Probiotics and atopic dermatitis

Le giornate della salute e del benessere: Innovazione e Ricerca
Milano, 30 Giugno - 1 Luglio

Luca Mogna, Ph.D.
Probiotical S.p.A. - Novara

Milan, July 1st, 2016
Atopic Dermatitis (AD) affects 15-30% of children, 2-10% of adults;

60% begin during the first year of life;

45% begin within the first 6 months;

85% begin before 5 years;

Up to 70% spontaneous remission before adolescence;

Predisposed to developing asthma later in childhood.
The atopic march
Clinical diagnosis of AD

♦ Pruritus;
♦ Eczematous skin lesions in typical age-specific distribution patterns;
♦ A chronic or chronically relapsing course;
♦ Early age at onset;
♦ A personal and/or family history of atopy.
Pathogenesis of Atopic Dermatitis

Immunologic Mechanisms

Epidermal Barrier Dysfunction

AD

Environmental

Genetic
The entities in the atopic triad cluster together in families. AD is a complex genetic disease, and both gene-gene and gene-environment interactions have pathogenic roles.
Filaggrin

Mutations in the filaggrin gene (FLG), which encodes a protein that aggregates keratin filaments during terminal differentiation of the epidermis.

The presence of the filaggrin variants is correlated with early-onset, relatively severe, “extrinsic” (specific IgE-associated) AD that tends to persist into adulthood.

Affected individuals have an increased risk of eczema herpeticum and peanut allergies as well as a propensity to later develop asthma.
Epidermal barrier dysfunction

The consequence of epidermal barrier dysfunction and an altered stratum corneum leading to increased transepidermal water loss.
Environmental factors

- **Food allergens (egg, milk, wheat, soy)**
  - Associate to infantile AD
  - Related to disease severity

- **Aeroallergens (pets, mites, pollen)**
  - Exacerbation of AD in other children
# Immunologic mechanisms

<table>
<thead>
<tr>
<th>Non-homeostatic element</th>
<th>Main component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanisms of inflammation in the absence of IgE-mediated sensitization</td>
<td>Increased epidermal protease activity</td>
</tr>
<tr>
<td>Role of Dendritic Cells (DCs)</td>
<td>Langerhans cells (LCs) and inflammatory dendritic epidermal cells: present allergens to Th1/Th2 cells</td>
</tr>
<tr>
<td>Impairment of the epidermal barrier</td>
<td>Degradation of corneodesmosomi, deficiency of Filaggrin</td>
</tr>
<tr>
<td>Epicutaneous sensitization</td>
<td>High levels of thymic stromal lymphoprotein (TSLP) by keratinocytes leads to Th2 polarization</td>
</tr>
<tr>
<td>Role of microbial colonization</td>
<td>Decreased levels of antimicrobial peptides, <em>S. aureus</em> adherence to skin</td>
</tr>
<tr>
<td>Role of autoimmunity</td>
<td>Circulating IgE antibodies</td>
</tr>
<tr>
<td>T-cell responses, cytokines and chemokines</td>
<td>Th2 predominates in acute, Th1 in chronic</td>
</tr>
</tbody>
</table>
The intestinal microbiota is the major external driving force in the maturation of the immune system after birth.

It is a prerequisite for normal development of oral tolerance.

The recent increase in allergic disorders has been attributed to a relative lack of microbial stimulation of the infantile gut immune system.

The Hygiene Hypothesis

A reduction in microbial antigenic stimulation brought by widespread vaccination, improvements in standards of hygiene, and extensive use of antibiotics has contributed to the dysregulation of T-helper 2 cell (Th2) type responsiveness that typifies allergy (Hygiene Hypothesis).
Some data

80% of our immune system is located in the digestive tract

Particularly prevalent in the small intestine

The overall area of the gut surface is approximately 200 m²
Barrier defects in AD

- The barrier defects in AD seem to go beyond the skin;
- Barrier defects seem to involve the intestinal mucosa;
- Indigenous intestinal bacteria (microbiota) contribute to stabilize the intestinal permeability and the mucosal barrier function.

Probiotic microorganisms with a biotherapeutic value seem to be able to modulate the mucosal immune responses, thus leading to a reduction in gastrointestinal inflammation.

The oral consumption of biotherapeutic microorganisms might reduce skin sensitivity, supporting the skin’s immune function.
Experimental part

- In vitro selection of the best biotherapeutic strain to be used in human clinical trials;

- A study on LS01 in adults with Atopic Dermatitis;

- A study on LS01 in children with Atopic Dermatitis;

- A study involving an association of LS01 and a strain with anti-inflammatory activity in adults with AD.
The selection of the best biotherapeutic

$L. salivarius$ LS01 significantly moved the Th1/Th2 ratio toward a Th1 response, whereas BNL1059 and RGS1746 led to a decrease of the Th1/Th2 ratio in favor of the proinflammatory cytokines.

Ratio between Th1 and Th2 responses. Data are means of 3 experiments ± SD. * p<0.05 versus LS01 strain.

The Th1/Th2 paradigm implied the existence of two different, mutually regulated, CD4(+) T helper subsets. A third member of the T helper set, IL-17-producing CD4(+) T cells, now called Th17 cells, was recently described as a distinct lineage that does not share developmental pathways with either Th1 or Th2 cells.
The importance of human clinical trials

An efficacy trial with a proper protocol is the basis for the demonstration of efficacy of a probiotic strain and to be hopefully able to get a health claim approved.

