IMMUNO-MODULATORY EFFECT OF PROBIOTIC AND SUBLINGUAL IMMUNOTHERAPY IN ASTHMATIC CHILDREN

Arijanto Harsono, Lisa Angraini Subrata, Anang Endaryanto
Department of Pediatrics
Faculty of Medicine
Airlangga University, Surabaya, Indonesia

ABSTRACT

Sublingual immunotherapy (SLIT) effectively influences the natural course of asthma in aero-inhalant allergy. Probiotic as immunomodulator seems decreases inflammatory proccess induced by food allergy. But the immunopathogenesis about how both immunomodulator reducing allergy inflammation are still unclear. The objective of this study was to investigate the ability of sublingual house dust immunotherapy and probiotic in improving lung function and regulating cytokines TH2 (IL-4, IL-5), IgE, eosinophil, TH1 (IFN-γ), and Tregulator (TGF-β) in asthmatic children. Study design was Randomized Single Blind clinical experimental trial of 6-17 years old asthmatic children sensitive to food and aero-allergens. Informed consent were obtained prior to the Study. Sample were alocated to Group 1 receiving SLIT, Group 2 receiving probiotic, Group 3 receiving SLIT and probiotic with systematic random sampling. All parameter were evaluated on week 0 until 14. Primary outcome was FEV1 reversibility, Secondary outcome were IL-4, IL-5, IgE level, Eosinophil count, IFN-γ and TGF-β. Local Ethic Committee approved this study. It was found that FEV1 reversibility improved in all Groups. IgE level decreased in Group 1 and 3, slightly inccreased in Group 2. Eosinophil counts decreased in all Group. IL-4 and IL-5 decreased significantly in Group 3. IFN-γ and TGF-β increased in all Groups. In conclusion, lung function test improved in patients receiving SLIT and probiotic suggestive modulation of cytokine balance, decreasing of eosinophil and IgE. Increasing IgE titer in patients receiving probiotic was compensated by increasing IgA, suggesting achievement of new equilibrium of Th1 and Th2 profile at upper level.

Keywords: *sublingual immunotherapy, probiotic, FEV1 reversibility, cytokines, eosinophil, IgE*

Correspondence: Ariyanto Harsono, Department of Paediatrics, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, Jl Mayjen Mustopo 6-8 Surabaya, Indonesia, phone 62-31-5501680

INTRODUCTION

Sublingual immunotherapy (SLIT) is very effective in the management of asthma in children sensitive to inhalant allergen. The efficacy of SLIT in allergic asthma due to food allergy is not yet proven. Probiotic bacteria, which beneficially affect the host by improving its microbial balance, may mediate antiallergenic effects by stimulating production of TH1-cytokines, TGF-ß, and gut IgA. This immunomodulator may reduce symptoms of allergy in children, but thus far few clinical trials exist. Observational studies in humans also support the importance of the intestinal micro biota in immune development, and have found a relationship with the development of hypersensitivity. Interestingly these differences in load and species composition of Bifidobacteria may have special anti-inflammatory modality as they have not been found in young children with wheeze and allergic sensitization (Ebner 1997; Isolauri 2000). It is expected that probiotic as potent immuno-modulator may acts synergistically improving the clinical efficacy of SLIT that limited to the aero-inhalant allergen.

MATERIALS AND METHODS

Asthmatic children controlled as Allergy and Immunology outpatient clinic Dr. Soetomo Hospital – Surabaya, from January to April 2006 were enrolled in this study. A written informed consent form would also be obtained prior to any study-related procedures, where practical. The subject must give an oral consent/assent to participate in the study. The inclusion criteria were as follows: Subject, of either gender, is 6 - 17 years of age and has a diagnosis of asthma as defined by the American Thoracic Society; subjects 6-11 years of age, must have a pre-bronchodilator FEV1 $\geq$ 75% and $\leq$ 90% of Polgar predicted normal value at Visit 1; subjects 12-17 years of age, must have a pre-bronchodilator FEV1 $\geq$ 60% and $\leq$ 90% of Polgar predicted normal value at Visit 1; subjects with an FEV1 > 90% and $\leq$ 95% predicted may be included if they have an absolute

