

Preliminary evidence of the efficacy of probiotic eye-drop treatment in patients with vernal keratoconjunctivitis

Alfonso Iovieno · Alessandro Lambiase ·
Marta Sacchetti · Barbara Stampachiacchiere ·
Alessandra Micera · Stefano Bonini

Received: 20 February 2007 / Revised: 11 July 2007 / Accepted: 25 August 2007
© Springer-Verlag 2007

Abstract

Background Probiotics have been shown to improve allergic inflammation. The aim of this study was to evaluate the efficacy of *Lactobacillus Acidophilus* eye-drops in controlling signs and symptoms of vernal keratoconjunctivitis (VKC).

Methods Seven patients (mean age 11.8 ± 4.3 ; five M, two F) with mild to moderate VKC were included in the study. *Lactobacillus Acidophilus* diluted in saline solution (2×10^8 CFU/ml) was administrated as eye-drops four times daily for 4 weeks in both eyes. Clinical signs (conjunctival hyperemia, chemosis, secretion, Trantas dots, superficial punctate keratitis) and symptoms (itching, photophobia, burning, tearing) were evaluated and scored from 0 to 3 at baseline, after 2 and 4 weeks of treatment. Total sign (TSS) and symptom (TSyS) scores were calculated. Conjunctival impression cytology was performed in three patients at baseline and after 4 weeks of treatment, in order to evaluate the expression of ICAM-1 and TLR-4.

Results In the six out of seven patients who completed the study, symptoms were significantly improved after both 2 weeks (TSyS: baseline 6.7 ± 0.9 vs 4.1 ± 1.2 ; $p=0.017$) and 4 weeks (TSyS: baseline 6.7 ± 0.9 vs 3.6 ± 1.2 , $p=0.011$) of

treatment. A significant improvement of clinical signs was observed after 4 weeks of treatment (TSS: baseline 7.5 ± 1.6 vs 3.9 ± 1.7 , $p=0.034$) but not after 2 weeks of treatment (TSS: baseline 7.5 ± 1.6 vs 5.3 ± 1.5 ; NS). In particular, photophobia was significantly reduced (2 ± 0.6 vs 1 ± 0.3 ; $p=0.023$) at 2 weeks, while at 4 weeks the scores for itching (1.8 ± 0.3 vs 1 ± 0.3), tearing (1.6 ± 0.4 vs 0.8 ± 0.2), conjunctival hyperemia (2.3 ± 0.2 vs 1.4 ± 0.5) and chemosis (1.2 ± 0.4 vs 0.4 ± 0.4) were significantly lower compared to baseline. A down-regulation of ICAM-1 and TLR-4 was observed in two patients showing clinical improvement after 4 weeks of treatment.

Conclusion Our open pilot study showed that 1-month treatment with probiotic eye-drops improves signs and symptoms in patients with VKC. Additional double-blind controlled clinical trials with a larger sample of patients are needed to confirm the effects of topical *Lactobacilli* on VKC patients.

Keywords Vernal keratoconjunctivitis · Allergy · Conjunctiva · *Lactobacillus Acidophilus* · Probiotic

Introduction

Vernal keratoconjunctivitis (VKC) is a chronic allergic eye disease affecting children in pre-puberal age. However, VKC is not associated with a positive skin test or RAST in 42–47% of patients, confirming that it is not solely an IgE-mediated disease, but that other mechanisms are involved in VKC pathogenesis [2, 3]. It is characterized by recurrent ocular surface inflammation leading to conjunctival hyperemia, mucus discharge and tarsal and/or bulbar papillary reaction. Patients complain of intense ocular symptoms such as itching, photophobia, burning and tearing, lasting all year with seasonal recurrences [2, 3].

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO), Fort Lauderdale, Florida, May 2006.

