 0.01 $\downarrow$	-	-	-	-	-
GX1		-	0.01 $\downarrow$	< 0.01 $\downarrow$	0.02 $\downarrow$	-	0.05 $\downarrow$	< 0.01 $\uparrow$
GX2		-	0.05 $\downarrow$	< 0.01 $\downarrow$	0.01 $\downarrow$	-	-	-
GX1	Seasonal period	< 0.01 $\uparrow$	-	-	-	< 0.01 $\downarrow$	-	-
GX2		< 0.01 $\uparrow$	-	-	-	< 0.01 $\downarrow$	-	-
GX1	Allergen	< 0.01 $\uparrow$	-	< 0.01 $\uparrow$	-	-	-	-
GX2		< 0.01 $\uparrow$	-	< 0.01 $\uparrow$	-	0.01 $\uparrow$	-	-

Cytokines were measured in the supernatants of mononuclear cells (MNC) cultured in the presence or absence of GX1 or GX2 pollen antigens. Igs were measured in plasma. Only *P*-values less than or ca. 0.05 are shown.

In changes due to 'treatment' the arrows show increases ($\uparrow$) or decreases ($\downarrow$) within the probiotic compared with placebo group.

In changes due to 'seasonal period' the arrows show increases ($\uparrow$) or decreases ($\downarrow$) at post-season compared with peak season.

In changes due to 'allergen' the arrows show increases ($\uparrow$) or decreases ($\downarrow$) compared with values derived in the absence of allergen.

allergen driven (Table 1, Fig. 1). TNF- α was produced spontaneously in the absence of allergenic challenge, at which time IL-1 β was virtually absent. In the presence of GX1 or GX2 allergens *in vitro*, TNF- α levels declined while those of IL-1 β increased. The implication of this observation is unknown, but it may suggest a differential regulation of IL- β and TNF- α in AR, or their participation in a reciprocal feedback control mechanism.

Allergen-specific immunoglobulin production is modified in *Lactobacillus casei* Shirota-treated volunteers

There were no differences in total IgE levels between the study groups, with a general increase in production from basal levels at both peak- and post-rhinitis periods (Tables 1 and 2). In contrast, both GX1- and GX2-specific IgE levels were lower in the probiotic group at both peak- and post-rhinitis periods, while the levels of GX1- and GX2-

specific IgG were higher. Owing to the high degree of individual variability, these differences only show statistical significance for GX1-specific IgE and IgG ratios (Table 2).

Lactobacillus casei Shirota-treated and placebo-treated volunteers can be separated by immunoglobulin and cytokines into two distinct groups

Canonical discriminant analysis was performed between four groups of observations, placebo/peak-season, placebo/post-season, probiotic/peak-season and probiotic/post-season, according to the Ig and cytokines ratios found to be affected by the probiotic drink and/or the seasonal period. The datasets used consisted of cytokines (IL-1 β , IL-5, IL-6, IFN- γ , TNF- α) measured in MNC cultures challenged with GX1 or GX2 allergens and allergen-specific plasma IgE and IgG. Cytokines and Igs with

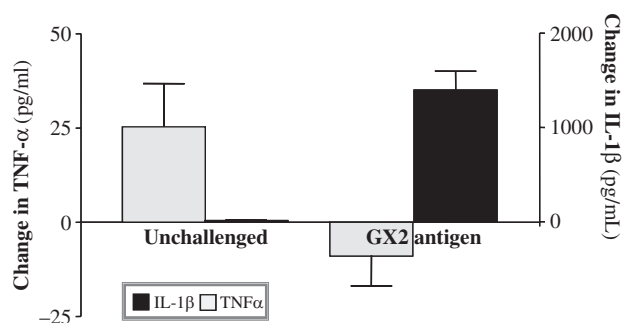


Fig. 1. Changes in TNF- α and IL-1 β secretion (compared with pre-season) were measured in mononuclear cells, supernatants following culture for 6 days in the absence (unchallenged) or presence (challenged) of GX2 pollen antigens. As the reciprocal relationship seen between the two cytokines was induced by allergenic challenge and not the treatment given (probiotic or placebo drink) data from the two groups were pooled and are shown here as pg/mL $\pm$ SEM.