FEV1/FVC ratio measured on screening spirometry of < 80%; FEV1 reversibility ≥ 12% , performed at Visit 1; treatment of orally inhaled corticosteroid for no more than 1 month (30 days) immediately preceding Visit 1. The exclusion criteria were as follows: Subjects with life-threatening asthma including severe asthma, any prior intubations, respiratory arrest or seizures within the past 5 years as a result of an exacerbation of asthma; subjects with = inpatient hospitalizations for asthma within 1 year of Visit 1 or any emergency room for asthma within 6 months of Visit 1; use of leukotriene modifiers, inhaled long acting β2 agonist, oral β2-agonists, theophyllines, anti-cholinergic, cromones or ketotifen (oral), within 2 weeks prior to Visit 1; history of smoking; acute exacerbation of asthma/respiratory tract infection within 30 days prior to Visit 1 that may affect the results of the study; previous randomization into this study; use of an experimental drug or device within 30 days of Visit; any subject who is unable to meet the concomitant medication restrictions should not be enrolled. Subject will be withdrawn if they have: Intolerable adverse event; decrease in FEV1 (L) ≥ 25 % from Visit 1 or to below 40% of predicted; using of ≥ 12 actuations of albuterol or salbutamol pMDI/day for 2 days within a 3 day period; decreasing in morning PEF> 25% from baseline (baseline defined as the mean of the last 7 days prior to randomization) on 3 days within a 5-day period; three nighttime awakenings requiring treatment with short-acting inhaled β2-agonist within a 5-day period; = 80% data entry compliance.

The study was designed in prospective-single blind randomized clinical trial. The patients were enrolled in three groups (SLIT, probiotic, and SLIT-probiotic) using computer software incorporating a random number generator data. Eligible subjects from Allergy and Immunology outpatient clinic will be randomized at Visit 2 to receive one of 3 possible treatments for 14 weeks: Group A (SLIT), Group B (Probiotic), and Group C (SLIT and Probiotic).

Table 1. Dosing Schedule:

Groups	N	Sublingual Immunotherapy	Probiotic
A	10	Drug	Placebo (2.5g skim milk)
B	10	Placebo (Dextrose 2.5%)	Drug
C	10	Drug	Drug

NOVO-HELISEN ORAL is an allergen extract for specific oral immunotherapy. The composition of the allergens is listed on the labels. Novo-Helisen® oral is standardized in TU (therapeutic units) or in PNU (protein nitrogen units). Strength 2 of Novo-Helisen oral is a 1:10 dilution of strength 3, strength 1 is a 1:10 dilution of strength 2 and strength 0 is a 1:10 dilution of strength 1. Initial treatment set: 3 vials of strength 1, 2, 3 vials of strength 1, 2, 3 with 30 ml each. Begin treatment with 1 drop of the weakest concentration (strength 1). This dose is increased by 1 drop daily. Once the dose of 28 drops a day has been reached, treatment is continued with 2 drops of the next highest concentration (strength 2). This dose is again increased by 1 drop daily until 28 drops of strength 2 are reached. Treatment is then continued with 2 drops of strength 3, again increased daily by 1 drop until the maximum dose of 28 drops of strength 3 is reached. In probiotic preparation, each capsule contains: Lactobacillus GG 40 mg (20 Billion). A dose of 1010 CFU of each strain was given twice daily. During the 14 week study period, the patients were asked to abstain from any fermented food products containing live microorganisms. Otherwise, none of the patients changed diet during the study period.

Visit I

All subjects will be asked to supply certain information and will undergo study tests and procedures as indicated below:

1. Medical/medication history, demonstrate or provide documentation of clinical history of asthma.
2. Pulmonary function testing. Including reversibility test of FEV1
3. Clinical chemistry, hematology, and cytokine
4. Complete physical examination.
5. Make an appointment for Visit 2 which should be 7 days (± 3 days) after Visit 1.
6. Urine pregnancy test, if applicable.

The subject/guardian will be instructed to record diary information on a daily basis.

Visit II to Visit XIII

1. Review diary data to ascertain eligibility.
2. The subject/guardian should be queried regarding concomitant medications and adverse events.
3. Pulmonary function testing (without FEV1 reversibility).
4. Mouth and throat exam.
5. If the subject has been compliant with study related procedures and wishes to continue, randomize the subject, collect and dispense study drug, and instruct the subject regarding the dosing regimen.
6. Make an appointment for Visit 3 (and etc.) which should be 1 week (± 3 days) after Visit 2.