A. Iovieno · A. Lambiase · M. Sacchetti · B. Stampachiacchiere ·
A. Micera · S. Bonini (✉)
Interdisciplinary Center for Biomedical Research (CIR),
Laboratory of Ophthalmology,
University of Rome "Campus Bio-Medico",
Via E. Longoni, 83,
00155 Rome, Italy
e-mail: s.bonini@unicampus.it

B. Stampachiacchiere · A. Micera
IRCCS-G.B. Bietti Eye Foundation,
Rome, Italy

Topical antiallergic treatment is commonly used to reduce signs and symptoms of the disease but is not always successful. Topical cyclosporine has shown good effectiveness in long-term maintenance of clinical remission in VKC patients, but it is not commercially available for the treatment of VKC [2, 3]. Topical steroids represent the most effective therapy for moderate to severe forms of VKC; however, their long-term use should be strictly limited as it exposes patients to potential ocular side effects. If untreated, VKC leads to severe corneal complications and visual impairment [2, 3].

A novel therapeutic approach to allergic diseases based on the oral administration of probiotic bacteria has been recently proposed [5, 18, 27, 30]. Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [11]. The best-known and commonly used probiotics are strains of *Bifidobacterium* and *Lactobacillus* species. *Lactobacilli* are nonpathogenic, gram-positive bacteria, and represent the major components of the commensal microbial flora of the small and large intestinal tract of both humans and animals.

Previous clinical trials demonstrated the efficacy of *Lactobacilli* therapy for the prevention and treatment of atopic dermatitis, food allergy and allergic rhinitis [14–16, 23, 28, 31]. The health-promoting properties attributed to *Lactobacilli* strains are multiple, and include their capacity to interact with the host immune system. The ingestion of *Lactobacilli* has been shown to enhance IL-10 serum concentrations in patients with atopic dermatitis, therefore displaying anti-inflammatory activities [24]. The chronic use of *Lactobacilli* may strengthen the mucosal immunological barrier of the gut through the promotion of local IgA production and the reduction of epithelial permeability to allergens [1, 9, 10, 17]. In vitro studies have demonstrated that *Lactobacilli* are able to induce Th1-type cytokines such

as IL-12 and IFN- γ ; the stimulation of the Th1 phenotype may normalize the Th1/Th2 imbalance present in allergic conditions [19, 26].

Since VKC is mainly characterized by the increase of Th2-type cytokines and by conjunctival immune cell infiltration, the aim of this study was to investigate the effects of the topical administration of *Lactobacillus Acidophilus* on signs and symptoms of patients with active VKC.

Materials and methods

Lactobacillus Acidophilus eye drops were prepared as follows: freeze-dried inactivated *Lactobacillus Acidophilus* (Lacteol forte, Bruschettini srl, Genova, Italy) was diluted in saline solution (NaCl 0.9% solution) to obtain a final concentration of 200 millions CFU/ml. The preparation was then aliquoted in eye-drop bottles at PH 7–7.4 and stored at -70°C until use. The eye-drop solution was prepared once for the 4 weeks of treatment under the hood in the hospital pharmacy using sterile eye-drop bottles, therefore preserving a sterile condition, aliquoted in eye-drop bottles at PH 7–7.4 and stored at -70°C . One bottle of eye-drop solution (7 ml) was given to the patient once every week, for 1 month. The patients were instructed to keep the eye-drops bottles at 4°C .

This study followed the guidelines of the Declaration of Helsinki for research involving human subjects, and approval was received from The Intramural Committee, in accordance with the ethical principles for medical research involving human subjects. Informed consent was obtained from each patient or his/her parents.

Seven patients with mild to moderate VKC in the active phase (mean age 11.8 ± 4.3 years; range: 7–17 years; sex: five M, two F; Table 1) were included in the study during

Table 1 Characteristics of patients enrolled in the study

Patient	Age/ gender	VKC form	Duration (ys)	Previous therapies	*TSyS (baseline)	*TSyS (post- <i>Lactobacilli</i> treatment)	**TSS (baseline)	**TSS (post- <i>Lactobacilli</i> treatment)
1	13/M	Tarsal/ perennial	4	Lodoxamide	12	8	10	8
2	13/M	Mixed/ perennial	4	Ketotifen	16	14	20	19
3	9/F	Tarsal/ perennial	4	Lodoxamide	16	12	25	19
4	7/M	Mixed/ perennial	6	Ketotifen	16	8	18	6
5	7/M	Tarsal/ seasonal	2	Ketotifen	6	6	6	6
6	17/M	Limbal/ seasonal	2	None	16	12	14	12
7	17/F	Tarsal/ seasonal	9	Ketotifen	12	0	12	0