reference to GX1 or GX2 antigens were analysed independently but due to their similarity only data generated for GX1 are shown in Fig. 2. The aim of this analysis was to find those linear combinations of the original variables that, if existent, discriminate between the groups of observations. The first two canonical correlations, CAN1 and CAN2, were significantly different from 0 ($P < 0.05$), indicating that the first two canonical variables are able to discriminate between the groups of observations. CAN1 discriminates between seasonal (peak- and post-rhinitis) responses. It shows that IL-1 β ratios were higher than TNF- α ratios at the post-rhinitis period, and that the corresponding decrease in TNF- α levels occurred at the end of the same period. CAN2 discriminates between

treatment (probiotic and placebo) groups. These analyses show that increases in IL-5, IL-6, IFN- γ and IgE were smaller within the probiotic group, while IgG levels were higher in the same subjects.

Discussion

It was the aim of this study to see whether probiotics can have an effect on some commonly measured immunological parameters of SAR (i.e. cytokines and Igs) as a prelude to further studies to elucidate the mechanism(s) of action. These parameters were not related to clinical symptoms. We reasoned that as the latter are mediated by the presence of IgE antibodies, cytokines and other mediators, by the time clinical symptoms are evident there is moderate to severe disease. Any immunomodulatory effects arising through the ingestion of probiotic organisms therefore may not have an immediate impact on observed clinical symptoms.

The main finding of this paper is that oral administration of live LcS modifies allergen-specific responses in AR. Selected strains of lactic acid bacteria have already been shown to influence aspects of immune function, but most of these studies have been performed in experimental mice. They nevertheless show that *L. casei* and other probiotic organisms can have systemic effects on antibody responses [14], modulate cytokine production [15] and augment non-specific immunity [16]. There are a few documented human studies that use probiotics for therapeutic intervention, such as *L. casei* to reduce the severity of pathological processes like diarrhoea [17]. However, its capacity to influence immune function is not as clearly documented. Probiotics hold much promise

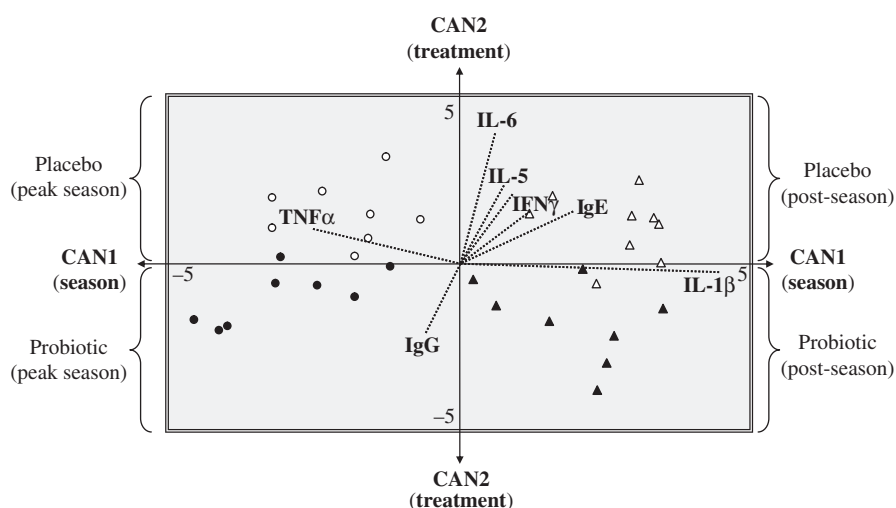


Fig. 2. Canonical discriminant analyses showing GX1-specific responses were performed between four groups of observations; placebo/peak-season ($\circ$), placebo/post-season ($\triangle$), probiotic/peak-season ($\bullet$) and probiotic/post-season ($\blacktriangle$) according to the Ig and cytokine ratios found to be significantly affected by the probiotic drink and/or the seasonal period. Cytokines were measured in culture supernatants from mononuclear cells challenged with pollen allergens and specific Igs were measured in plasma. CAN1 discriminates between peak- and post-rhinitis periods and CAN2 discriminates between probiotic and placebo groups.

as a functional food and well-controlled human studies could clarify the influence probiotics may have, particularly in common systemic inflammatory disorders such as SAR.