Visit XIV

1. Complete physical examination including mouth and throat examination.
2. Clinical chemistry, hematology, and cytokine.
3. Concomitant medication and adverse event query.

4. Pulmonary function testing (including FEV1 reversibility).
5. Review diary data.
6. Subject will resume pre-study asthma therapy or receive other appropriate asthma treatment at the discretion of the investigators.

Pulmonary function tests will consist of triplicate forced expiratory maneuvers in which the subject expires forcefully from total lung capacity to residual volume. A Spiro-meter, which meets ATS standards and is calibrated daily, will be used. Forced vital capacity and FEV1 are to be obtained from the full expiratory flow-volume-time curve. At least 3 technically satisfactory FVC maneuvers should be performed and the largest value from the three technically satisfactory attempts will be selected and recorded. The difference between the largest and second largest FVC and FEV1 should not vary by more than 0.2 L. A maximum of eight maneuvers can be performed until the reproducibility criteria are met. If the test maneuvers induce bronchoconstriction, so that consecutive measurements become lower, this trend should be noted and the largest FVC and FEV1 should be reported. The test must be conducted between 6:00 and 9:30 AM + 30 minutes. The timing of the test should then be standardized and remain approximately the same throughout the study, starting with Visit 1. Polgar predicted normal will be used.

Airway reversibility will be tested using spirometry before and after administration of inhaled albuterol (90 ug/actuation), 2 actuations, or after up to 2.5 mg of nebulized albuterol (Visit I & XIV). The second spirometry will be obtained between 15 to 30 minutes after albuterol administration. An improvement of > 12% in FEV1 from baseline will be considered diagnostic of reversible airway obstruction. Markers of atopy (total IgE and eosinophil count) and cytokines (IFN-γ, TGF-β, IL-4, and IL-5) examination was performed at Visit I and XIV. Sample size was calculated from a formula with 10 subjects in each treatment group the study had a power of 80% to detect a difference in change in efficacy and safety between any two treatments of 0.5 SD at the 5% level of significance. The analysis for all data will be performed using the SPSS. Values were expressed as means + SD unless otherwise noted. Differences between the groups were tested for significance with test of Manova. Differences between proportions for both groups were analyzed with either the two tailed Chi-square test or Fisher's exact test. Primary outcome was FEV1 reversibility, Secondary outcome were IL-4, IL-5, IgE level, Eosinophil count, IFN-γ and TGF-β.

RESULT

A total 34 of asthmatic children with asthma bronchial with the onset less than 2 years controlled to our institution during the study period. The remaining 34 patients en-rolled; 11 patients were treated by SLIT (group A), 12 patients were treated by probiotic (group B), and 11 patients were treated both by SLIT and probiotic (group C). Two patients were dropped out from the study because their family moved to other city, but all were in good condition.

Table 2. Baseline characteristics of subjects from all groups

	SLIT: (n=11)	Probiotic: (n=10)	Combination: (n=11)	p
1. Age (months)	63.8 ± 9.40	67.6 ± 9.06	29.4 ± 11.73	0.945
2. Gender				
- ♂	6 (54.5%)	4 (40.0%)	8 (72.7%)	0.817
- ♀	5 (45.5%)	6 (60.0%)	3 (27.3%)	0.916
3. Body Weight (kg)	28.6 ± 11.83	28.5 ± 5.02	29.4 ± 11.73	0.976
4. Height (cm)	131.7 ± 17.54	131.3 ± 1.99	130. 5 ± 12.77	0.978
5. Atopy	10 (90%)	9 (90%)	11 (100%)	0.513
6. Total IgE (IU/ml)	414.9 ± 358.15	426.7 ± 277.5	220.5 ± 244.30	0.213
7.Eosinophil (/cmm)	669.8 ± 349.35	679.5 ± 365.39	588.7 ± 281.44	0.798
8. FEV1 reversibility (%)	20.3 ± 8.62	20.6 ± 12.03	17.6 ± 4.95	0.699

There was no significant difference in age, gender, body weight, history of atopy, level of IgE, eosinophil count, and FEV1 rev between the groups (Table 2). All groups started with the homogeny performance. There was no

asthmatic; non asthmatic or medication related adverse event at all groups. Clinical improvement as the regulation of balance TH1-TH2 pro-file via TREGULATOR cells has been proved by decreasing of reversibility FEV1 that reflected bronchial hyper reactivity (Table 3).