*TSyS = total symptoms score; **TSS = total sign score.

springtime. Diagnosis of VKC was based on history, clinical presentation (presence of mild to severe cobblestone-like appearance in the upper tarsal and/or limbal papillary reaction) and the presence of eosinophils in upper tarsal conjunctival scrapings. None of the patients were using any form of systemic therapy or contact lenses, had corneal ulceration, had a history of infectious conjunctivitis, or had undergone eye-surgery in past three months.

After 3 days of wash-out, in which patients were only treated with artificial tears and cold compresses, patients received probiotic eye-drops four times daily in both eyes for 1 month.

Clinical symptoms (itching, photophobia, burning and tearing) and signs (conjunctival hyperemia, chemosis, secretion, Trantas dots and superficial punctate keratitis) were evaluated according to slit lamp examination and fluorescein staining, and scored from 0 to 3 (0=absent, 1=mild, 2=moderate, 3=severe) at baseline, after 2 weeks and after 4 weeks of treatment with probiotic eye-drops. Total sign (TSS; range: 0–24) and symptom (TSyS; range: 0–30) scores were calculated as the sum of each symptom and sign score. At each time point, patients were asked about the tolerability of *Lactobacillus* eye-drops. Tolerability was expressed as a score from 0 to 3 (0=no discomfort, 1=mild discomfort, 2=moderate discomfort, 3=severe discomfort, cannot use study drug).

Conjunctival impression cytology (Millicell 0.22 µm filters, Millipore, Milan, Italy) was performed at baseline and after 1 month of *Lactobacillus Acidophilus* eye drop treatment in the three patients who had given their written consent to sampling (patients n° 4–5–6; see Table 1).

Millicell metilcellulose membranes were removed from plastic supports with a surgical blade and placed in an autoclaved microtube containing 300 µl cell lysis solution (Puregene RNA purification kit; Gentra Systems, MI, USA). Membranes were immediately processed to obtain maximum recovery of total RNA from each sample. Total RNA was reverse transcribed, and amplification reaction

was carried out in a programmable PTC100 thermocycler and an Opticon2 machine respectively (MJ Research, Watertown, MA, USA) as described elsewhere [4] (Table 2).

Samples were amplified in duplicate and from these replicates, averages were calculated and differences in C(t) data were evaluated by the REST software package [25]. Target gene expressions were shown in the graph as percentages of increase/decrease values that were obtained after calculating differences between real pre and post Ct values.

All sterile RNase-Free plasticware and analytical reagents were purchased from NUNC (Roskilde, Denmark), Euroclone (Milan, Italy) and GIBCO-Invitrogen (Carlsbad, CA, USA), unless otherwise specified in the text.

The non-parametric Mann-Whitney *U*-test was used to compare signs and symptoms at different time-points. Analysis was performed by using the statistical package StatView II for PC (Abacus Concepts Inc., Berkley, CA, USA) [32]. A probability <0.05 was considered statistically significant. All data is reported as mean ± SEM.

Results

Six out of seven patients (86 %) completed the entire study. Patient 3 discontinued treatment because there was no improvement in his clinical condition, and dropped out from the study at day 14. No side-effects were observed during clinical examination, and all patients tolerated probiotic eye-drop administration (0 score at each time point).