Our study reports lower levels of allergy-related cytokines IL-5, IL-6 and IFN- γ , which are assigned to both Th1 and Th2 functional subsets, from cultured MNC of volunteers treated with LcS. These changes were accompanied by lower and higher levels of pollen-specific IgE or IgG, respectively. The systemic release of IL-5, IL-6 and allergen-specific IgE after allergen inhalation is crucial to the generation of allergic symptoms throughout the entire airway [18]. Their decline in our probiotic-treated group would therefore be expected to impact on the symptoms of SAR. IL-5 is an eosinophil-activating cytokine [19], and the development of SAR is associated with a dramatic increase in eosinophils and their augmented degranulation within the nasal mucosa [20]. IL-5 is believed to direct a preferential growth of the eosinophil lineage [21], and may represent the systemic link between antigen deposition onto the airway mucosa, eosinopoiesis in the bone marrow and airway eosinophilia.

In both tissues and biological fluids from patients with asthma, eosinophilia is accompanied by the presence of exaggerated quantities of IL-6. When applied directly to the nasal mucosa, IL-6 reproduces the symptoms of rhinitis [22], while its down-regulation is associated with effective treatment [23]. Endogenous IL-6 plays a role in plasma cell differentiation and it is an important co-factor for the production of IgE [24]. Once produced, the IgE synergizes with antigen to stimulate further IL-6 synthesis. This serves to amplify and perpetuate the allergic inflammatory response, partly through the development of Th17 cells, a distinct effector lineage that up-regulates pro-inflammatory cytokines such as IL-6, IL-1 and TNF- α . Th17 cells have been implicated in chronic inflammatory conditions such as arthritis and inflammatory bowel disease [25].

In our study, pollen-specific plasma IgE levels were significantly lower and plasma IgG levels were higher in the probiotic group, but their specificity was restricted to GX1 (early) antigens, at both peak- and post-rhinitis periods. This may simply reflect the small study size with a high degree of individual variability. It may equally suggest a complexity in the mechanism of probiotic action, where the nature of the challenging allergen and the immune responses evoked may play a role in the subsequent outcome. An intriguing and selective influence of probiotic strains on components of non-specific immunity has already been described, where probiotics are shown to exert both stimulatory and down-regulatory influences in healthy or hypersensitive subjects, respectively [26]. Furthermore, increases in allergen-specific IgG and decreases in allergen-specific IgE levels are generally associated with hypo-sensitization and resolution [27–29].

The resolution of allergic responses is widely thought to be associated with a shift from Th2 to Th1 cytokine production and in this context IFN- γ is considered to be a signal cytokine for Th1 responses, to act as an inhibitory factor in Th2-mediated allergic reactions [30]. However, the roles of IFN- γ in allergic inflammation are controversial. Eosinophils bear receptors for IFN- γ [31] and uptake of this cytokine serves to promote eosinophil survival and enhance certain functions, such as histamine release, Fc receptor and adhesion molecule expression [32, 33]. In our study, there was a significant decline in IFN- γ production by volunteers in the probiotic-treated group. Some other studies have associated similar findings with lower allergic reactivity. For example, in patients undergoing treatment for SAR due to Japanese cedar pollens, the levels of IFN- γ were significantly lower in the 'cure' group than in the non-atopic group [34]. Similarly, immunotherapy for perennial AR resulted in lower IFN- γ synthesis [35], while intramuscular administration of IFN- γ to patients with pollinosis failed to improve symptoms or reduce serum IgE levels [36]. These observations give rise to the possibility that Th1/Th2 dysregulation may not be the sole cause of nasal allergy and that it can arise through a Th2-independent, possibly IL-18-mediated pathway [37]. IL-18 has potent IFN- γ -inducing activity and, once produced, IFN- γ could participate in the allergic response as described earlier. Thus, suppression of both Th1 and Th2 cells may be an immunological indication of cure [34, 35].

The importance of commensal intestinal bacteria is demonstrated clearly by the multiple structural and functional immunological abnormalities present in germ-free mice [38]. In humans, epidemiological associations have been made between excessive hygiene and increased incidence of allergy and autoimmunity. Probiotics may have a therapeutic potential in the management of these. There are reports of their success in the prevention and treatment of food allergies in children, but not adults [39]. This may be due to the heterogeneous and strain-specific effects of Lactobacilli that have been suggested by several studies. It has also become apparent that despite similar *in vitro* probiotic properties, distinct Lactobacillus strains may colonize the gut differently and generate divergent immune responses. Future human studies are therefore needed to elucidate the mechanisms that operate in the complex GI macroenvironment *in vivo* and how these mechanisms are related to the clinical effects in a dose-dependent manner.