Table 3. The Improving of FEV1 reversibility from all groups

	Mean FEV1 reversibility (%)		Delta	*p*
	Visit I	Visit XIV		
SLIT	20.25%	3.83%	-16.43%	0.0001
Probiotic	20.59%	4.84%	-15.75%	0.002
SLIT+Probiotic	17.61%	2.91%	-14.70%	0.0001

Mean value of total IgE from all groups before intervention were above 200 IU/ml (SLIT group: 414.91 IU/m; Probiotic group: 426,70 IU/ml; Combination group: 220,45 IU/ml); decreasing of 50% after intervention. Slight increasing of IgE level was seen on Probiotic group (p=0.622). Mean Eosinophil count from all groups before intervention were above 500/cmm (Figure 2). Serum eosinophillia that correlated with tissue eosinophillia decreased in all treated groups.

Table 4. Alteration of variables pre and post treatment

Groups	Pre and Post alteration	*Mean*	SD	P (between groups A,B,C)
A	Δ IgE	-138.09	237.69	0.245
(n = 11)	Δ Eosinophil	-161.18	164.06	0.067
	Δ IL-4	-28.60	52.99	0.780
	Δ IL-5	-42.07	33.11	0.877
	Δ IFN-□	55.30	30.06	0.958
	Δ TGF-β	3.72	1.22	0.985
	Δ FEV1 rev	-16.43	8.77	0.895
B	Δ IgE	69.70	431.81	0.245
(n = 11)	Δ Eosinophil	-56.2	159.88	0.067
	Δ IL-4	-28.1	56.19	0.780
	Δ IL-5	-33.89	49.05	0.877
	Δ IFN-□	59.58	50.25	0.958
	Δ TGF-β	3.84	1.71	0.985
	Δ FEV1 rev	-15.75	11.43	0.895
C	Δ IgE	-77.55	90.91	0.245
(n = 10)	Δ Eosinophil	-239.00	188.73	0.067
	Δ IL-4	-41.45	38.78	0.780
	Δ IL-5	-38.68	24.86	0.877
	Δ IFN-□	58.87	25.45	0.958
	Δ TGF-β	3.75	1.92	0.985
	Δ FEV1 rev	-14.70	4.59	0.895

Although the level of IL-4 decreased in all groups, significantly decreasing only seen on the last group. Combination group showed the most significant decreasing of IL-5 serum. Surprisingly, all groups revealed significantly in-creasing of IFN-γ. Perfect improving of TGF-ß level occurred at all groups, with p=0.0001.

DISCUSSION

The potential of mucosal approach via gastrointestinal tract in modulation of broad spectrum systemic hyporesponssiveness has been increasing to modify natural course of allergic disease (Togias 2000). The gastrointestinal tract is the largest immunologic organ in the body. It is lined by a single layer of epithelium. It is constantly bombarded by a myriad of dietary proteins. Despite the large extent of dietary antigenic exposure, only small percentage of individuals has allergic reaction. This is due to development of oral tolerance to antigenic proteins (Nasser 2001). Oral tolerance, as characterized by Chase in 1946, refers to a state of active inhibition of immune response to an antigen by means of prior ex-pose of that antigen through the oral route. Oral delivery is effective in inducing both systemic and generalized mucosal immune responses. This property has been used to develop antigen-specific therapy in allergy. [11] The exact mechanisms of induction of oral tolerance are still under debate, but some hypotheses can be proposed: (i) antigen-driven suppression; (ii) clonal anergy; (iii) clonal deletion; and (iv) bystander suppression (Bellinghausen 1997, 2001). Recently, the World Health Organization has reported that sublingual-swallow therapy shows evidence of clinical efficacy in the treatment of respiratory allergies. In particular, it has been demonstrated that, in atopic patients, the allergen can cross the gastrointestinal mucosa, leading to a desensitization of the immune system (Holt 1981).