All six patients (100%) reported improvement of clinical conditions after 2 weeks of treatment, and five out of six patients (83%) reported improvement of clinical conditions after 4 weeks of treatment. Accordingly, total symptom score (TSyS) was significantly lower after both 2 weeks (TSyS 6.7±0.9 vs 4.1±1.2; *p*=0.017) and 4 weeks (TSyS: 6.7±0.9 vs 3.6±1.2, *p*=0.011) of treatment when compared to baseline (Fig. 1a). In particular, photophobia was

Table 2 Sequence of primers used for gene amplification in real-time PCR

Gene (amplicon size)	Sequence	Reference
ICAM-1 (107 bp)	Sense: 5'-ATGAGTGCCCGAGGAATATG-3' Antisense: 5'-CCCTGATCACTGCAGGAAA -3'	J03132
TLR-4 (102 bp)	Sense: 5'-AAT CCC CTG AGG CAT TTA GG-3' Antisense: 5'- CAG GGC TAA ACT CTG GAT GG-3'	U88880
GAPDH (100 bp)	Sense: 5'-GAA GGG GTC ATT GAT GGC AAC-3' Antisense: 5'- GGG AAG GTG AAG GTC GGA GTC-3'	BC013310

cDNAs were run for amplification with primers (10 pM, prepared by MWG, Biotech, Ebersberg, Germany) specific for ICAM-1 and TLR4 designed by the primer3 software available online at http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi [29]. GenBank software was used to select the mRNA complete sequence of each gene investigated (<http://www.ncbi.nlm.nih.gov/> Genbank; provided to the public domain by the National Center for Biotechnology Information, Bethesda, MD, USA). The PCR amplification profile included: 1 cycle of activation 95°C/15 min followed by 43 cycles of denaturation at 94°C/30 sec, annealing at 60°C/25 sec and extension at 75°C/30 sec, completed by a further extension at 75°C/5 min.

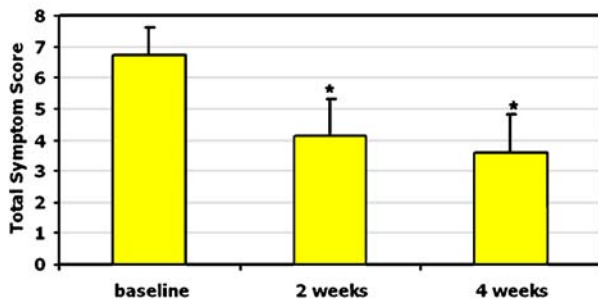
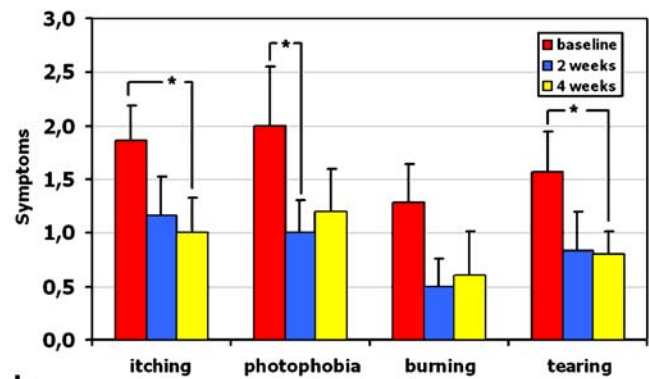
**a**

Fig. 1 Effect of *Lactobacillus Acidophilus* eye-drops on symptoms of VKC patients. **a** Total symptom score at different time-points. A significant improvement (*) in TSyS was observed after 2 and 4 weeks of treatment. **b** Symptom scores at different time-points. Photophobia

**b**

was significantly improved (*) after 2 weeks of treatment while itching and tearing were significantly improved after 4 weeks of treatment

significantly improved (2 ± 0.6 vs 1 ± 0.3 ; $p=0.023$) at two weeks time-point, while at 4 weeks time-point itching (1.8 ± 0.3 vs 1 ± 0.3) and tearing (1.6 ± 0.4 vs 0.8 ± 0.2) were significantly improved (Fig. 1b).

A significant improvement in total sign score (TSS) was observed after 4 weeks of treatment with respect to baseline (TSS: 7.5 ± 1.6 vs 3.9 ± 1.7 , $p=0.034$) but not after 2 weeks (TSS: 7.5 ± 1.6 vs 5.3 ± 1.5 ; NS) (Fig. 2a). In particular, conjunctival hyperemia (2.3 ± 0.2 vs 1.4 ± 0.5) and chemosis (1.2 ± 0.4 vs 0.4 ± 0.4) scores were significantly lower at the 4-week time-point compared to baseline (Figs. 2b, 3).

