

Short Report

Cytokines evaluation in nasal lavage of allergic children after *Bacillus clausii* administration: A pilot study

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Respiratory infections are very frequent in children. *Bacillus clausii* has been demonstrated to exert some immunomodulatory activities and to be safe. We conducted a study to investigate whether *B. clausii* administration in allergic children with recurrent respiratory infections might modulate cytokine pattern. Ten children (mean age 4.4 yr) attending the nursery school were enrolled at the end of school year (i.e. in the summer). *Bacillus clausii* spores (Enterogermina®: 2 billion spores per vial) were administered at the dosage schedule of two vials a day for 4 wk. A panel of cytokines, including interleukin (IL)-1, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, interferon (IFN)- γ , transforming growth factor (TGF)- β , and tumor necrosis factor (TNF)- α , was measured by immunoassay in the fluid recovered from nasal lavage, performed before and after the treatment. *Bacillus clausii* treatment induced a significant decrease of IL-4 levels ($p < 0.01$) and a significant increase of IFN- γ ($p < 0.05$), IL-12 ($p < 0.001$), TGF- β ($p < 0.05$), and IL-10 ($p < 0.05$) levels. Other cytokines were not significantly modified. In conclusion, this study shows that the *B. clausii* may exert immunomodulating activity by affecting cytokine pattern at nasal level in allergic children with recurrent respiratory infections.

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Respiratory infections are very frequent in children and their recurrence constitute a demanding challenge for the pediatrician, mainly concerning the diagnosis, the therapeutic strategies, and the preventive approach. As prevention should reduce the need for treatment, the prophylactic use of a probiotic milk has been recently demonstrated to be able to reduce respiratory infections and their severity among children attending daycare centers (1). Probiotics are microorganisms that exhibit a beneficial effect on the health of the host. The interaction between probiotics and the host has a profound impact in many ways. One is to stimulate immune system by promoting T-helper 1 (Th1) and T-regulatory 1 (Tr1) immunity, and by decreasing T-helper 2 (Th2) activity (2). Allergic children show a Th2 polarization characterized

by high levels of interleukin (IL)-4 and low levels of interferon (IFN)- γ (3). Therefore, probiotics could represent an important therapeutic advance concerning the prevention of infections and atopy.

In this context, *Bacillus clausii* is a safe and frequently prescribed probiotic. A recent review reports some studies showing its role in preventing gastroenteric and respiratory infections in children, and in modulating immune response (4).

Therefore, we aimed at investigating the potential effects exerted by *B. clausii* on cytokine pattern, including IL-1, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, IFN- γ , transforming growth factor (TGF)- β , and tumor necrosis factor (TNF)- α , in allergic children with recurrent respiratory infections.

Material and methods

Subjects

Ten allergic children attending the nursery school were evaluated. A detailed clinical history and a complete physical examination, including allergy evaluation, were carried out for each patient. Demographic data are shown in Table 1.

Study design

In order to evaluate cytokine pattern, we included a group of 10 children with the diagnosis of allergy and the recurrence of respiratory infections. The diagnosis of allergy was made on the basis of clinical history, physical examination, and skin prick test for a panel of common allergens described elsewhere (5). Recurrence of respiratory infections was arbitrarily defined as many as six infections per year. We excluded all the children who used antihistamines, nasal, inhaled or oral corticosteroids within the previous 4 wk.

A placebo group was not considered, as the main scope of this study was to evaluate the immunopathologic phenomena appearing after the *B. clausii* administration. Anyway, the study was blind to the investigator who performed the cytokine dosages.

All children were treated with oral *B. clausii* (Enterogermina®; Sanofi-Synthelabo OTC, Milan, Italy) spores (2 billion spores per vial) at the dosage schedule of two vials a day for 4 wk. The study was performed at the end of the school year, i.e. in the summer: period with very low incidence of respiratory infections in our area.

Nasal lavage was performed in all subjects before and directly after *B. clausii* administration.