Our own study demonstrates for the first time the ability of LcS to down-regulate both Th1- and Th2-type cytokines and to beneficially alter the balance of pollen-specific IgG and IgE levels in SAR. Although several studies show beneficial effects of probiotics in the prevention of atopic eczema [40], this is the first study to support their use in another allergic disease. LcS is a good

candidate for further investigation into its mechanism of action and immunomodulatory effects in allergic inflammation, in order to fully elucidate its health-promoting potential and applications in therapy. This was a pilot study that aimed to see whether probiotics altered allergen-induced immune responses, and was not designed to evaluate whether those changes affected symptoms of SAR. In the next phase of the study, we will assess changes in clinical symptoms and elucidate some of the mechanisms by which probiotics exert their effects.

Acknowledgements

We wish to thank Aliceon Blair, Lesley Maloney, Yvonne Clements, Trudy Harrison, Dr Lisa Jackson and Dr David Hughes from our Human Nutrition Unit for their contribution to this human study. This work was sponsored by Yakult Honsha of Japan and a core strategic grant from the British Biotechnology and Science Research Council. Dr Juan L. Arqués was funded by a fellowship from Ministerio de Educación y Ciencia, Spain.

References

- Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; 122:107–18.
- Yazdanbakhsh M, Kretsinger PG, van Ree R. Allergy, parasites and the hygiene hypothesis. *Science* 2002; 296:490–4.
- Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001; 108:516–20.
- Powell N, Huntley B, Beech T, Knight W, Knight H, Corrigan CJ. Increased prevalence of gastrointestinal symptoms in patients with allergic disease. *Postgrad Med J* 2007; 83:182–6.
- Smith DW, Nagler-Anderson C. Preventing intolerance: the induction of nonresponsiveness to dietary and microbial antigens in the intestinal mucosa. *J Immunol* 2005; 174:3851–7.
- Sudo N, Sawamura S, Tanaka K *et al.* The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159:1739–45.
- Feleszko W, Jaworska J, Rha R-D *et al.* Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy* 2007; 37: 498–505.
- Isolauri E. Probiotics for infectious diarrhoea. *Gut* 2003; 52:436–7.
- Rautava S, Kalliomaki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002; 109:119–21.
- Foley S, Hamid Q. Inflammatory patterns in allergic rhinitis. *Clin Exp Allergy Rev* 2006; 6:91–5.
- Rosenfeldt V, Benfeldt E, Valerius NH, Pærregaard A, Michaelsen KF. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. *J Pediatr* 2004; 145:612–6.
- Borruel N, Carol M, Casellas F *et al.* Increased mucosal tumour necrosis factor α production in Crohn's disease can be down-regulated ex vivo by probiotic bacteria. *Gut* 2002; 51:659–64.
- Ogawa M, Shimizu K, Nomoto K *et al.* Protective effect of *Lactobacillus casei* Strain Shirota on Shiga toxin-producing *Escherichia coli* O157: H7 infection in infant rabbits. *Infect Immunity* 2001; 69:1101–8.
- Erickson KL, Hubbard NE. Probiotic immunomodulation in health and disease. *J Nutr* 2000; 130:403S–9S.
- Bai A-P1, Ouyang Q, Xiao X-R, Li S-F. Probiotics modulate inflammatory cytokine secretion from inflamed mucosa in active ulcerative colitis. *Int J Clin Pract* 2006; 60:284–8.
- Kima Y-G, Ohtab T, Takahashib T *et al.* Probiotic *Lactobacillus casei* activates innate immunity via NF- κ B and p38 MAP kinase signaling pathways. *Microbes Infect* 2006; 8:994–1005.
- de Vrese M, Marteau PR. Probiotics and prebiotics: effects on diarrhea. *J Nutr* 2007; 137:803–11S.
- Hamelmann E, Oshiba A, Schwarze J *et al.* Allergen-specific IgE and IL-5 are essential for the development of airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 1997; 16:674–82.
- Temple R, Allen E, Fordham J *et al.* Microarray analysis of eosinophils reveals a number of candidate survival and apoptosis genes. *Am J Resp Cell Mol Biol* 2001; 25:425–33.
- Ahlström-Emanuelsson CA, Greiff L, Andersson M, Persson CGA, Erjefält JS. Eosinophil degranulation status in allergic rhinitis: observations before and during seasonal allergen exposure. *Eur Respir J* 2004; 24:750–7.
- Hitoshi Y, Yamaguchi N, Korenaga M, Mlta S, Tominaga A, Takatsu K. In vivo administration of antibody to murine IL-5 receptor inhibits eosinophilia of IL-5 transgenic mice. *Int Immunol* 1991; 3:135–9.
- Fireman P. Virus-provoked rhinitis and asthma in allergic patients. *Clin Exp Allergy Rev* 2003; 3:33–7.
- Benson M, Strannegård IL, Strannegård Ö, Wennergren G. Topical steroid treatment of allergic rhinitis decreases nasal fluid TH2 cytokines, eosinophils, eosinophil cationic protein, and IgE but has no significant effect on IFN- γ , IL-1 β , TNF- α , or neutrophils. *J Allergy Clin Immunol* 2000; 106:307–12.
- Jego G, Palucka AK, Blanck J-P, Chalouni C, Pascual V, Banchereau J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* 2003; 19:225–34.
- Cooke A. Th17 cells in inflammatory conditions. *Rev Diabetes Study* 2006; 3:72–5.
- Pelto L, Isolauri E, Lilius EM, Nuutila J, Salminen S. Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. *Clin Exp Allergy* 1998; 28: 1474–9.
- Deuschl H, Johansson SGO, Fagerberg F. IgE, IgG and IgA antibodies in serum and nasal secretion during parenteral hyposensitization. *Clin Exp Allergy* 1977; 7:315–24.
- Urbanek R, Forster J, Karitzky D, Ziupa J. The prognostic significance of specific IgG antibodies in insect sting allergy. *Eur J Paediatr* 1981; 136:31–4.
- Jeal H, Harris J, Draper A, Newman Taylor A, Jones M. Specific IgG and IgG4 antibodies to rat urinary allergen in sensitized and