These observations led to the formulation of the 'hygiene hypothesis', which suggested that microbial infections induce TH1-biased immune responsiveness, which, in turn, protects the host from TH2-biased allergic diseases. These observations suggest that microbe induced protection against atopy is not simply the result of immune deviation. Because the cytokines secreted by TH1 cells cross-regulate TH2 cells at least partially, it was assumed that TH1 cells had a beneficial role in allergic disease and asthma. However, direct examination of the role of antigen-specific TH1 effectors cells in asthma demonstrated that although

TH1 cells were able to reduce mucus production and airway eosinophilia, they failed to inhibit TH2 cell-induced airway hyper reactivity. This finding suggests that other forms of immunity might be effective in down regulating both inflammation and allergic diseases and asthma (Holt 1981, 1989; Swarbrick 1979; Mowat 1987). Wills-Karp has proposed an alternative paradigm, which suggests that all types of microbial stimulation (both TH1 and TH2 polarizing) induce T regulatory cells that control immune responsiveness through the production of immunosuppressive cytokines [IL-10 and TGF-ß] (Tari 1990). The old TH1/TH2 paradigm regards TH1 cells as regulator of TH2 cells inducing positive immune balance leading to clinical improvement of children with asthma (Mowat 1987). These analysis has confirmed the new paradigma of TREGULATOR cells as foccus in TH1/TH2 balancing through principle regulator cytokine especially TGF-ß. In line with new paradigma, the potent immune-stimulation observed by the use of SLIT, probiotic, or SLIT-probiotic with mucosal approach proved enhance production of TGF-ß significantly (p= 0.0001) as precursor of IgA, potentially excluded alergen for sensitizing IgE receptor in GALT system. The result of all do not differ significantly. It means that probiotic can use as alternative immunomodulator for treating asthma bronchiale in children (Nasser 2001, Nelson 1993).

Modulation TREG cells by SLIT or probiotic stimulate IL-12 as the principle cyto-kine for generating TH1 cells profile. Interleukine 12 induces IFN-γ and directly cross supresses IL-13 as primary cytokine of TH2 responses. It is followed by switching of transcription factor of genes that code e chain to γ chain leading to increasing of IgG and decreasing of IgE. Oetgen said that there ia also decreasing of proliferation signal of B cell to plasma cell. TREG cells modulate IL-12 and supress IL-13, lowering TH2 cells proliferation . Finally it causes decreasing of IL-4 level. Immunotherapy also induces apoptosis of TH2 cells. Leung said that NFk-B blocking suppresses gen transcription for IL-4 in TH2 cell nucleus (Casanovas 1994, Troise 1995, Fanta 1999).

Probiotic decreased IL-4 level not significantly. This immunomodulator blocked B cell proliferation to plasma cell with result of decreasing serum IgE but not incompanni-ed with increasing of IL-2 so there was no production of IgG from IgG plasma cell. [21] Beside that, compared to others both groups, the smallest improvement of IFN-γ value as the principle cytokine for generating TH1 cytokine profiles was seen at probiotic group (p=0.005) . In other hand we can say that immune response balance still skewed to TH2. It was found highly significant differences of p value for IFN-γ before and after intervention (p=0, 0001). Deblic found very low ratio of IFN-γ/IL-4 in severe asthmatic children (Holt 1981). Ratio of IFN-γ/IL-4 was 0.205 (before intervention) to1.814 (SLIT group); 0.246 became 1.088 (Probiotic group), and 0.205 became 1.814 (Combination group) after intervention. All groups prove that immune response balance has moved to TH1 profile, but probiotic group was the smallest one. Ratio of IFN-γ/IL-4 was nearly 1; it means TH2 cytokine includes IL-4 has been suppressed but modulation to TH1 profile is still early beginning. Probiotic need longer period intervention for the same result.

Level of IL-5 decreased significantly at SLIT group (p=0.002) and Combination group (p=0.0001). Interleukin-5 is thought to play a pivotal role in the pathogenesis of asthma. High levels of circulating IL-5 have been documented in acute asthma. Interleukin 5 is a key mediator in eosinophil activation. [22] Our analysis showed positive correlation of IL-5 and FEV1rev. It can be explained through influence of eosinophil to bronchus hyper reactivity. Interleukin 5 itself is a differentiating factor for eosinophil in the bone marrow (Nasser 2001).