Figure 4 shows modifications of conjunctival epithelial expression of ICAM-1 and TLR-4 after 4 weeks of treatment with *Lactobacillus Acidophilus* eye-drops. Interestingly, a down-regulation of both ICAM-1 and TLR-4 was observed in patient 4 and patient 6, and both these patients showed clinical improvement after 4 weeks of treatment. On the other hand, up-regulation of both conjunctival markers was observed in patient 5, who showed no improvement after treatment.

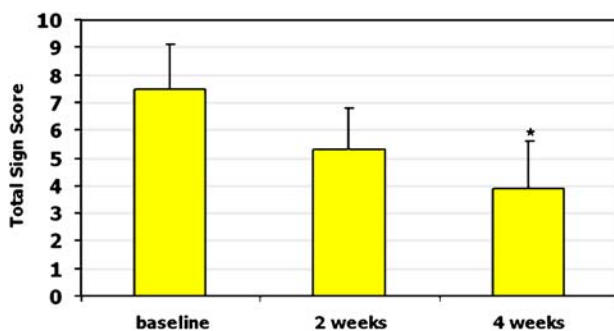
**a**

Fig. 2 Effect of *Lactobacillus Acidophilus* eye-drops on clinical signs of VKC patients. **a** Total sign score at different time-points. A statistically significant improvement (*) of TSS was observed after

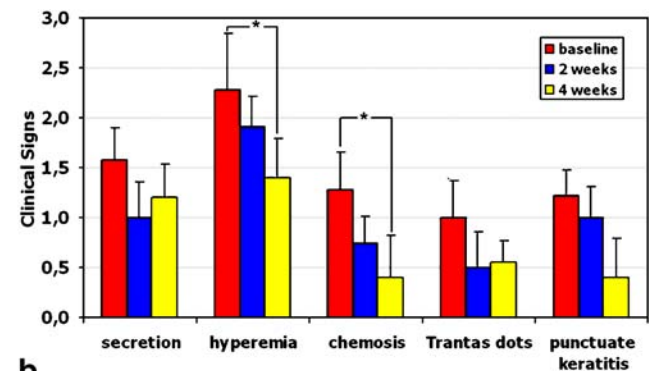
Discussion

This pilot open study demonstrated that 4-week treatment with *Lactobacillus Acidophilus* eye-drops was effective in reducing signs and symptoms of patients with mild to moderate vernal keratoconjunctivitis in the active phase.

Lactobacilli eye-drops did not cause any side effects and were well-tolerated by patients, as reported after systemic administration.

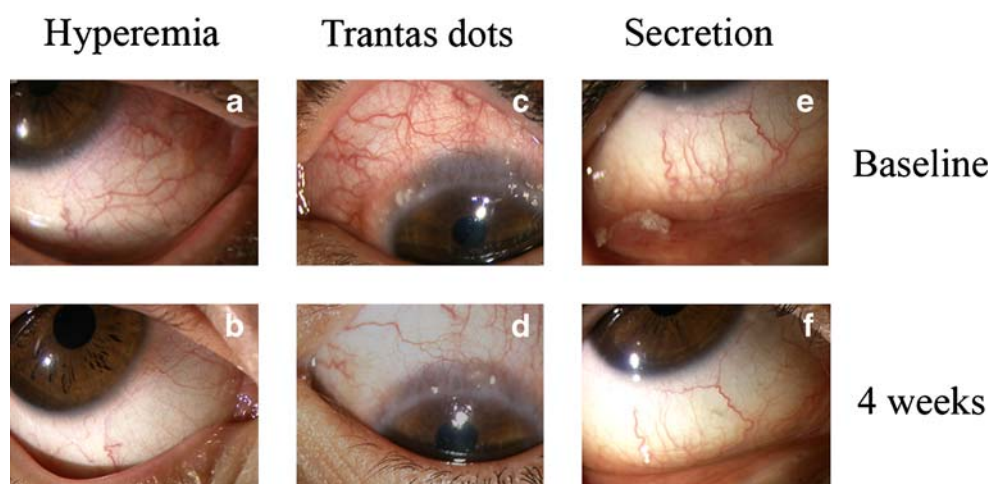
All patients reported improvement of symptoms, except one who dropped out of the study because his clinical condition did not improve after 2 weeks of treatment. This patient had the most severe form of VKC included in the study, with the highest total symptom score, total sign score and punctuate keratopathy scores at baseline.