The study was approved by the Institutional Review Board and an informed oral consent was obtained from the parents of the children.

Nasal lavage

A lavage of the nasal cavity was performed using 5 ml of physiologic saline, according to standard methods described elsewhere (5). This was performed with the patient's head bent backwards during closure of the soft palate. The recovered fluid was comparable in all subjects, it was collected to dose cytokine levels and stored at -20°C (5).

Cytokine assessment

The cytokines were measured with ELISA method (R&D Systems, Minneapolis, MN, USA). This assay employed the quantitative sandwich enzyme immunoassay technique. The methodology was extensively reported elsewhere (5).

Statistical analysis

The data collected were subjected to statistical analysis by M-ANOVA and *post hoc* comparison by Duncan test. The Th2/Th1 ratio was calculated using the *t*-test. All results with *p*-values less than 0.05 were considered statistically significant.

Results

All children assumed the prescribed treatment with *B. clausii* spores without any side-effect. Figures show the cytokine pattern before and after *B. clausii* treatment.

The IL-4 levels significantly diminished after the treatment ($p < 0.001$; mean basal values 4.49 ± 1.45 s.d. and mean post values 3.52 ± 0.9 s.d.). *Bacillus clausii* significantly increased IFN- γ levels ($p < 0.05$; mean basal values 1.81 ± 0.79 s.d. and mean post values 2.65 ± 0.69 s.d.) and IL-12 ($p < 0.001$; mean basal values 0.76 ± 0.43 s.d. and mean post values 1.36 ± 0.81 s.d.). TGF- β levels were significantly augmented by *B. clausii* ($p < 0.05$; mean basal values 0.9 ± 0.34 s.d. and mean post values 1.33 ± 0.51 s.d.) as well as IL-10 levels ($p < 0.05$; mean basal values 1.45 ± 0.75 s.d. and mean post values 2.05 ± 0.72 s.d.).

The Th2:Th1 ratio (IL-4/IFN- γ) was significantly reduced from 2.92 ± 1.71 before treatment to 1.44 ± 0.57 after treatment ($p = 0.018$).

The other evaluated cytokines (IL-1, IL-3, IL-6, and IL-8) were not significantly modified by *B. clausii* (data not shown).

Table 1. Subjects characteristics

Subjects	Gender	Age	Allergic disease	Sensitization	Asthma severity	Type RI	Frequency
1	F	4	A	HDM	1	URI	7
2	M	3	A, R	HDM	1	URI	6
3	M	5	A	HDM	1	URI	7
4	M	5	A, R	HDM	1	URI	6
5	F	4	A, R	HDM	1	URI	7
6	M	4	A, R	HDM	1	URI, LRI (2)	8
7	M	4	A	HDM	1	URI	8
8	F	5	A, R	HDM	1	URI, LRI	7
9	F	5	A	HDM	1	URI	8
10	M	6	A, R	HDM	1	URI	7

A, asthma; R, rhinitis; HDM, house dust mite; Asthma severity according to Global Initiative for Asthma (GINA) classification; Type RI, type of respiratory infections: URI, upper respiratory infections (rhinopharyngitis, sinusitis, tonsillitis, adenoiditis, otitis media); LRI, lower respiratory infections (tracheobronchitis and pneumonia); Frequency in the last year.

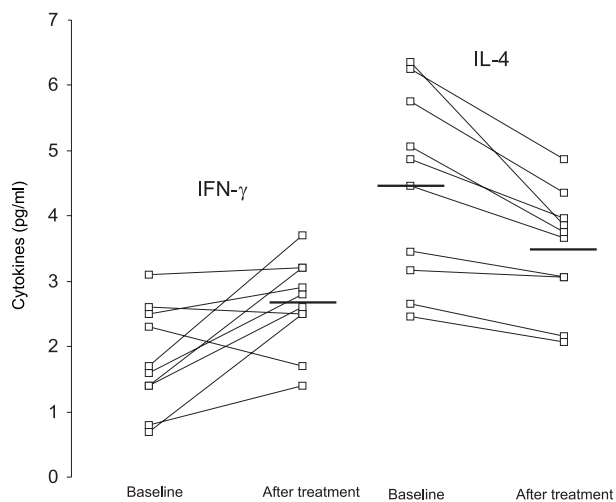


Fig. 1. Cytokine levels before and after *B. clausii* treatment.