The increasing of serum IgE at probiotic group can be review from many immunology aspect. Improvement of clinical features in asthmatic children are not followed by changing of serum IgE. Contrast to IgE, IgG1 dan IgG4 have strong positive corellation to the clinical improvement (Bellinghausen 2001, Rautava 2002). THELPER1- THELPER2 balance is importance in B cell isotype switching. Production of IgG1 and IgE are influenced by IL-4 and IL-13; and downregulated by IFN-γ and IL-2. Beside that, expression of IgG4 is influenced by IFN-γ. Based on this fact, theory of skewing TH2 to TH1 only can not explain increasing of IgG1 and IgG4 simultanneously without changing of IgE titer (Borish 2003). Barnes and William observed the “IgE overshot phenomenon” in the early phase of immunomodulation. Haaktela T, Kankaanranta H, and Moilanen E had proven that IgE level did not correlate with bronchial hyperreactivity (Frew 2003, Borish 2003). Our analysis with correlation test confirmed that there was no corellation of FEV1 reversibility as clinical improvement indicator with IgE titer in all groups (p>0.05). In randomyzed double blind multicentre study Mothes et all had compared clinical improvement and the titer of IgG, IgM, and IgE. There is causative relationship between improvement of clinical features, functional blocking of IgG, and titer of specific IgG. Immunotherapy will inhibit early and late phase immune responses by preventing the crosslink of IgE – mast cell and antigen presentation to T cell that facilitated by IgE. Immunoglobulin G4 is a bivalent structure that will able to form immune complex with

antigen and Fc receptor on mastcell without sensitizing. Increasing of IgG value after immunotherapy can be used as predicting factor for successfull of immunotherapy . Immunoglobulin G4 still use as gold standard in measuring efficacy of immunotherapy. In the early phase immunotherapy will induce IgG1 forming immune complex and binding with Fc receptor on T cell suppressor (Fcγ-receptor) . The complex will proliferate directly maintaining peripheral immune tolerance from allergen sensitization. Beside that, Ig G4 crosslinks with FceRI, a high affinity of IgE receptor, blocking transmission signal from FceRI, release of inflammatory mediator,and finally downregulate inflammation process (Nasser 2001, Swarbrick 1979, Nelson 1993, Troise 1995). In this study, increasing IgE titer in patients receiving probiotic was compensated by increasing IgA, suggesting achievement of new equilibrium of Th1 and Th2 profile at upper level.

Immunoglobulin E production is controlled strikely by molecular and cellular sig-nals. Immunoglobulin M fasilitate up take of allergen by dendritic cell, that followed by peptide presentation to TNAIVE cell through MHC class II . Peptide - T cell receptor com-plex induces expression of CD40L, forming CD40-CD40L and inducing B7 expression (CD80). Interaction of B7 and CD28 on T lymphocyte produce costimulation signal that will increase IL-4 synthesis. Conjugation of IL 4 to IL-4R on B lymphocyte and CD40-CD40L trigerred IgE isotype switching , B lymphocyte proliferation, and IgE clonal ex-pansion (Togias 2000, Nasser2001). This fact can explain the positive correlation betwen IgE and IL-4 at SLIT group (Pearson correlation p=0.001). Interleukin 5 has a highly selective effect on eosinophil differentiation , adhesion, and effector modulate eosinophil migratory res-ponses. The capacity of IL-5 to prime eosinophils in directional locomotion suggests that this cytokine may have a crucial influence on the preferential accumulation of this cell type in allergic inflammation. Eosinophil infiltration to the airway determines bronchial hyper-reactivity of asthmatic children (Holt 1981, Mowat 1987).

Level of IFN-γ increased 400-700% form all groups. Under IL-12 millieu, TH1 cells will produce IFN-γ. IFN-γ induces IgG2a and IgG4 from B cell and blocks IgE. IFN-γ with IL-2 within context of T cell receptor-MHC class II will trigger differentiation of B cell to IFN-γ producing plasma cells. Interleukin 12 is a central motion for TH1 cell polarisation. Manipulation of IL-12 in animal model caused de-creasing of IFN-γ and also increasing of IL-4. Twenty four hours after giving specific stimulation could be seen increasing IL-12 mRNA-positive cells followed by increasing of IFN-γ mRNA - positive cells and decreasing of IL-4 mRNA-positive cells (Durham 1996, Frew 2003, Togias 2000, Bellinghausen 2001).