Thus, total symptom score of the remaining patients was significantly improved after 2 and 4 weeks of treatment, demonstrating that *Lactobacillus Acidophilus* eye-drops can improve symptoms in patients with VKC in the active phase. A statistically significant improvement of clinical signs was also observed after 1 month of treatment; in

**b**

4 weeks of treatment. **b** Symptom scores at different time-points The improvement in hyperemia and chemosis reached statistical significance (*) at the 4-week time-point

Fig. 3 Patient characteristics at slit-lamp examination. An improvement of conjunctival hyperemia (A, B; $p < 0.05$), Trantas dots (C, D; $p > 0.05$) and secretion (E, F; $p > 0.05$) was observed after 4 weeks of *Lactobacilli* eye-drop treatment



particular, hyperemia and chemosis scores were significantly lower with respect to baseline.

Little is known regarding the precise mechanisms by which lactobacilli may improve the clinical conditions of allergic patients. The therapeutic effects of *Lactobacillus Acidophilus* on VKC patients after 1 month of therapy could be related to its antiinflammatory properties. Lactobacilli act by inducing antiinflammatory cytokines such as IL-10 and TGF- β , that play a crucial role in down-regulating inflammatory cascades. Pessi et al. showed that serum IL-10 concentrations were significantly elevated after giving oral *Lactobacillus rhamnosus* GG at a daily dose of 2×10^{10} CFU for 4 weeks to nine children affected by atopic dermatitis [24]. Peripheral blood mononuclear cells, monocytes and lymphocytes from healthy donors, if stimulated with lactobacilli and with polyclonal T cell stimulator

phytohaemagglutinin (PHA), produce high levels of IL-10 and low levels of Th2-type cytokines [20]. In line with this hypothesis, the reduction of conjunctival epithelial ICAM-1 expression observed in two patients showing clinical improvement supports the hypothesis of an antiinflammatory effect of topical *Lactobacillus* on the ocular surface.

Another hypothesis is based on a direct effect of *Lactobacilli* eye-drops in modulating the allergic reaction. The concept of probiotics as possible means of antiallergic therapy emerged from substantial but indirect evidence linking low incidence rates of allergies in poor countries with a certain composition of intestinal flora. These findings are in line with the “hygiene hypothesis”, reporting that the exposure to microbial products early in life could be essential for the proper development of the immune system [30]. The greatest microbial exposure throughout life, and the newborn’s first major challenge, is the intestinal microbiota. Bjorkstein et al. observed that *Lactobacilli* are more commonly found in the intestinal microflora of populations (eg. Estonian) with low incidence of allergic diseases [6]. Besides, Kalliomaki et al. showed that children who developed allergic diseases were less often colonized by lactic acid producing bacteria, but had higher counts of *Clostridia* when compared with healthy children [13].

There is evidence that probiotic microorganisms preferentially elicit T-helper 1 cells (Th1) and T-helper3/T regulatory cells (Treg) that counterbalance the predominance of T-helper 2 (Th2) direct immune responses in allergic disease. The ability of probiotic bacteria to induce Th1 and Treg responses is based on the recognition of bacterial cell components by cell receptors such as Toll-like receptors (TLR), a family of glycoproteins expressed by epithelial cells, dendritic cells, monocytes and mast cells/basophils [8, 12]. TLRs are involved in the production of inflammatory cytokines, cell stimulation and activation of the specific immune response [12]. The expression of TLR-4 has been correlated with the allergic phenotype. Dabbagh