- non-sensitized individuals. *J Allergy Clin Immunol* 2004; 113: S336.
- 30 Tang C, Inman MD, van Rooijen N *et al*. Th type-1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN- γ -dependent mechanism. *J Immunol* 2001; 166:1471–81.
- 31 Ishihara C, Ochiai K, Kagami M *et al*. Human peripheral eosinophils express functional interferon- γ receptors (IFN- γ -maR). *Clin Exp Immunol* 1997; 110:524–9.
- 32 Yanagida M, Fukamachi H, Takei M *et al*. Interferon- γ promotes the survival and Fc epsilon RI-mediated histamine release in cultured human mast cells. *Immunology* 1996; 89: 547–52.
- 33 Lopez JA, Newburger PE, Condino-Neto A. The effect of IFN- γ and TNF- α on the eosinophilic differentiation and NADPH oxidase activation of human HL-60 clone 15 cells. *J Interferon Cyt Res* 2003; 23:737–44.
- 34 Nakai Y, Ohashi Y, Tanaka A *et al*. Cry j 1-induced synthesis of interleukin-5 and interferon- γ by peripheral blood mononuclear cells of patients with seasonal allergic rhinitis due to Japanese cedar pollens. *Acta Otolaryngol* 1998; 538 (Suppl.): 143–51.
- 35 Tanaka A, Ohashi Y, Kakinoki Y *et al*. The herbal medicine Shoseiryu-to inhibits allergen-induced synthesis of tumour necrosis factor alpha by peripheral blood mononuclear cells in patients with perennial allergic rhinitis. *Acta Otolaryngol* 1998; 118:118–25.
- 36 Horiguchi S, Okamoto Y. Role of T cells in allergic rhinitis. *Clin Exp Allergy Rev* 2005; 5:64–7.
- 37 Tsutsui H, Yoshimoto T, Nobuki H, Hitoshi M, Kenji N. Induction of allergic inflammation by interleukin-18 in experimental animal models. *Immunol Rev* 2004; 202:115–38.
- 38 Lupp C, Finlay BB. Intestinal microbiota. *Curr Biol* 2005; 15:R235–6.
- 39 Helin T, Haahtela S, Haahtela T. No effect of oral treatment with an intestinal bacterial strain, *Lactobacillus rhamnosus* (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. *Allergy* 2002; 57:243–6.
- 40 Prescott SL, Bengt Björkstén B. Probiotics for the prevention or treatment of allergic diseases. *Allergy Clin Immunol* 2007; 120:255–62.