Skewing specific allergen- effectors T cells to the phenotype of TREGULATOR cells that produce immunoregulator cytokine such as TGF-ß is very crucial in determining successful of immunomodulation. Transforming Growth Factor-ß suppresses IgE production and simultaneously increasing non-inflammatory antibody isotype such as IgG4 and IgA. Effectors cells such as mast cell, basophile, and eosinophil that contribute to asthma remodeling were suppressed by TREG cell. THELPER3 cell is one of TGF-ß source that induce bystander immune suppression. TREG cell participates in T cell hyper reactivity apoptotic, maintaining the peripheral immune tolerance (Kapsenberg 2003, Togias 2000, Nasser 2001).

Finally a non invasive, accurate, applicable, and repeatable procedure was used to measured clinical improvement of asthmatic children. We used spirometer to measure variability of lung capacity all subjects. These results were very excellent. At the final intervention, none subject had FEV1 rev more than 12%. Other variables such as body weight, height, onset of disease, severity of asthma were able to control. In our analyzing, FEV1 reversibility had a strong correlation with Eosinophil (p=0,008) and IgE (p=0,050).

Over the last few decades, several studies from different parts of the world have shown an increasing prevalence of allergic diseases. It has been suggested that a reduced microbial stimulation during infancy and early childhood would result in a slower postnatal maturation of the immune system and development of an optimal balance between TH1- and TH2-like immunity (Durham 1996; McHugh 1995). The gastrointestinal tract is the largest reservoir of microorganisms, also containing up to 70–80% of all immunoglobulin-producing cells. The normal micro biota of the gastrointestinal tract is a major stimulus for the post-natal maturation of T cell function. The intestinal micro biota stimulate development of the lymphoid tissue (the GALT), by providing constant microbial stimulation of local and systemic immune responses via pattern recognition molecules such as toll-like receptor a series of receptors, e.g. Toll-like and CD14 receptors (Ebner 1997). These studies provide circumstantial evidence that alterations in immune stimulation by the intestinal micro biota are important in the development of allergic asthma (Frew 2003).

Previous studies have demonstrated differences in the composition of the intestinal micro biota between

allergic and non-allergic children in early childhood (Ebner 1997). In a prospective study, performed in Estonia, with a low, and Sweden with a high prevalence of allergic disease, differences were shown in the composition of intestinal micro biota al-ready during the first month of life between infants who developed or did not develop allergic manifestations. In the present study, bifidobacteria showed a lower proportion in the faecal micro biota of 5-year-old allergic children as compared with healthy ones (Kapsenberg 2003). The development of allergic diseases seems to be associated with an imbalance of the gut microbial ecosystem (Isolauri 2000; Borish 2003).

Administration of bifidobacteria enhanced antigen-specific IgA antibody production against antigen sensitization and protected against infections in mucosal tissues .Bacteroides become one of the major components of the cultivable gut micro biota from the second year of life (Togias 2000). The present study extends our previous observations, showing a positive correlation between total serum IgE levels and bacteroide counts in non-allergic children at 5 years. Possibly, high numbers of bacteroides may provide an anti-inflammatory stimulus, which protects the child against allergic manifestations, despite elevated serum IgE. Several studies hint that bacteroides promote isotype switch to IgA in B cells and induces oral tolerance via the activation of an antigen-specific non-response to an antigen. Recently the induction of transforming growth factor-ß by T-regulatory cells was shown by Bacteroides. Bacteroides seems to be associated with down-regulation of allergic response in non-allergic children with high serum IgE level (Durham 1996).

CONCLUSION

Lung function test improved in patients receiving SLIT and probiotic suggestive modulation of cytokine balance, decreasing of eosinophil and IgE. Increasing IgE titer in patients receiving probiotic was compensated by increasing IgA, suggesting achievement of new equilibrium of Th1 and Th2 profile at upper level.

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