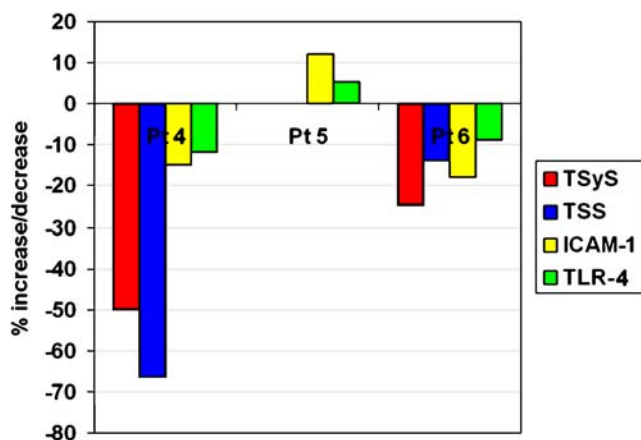


Fig. 4 Characteristics of the patient that underwent TLR4/ICAM-1 analysis. A significant down-regulation in ICAM-1 and TLR-4 mRNA was observed in patients (Pt4 and Pt6) showing a reduction in total symptoms score (TSyS) and total signs score (TSS) after 4 weeks of treatment with *Lactobacilli* eye-drops. A slightly increase of both ICAM-1 and TLR-4 mRNA was detected in the patient (Pt5) that did not show an improvement of TSyS and TSS following *Lactobacilli* treatment

et al. showed that TLR4-defective mice subjected to sensitization and pulmonary challenge with a protein allergen had reductions in airway inflammation by eosinophils, allergen-specific IgE levels, and Th2 cytokine production, compared with wild-type mice [7]. In addition, in vitro studies observed that TLR-4 activation on mast cells resulted in the induction of a subset of genes including Th2 cytokines and chemokines [21, 22]. In a recent study, VKC conjunctiva displayed upregulation of TLR-4, when compared to healthy conjunctiva [4]. Thus, the reduction of conjunctival expression of TLR-4 observed in patients after 4 weeks of therapy could be indirect evidence of the immunomodulating effects of *Lactobacillus Acidophilus*.

Our preliminary data could suggest that topical treatment with *Lactobacillus Acidophilus* may offer a safe and well-tolerated support to standard antiallergic therapy for the treatment of mild to moderate cases of vernal keratoconjunctivitis. However, it is not possible to exclude that the beneficial effects referred by patients after 2 weeks of treatment might be due to washing out of allergens from the ocular surface rather than the effect of *Lactobacillus* administration. The topical administration of *Lactobacillus Acidophilus* may require at least 4 weeks of treatment to exert a clinically significant effect. Additional double-blind controlled clinical trials with a larger sample of patients are needed to confirm the therapeutic effects of topical *Lactobacilli* on VKC patients.

In conclusion, the administration of *Lactobacilli* eye-drops in VKC patients represents the first description of a topical use of probiotic bacteria in humans.