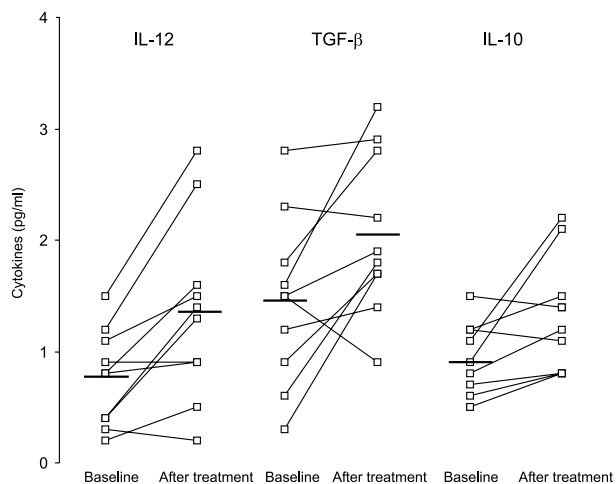


Fig. 2. Cytokine levels before and after *B. clausii* treatment.

Discussion

The recurrence of respiratory infections in children constitute a significant burden for society in general and exert a critical impact on families.

Allergic children are characterized by a Th2 polarization (3). As Th2 cytokines have a pivotal role on development of allergic inflammation, there is accumulating evidence that the Th1-related cytokine IFN- γ exerts a crucial role to reverse ongoing allergic reaction (6).

Probiotics may stimulate immune system at all mucosal surfaces and promote potentially anti-allergenic and anti-inflammatory processes: Th1 immunity, counter-regulatory Tr1 immune responses, and immunoglobulin (IgA) synthesis (2). Therefore, probiotics reversing the Th2 polarized immunologic memory could represent

an important therapeutic advance concerning the prevention of infections and atopy in children.

Although the exact mechanisms by which probiotics may affect immune response remain speculative, there is increasing evidence that specific input from fecal flora to the innate immune system is essential for the establishment and maintenance of mucosal immune tolerance (7). Specific oral tolerance is critically dependent on inhibition of potential lymphocyte reactivity, and the recently recognized Tr1 cells, which produce TGF- β and IL-10, have been shown to downregulate mucosal inflammatory responses (8).

Bacillus clausii spores have been demonstrated safe at any age and effective in the treatment of several disorders (4). Particularly, Novelli et al., in an open placebo-controlled study, showed prevention of recurrent respiratory infections in children treated with *B. clausii* (9). Galli et al. reported a beneficial effect exerted by *B. clausii* in children with recurrent otitis media (10).

Moreover, *B. clausii* has been shown to exert widely documented health effects especially on immune system. Its immunomodulating activity concerns both specific stimulation of production of secretory IgA in humans (11) and non-specific enhancement of IFN- γ synthesis in animals (12). In addition, normal subjects treated with spores of *B. clausii* showed an increased percentage of T lymphocytes positive for histocompatibility lymphocyte associated-DR (HLA-DR) as a marker of T-cell activation (13).

Our findings may suggest that *B. clausii* administration in allergic children with recurrent respiratory infections could exert some immunomodulating effects.

First, there is a reverse of Th2:Th1 ratio; IL-12 increased production stimulated Th1 function as evidenced by the augmented IFN- γ levels in nasal lavage fluids. Moreover, the stimulated Th1 response caused a reduction of IL-4 synthesis after *B. clausii* administration, confirming previous studies (14). In addition, *B. clausii* should affect the Tr1 cells as provided by increased levels of TGF- β and IL-10.