References

- Ait-Belgnaoui A, Han W, Lamine F, Eutamene H, Fioramonti J, Bueno L, Theodorou V (2006) *Lactobacillus farciminis* treatment suppresses stress induced visceral hypersensitivity: a possible action through interaction with epithelial cell cytoskeleton contraction. *Gut* 55:1090–1094
- Bonini S, Bonini S, Lambiase A, Marchi S, Pasqualetti P, Zuccaro O, Rama P, Magrini L, Juhas T, Bucci MG (2000) Vernal keratoconjunctivitis revisited: a case series of 195 patients with long-term followup. *Ophthalmology* 107:1157–1163
- Bonini S, Coassin M, Aronni S, Lambiase A (2004) Vernal keratoconjunctivitis. *Eye* 18:345–351
- Bonini S, Micera A, Iovieno A, Lambiase A, Bonini S (2005) Expression of Toll-like receptors in healthy and allergic conjunctiva. *Ophthalmology* 112:1528; discussion 1548–1549
- Boyle RJ, Tang ML (2006) The role of probiotics in the management of allergic disease. *Clin Exp Allergy* 36:568–576
- Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M (2001) Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 108:516–520
- Dabbagh K, Dahl ME, Stepick-Biek P, Lewis DB (2002) Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* 168:4524–4530
- Feleszko W, Jaworska J, Hamelmann E (2006) Toll-like receptors—novel targets in allergic airway disease (probiotics, friends and relatives). *Eur J Pharmacol* 533:308–318
- Galdeano CM, Perdigon G (2006) The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity. *Clin Vaccine Immunol* 13: 219–226
- Heyman M, Terpend K, Menard S (2005) Effects of specific lactic acid bacteria on the intestinal permeability to macromolecules and the inflammatory condition. *Acta Paediatr Suppl* 94:34–36
- Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food London, Ontario, Canada, April 30 and May 1, 2002
- Kaisho T, Akira S (2006) Toll-like receptor function and signaling. *J Allergy Clin Immunol* 117:979–987
- Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E (2001) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 107:129–134
- Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E (2001) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 357:1076–1079
- Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E (2003) Probiotic and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 361:1869–1871
- Majamaa H, Isolauri E (1997) Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 99:179–185
- Malin M, Suomalainen H, Saxelin M, Isolauri E (1996) Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus GG*. *Ann Nutr Metab* 40: 137–145
- Matricardi PM, Bjorksten B, Bonini S, Bousquet J, Djukanovic R, Dreborg S, Gereda J, Malling HJ, Popov T, Raz E, Renz H, Wold A; EAACI Task Force 7 (2003) Microbial products in allergy prevention and therapy. *Allergy* 58:461–471
- Miettinen M, Matikainen S, Vuopio-Varkila J, Pirhonen J, Varkila K, Kurimoto M, Julkunen I (1998) *Lactobacilli* and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. *Infect Immun* 66:6058–6062
- Niers LE, Timmerman HM, Rijkers GT, van Bleek GM, van Uden NO, Knol EF, Kapsenberg ML, Kimpfen JL, Hoekstra MO (2005) Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clin Exp Allergy* 35:1481–1489
- Nigo YI, Yamashita M, Hirahara K, Shinnakasu R, Inami M, Kimura M, Hasegawa A, Kohno Y, Nakayama T (2006) Regulation of allergic airway inflammation through Toll-like receptor 4-mediated modification of mast cell function. *Proc Natl Acad Sci USA* 103:2286–2291
- Okumura S, Kashiwakura J, Tomita H, Matsumoto K, Nakajima T, Saito H, Okayama Y (2003) Identification of specific gene expression profiles in human mast cells mediated by Toll-like receptor 4 and FcεR1. *Blood* 102:2547–2554
- Peng GC, Hsu CH (2005) The efficacy and safety of heat-killed *Lactobacillus paracasei* for treatment of perennial allergic rhinitis induced by house-dust mite. *Pediatr Allergy Immunol* 16:433–438
- Pessi T, Sutas Y, Hurme M, Isolauri E (2000) Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus GG*. *Clin Exp Allergy* 30:1804–1808
- Pfaffl MW, Horgan GW, Dempfle L (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* 30(9):e36

26. Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tiittanen M, Vaarala O, Savilahti E (2004) Lactobacillus GG effect in increasing IFN-gamma production in infants with cow's milk allergy. *J Allergy Clin Immunol* 114:131–136
27. Rautava S, Kalliomaki M, Isolauri E (2005) New therapeutic strategy for combating the increasing burden of allergic disease: Probiotics-A Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota (NAMI) Research Group report. *J Allergy Clin Immunol* 116:31–37
28. Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, Paerregaard A (2003) Effect of probiotic Lactobacillus strains in children with atopic dermatitis. *J Allergy Clin Immunol* 111:389–395
29. Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 132:365–386
30. Strachan DP (1989) Hay fever, hygiene, and household size. *BMJ* 299:1259–1260
31. Vanderhoof JA, Young RJ (2004) Current and potential uses of probiotics. *Ann Allergy Asthma Immunol* 93:S33–S37
32. Wilcox RR (2007) New statistical procedures for the social sciences: modern solution to basic problems. Lawrence Erlbaum Associates, Hillsdale (NJ)