IL-4 has been recently demonstrated to be able to disrupt the intestinal epithelial barrier function and IL-10 and TGF- β were able to partially inhibit this deleterious effect of IL-4 on the epithelium (15). Our findings indirectly confirm this regulatory activity exerted by the Tr1-related cytokines IL-10 and TGF- β toward the Th2-related cytokine IL-4.

Of course, our study was performed on a very small cohort of children and the scheduled time of administration and observation was limited.

In addition, there is no reference about the evaluation of *B. clausii* germination during this study, so its direct effect as immunomodulator may not be considered definitive. On the contrary, we investigated the *B. clausii* effects at the level of the target organ, such as the upper airways, in allergic children with a history of recurrent respiratory infections. These data are supported by the concept of the recirculation of immunocompetent cells as the *B. clausii* administration occurred *per os*.

Anyway, our findings provide possible mechanisms of action exerted by *B. clausii* in modulating immune functions.

Further studies are necessarily required to confirm this hypothesis, enrolling wide cohorts of children and using long-term administration schedules.

References

1. HATAKKA K, SAVILAHTI E, PONKA A, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centers: double blind, randomised trial. *BMJ* 2001; 322: 1–5.
2. ELSON CO, CONG Y. Understanding Immune-Microbial homeostasis in intestine. *Immunol Res* 2002; 26: 87–94.
3. MURCH SH. Toll of allergy reduced by probiotics. *Lancet* 2001; 357: 1057–9.
4. CIPRANDI G, TOSCA MA. Probiotics in paediatric allergy: evidence and perspectives for *Bacillus clausii*. *Ital J Paed Allergy Immunol* 2002; 2: 49–57.
5. RICCIO AM, TOSCA MA, COSENTINO C, et al. Cytokine pattern in allergic and non-allergic chronic rhinosinusitis in asthmatic children. *Clin Exp Allergy* 2002; 32: 422–6.
6. KALLIOMAKI M, KIRJAVAINEN P, EEROLA E, et al. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Clin Allergy Immunol* 2001; 107: 129–34.
7. MURCH SH. The immunologic basis for intestinal food allergy. *Curr Opin Gastroenterol* 2000; 16: 552–7.
8. LEVINGS MK, BACCHETTA R, SCHULZ U, RONCAROLO MG. The role of IL10 and TGF- β in the differentiation and effector function of T regulatory cells. *Int Arch Allergy Immunol* 2002; 129: 263–76.
9. NOVELLI A, ULIVELLI A, REALI EF, et al. *Bacillus subtilis* spores as a natural pro-host oral agent. Preliminary data in children. *Chemioterapia* 1984; 3: 152–5.
10. GALLI E, CORGIOLO M, FIORE L, et al. Valutazione di alcuni parametri immunologici in bambini con otite ricorrente trattati con spore di *B subtilis*. *Clin Ter* 1984; 109: 329–34.
11. FIORINI G, CIMMINIELLO C, CHIANESE R, et al. *B. subtilis* selectively stimulates the synthesis of membrane bound and secreted IgA. *Chemioterapia* 1985; 4: 310–2.
12. GRASSO G, MIGLIACCIO P, TANGANELLI C, et al. Restorative effect of *B. subtilis* spores on interferon production in aged mice. *Pharm Aging Proc* 1994; 717: 198–208.
13. CARUSO A, FLAMMINIO G, FOLGHERA S, et al. Expression of activation markers on peripheral-blood lymphocytes following oral administration of *B. subtilis* spores. *Int J Immunopharmacol* 1993; 15: 87–92.
14. KAUR IP, CHOPRA K, SAINI A. Probiotics: potential pharmaceutical applications. *Eur J Pharm Sci* 2002; 15: 1–9.
15. DI LEO V, YANG P, BERIN MC, PERDUE MH. Factors regulating the effect of IL-4 on intestinal epithelial barrier function. *Int Arch Allergy Immunol* 2002; 129: 219–27.