Protocol definition for human clinical trials

- Randomized, double-blind, placebo controlled (DBPC)
- Group dimensions calculated for each clinical output
- Protocol set able to register possible adverse effects
The first study on LS01 in adults

- 38 patients aged between 18 and 46 years with moderate/severe AD
- Allergy and Clinical Immunology Unit of L. Sacco Hospital of Milano
- Double-blind, placebo controlled trial
- Clinical severity was evaluated using the SCORAD index
- All subjects had to answer to a Dermatology Life Quality (DLQ) questionnaire
- A single investigator performed all SCORAD assessment at the beginning (T0), out of the pollen season, and at the end of treatment (T16) during the pollen season.

Patients received *Lactobacillus salivarius* LS01 (DSM 22775) at a dose of $1 \times 10^9$ CFU/sachet in maltodextrin, twice daily for 16 weeks.

Epidemiological data

<table>
<thead>
<tr>
<th></th>
<th>Active treatment (Group A)</th>
<th>Placebo (Group B)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>19</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>$32.07 \pm 1.79$</td>
<td>$28.86 \pm 2.15$</td>
<td>n.s.</td>
</tr>
<tr>
<td>Respiratory allergy</td>
<td>16/19</td>
<td>14/19</td>
<td></td>
</tr>
<tr>
<td>Food allergy</td>
<td>3/19</td>
<td>9/19</td>
<td></td>
</tr>
<tr>
<td>Contact dermatitis allergy</td>
<td>3/19</td>
<td>2/19</td>
<td></td>
</tr>
<tr>
<td>Other allergies</td>
<td>5/19</td>
<td>5/19</td>
<td></td>
</tr>
<tr>
<td>No allergy</td>
<td>3/19</td>
<td>3/19</td>
<td></td>
</tr>
<tr>
<td>SCORAD index</td>
<td>$27.57 \pm 3.40$</td>
<td>$24.28 \pm 3.54$</td>
<td>n.s.</td>
</tr>
<tr>
<td>DLQ</td>
<td>$8.28 \pm 1.79$</td>
<td>$5.78 \pm 1.81$</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

No significant differences were detected between the two groups of patients in any of the baseline characteristics.
After 4 months, a significant reduction in the SCORAD score was observed in the probiotic-treated grupo only (T0: 27.57 ± 3.4 vs. T16: 13.14 ± 0.27, p<0.001). No changes were reported in the placebo.
DLQ progressively decreased in probiotic patients during treatment. This significant modification was observed after 8 weeks of treatment (T8) and was also maintained 4 weeks after the end of treatment (T20).
Plasmatic LPS

Plasma LPS is an indicator of microbial translocation. Patients receiving biotherapeutic treatment showed a reduction in LPS plasma concentration that was maintained after the suspension of treatment (T0 vs. T16: p=0.050; T0 vs. T24: p<0.001).
Modifications of fecal microbiota

Bacterial counts are expressed as mean ± SD of $\log_{10}$ per gram of wet feces. 
A, Active group at baseline (grey bars), after treatment (open bars), and 1 month after treatment (black bars).
B, Placebo group at baseline (grey bars), after treatment (open bars), and 1 month after treatment (black bars).
* Significant decrease ($p<0.05$) compared with baseline.
△ Significant difference ($p<0.05$) compared with placebo patients at the same time.
Recovery of *L. salivarius* LS01

<table>
<thead>
<tr>
<th></th>
<th>Recovery in feces (% of treated patients)</th>
<th>Range of counts (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After treatment</td>
<td>100%</td>
<td>$10^3 - 10^6$</td>
</tr>
<tr>
<td>1 month after suspension</td>
<td>60%</td>
<td>$10^2 - 10^4$</td>
</tr>
</tbody>
</table>

All colonies with the typical morphology, identified using PCR and PFGE, corresponded to the previously identified *L. salivarius* LS01.
The clinical study in children

- 43 patients from 0 to 11 years (M/F ratio: 1:1) with AD
- Pediatric Allergology Unit of the Hospital of Spoleto and Foligno
- Enrolment carried out from December 2012 to February 2013
- Patients were visited at the beginning of the study (T0), and every 4 weeks during the treatment (T4, T8, T12, T16). Finally, they were visited 4 weeks after the end of treatment (T20)
- The clinical severity of AD was evaluated using the objective SCORAD and the SCORAD index

Patients took 2 sachets/die of a lyophilized form of *Lactobacillus salivarius* LS01 (DSM 22775) (1x10⁹ CFU/sachet) for 8 weeks, and 1 sachet/die for the following 8 weeks.

Patients receiving the active formulation showed a significant reduction of SCORAD during the treatment (p=0.001). After 4 weeks a significant decrease in SCORAD value has been observed and it continued until the end of treatment.
Itch intensity showed a significant decrease after lactobacillus have been given to patients and this reduction persisted after suspension of supplementation.
Immunomodulation studied in PBMCs

PBMCs are isolated from human peripheral blood and co-cultured with two selected probiotic strains LP01 and LPS01. The specific stimulation of different immune cell populations and cytokines secretion could be monitored by monoclonal fluorescent antibody staining (FACS) and by enzyme-linked immunosorbent assay (E.L.I.S.A.), respectively.
The anti-inflammatory activity of *B. breve* BR03

Averages ± S.E.M. of 10 independent experiments

Study on the association of LS01 and BR03

✓ 48 adults patients suffering from AD
✓ Allergy Unit of L. Sacco Hospital in Milano
✓ Double-blind, placebo controlled trial
✓ Patients randomized according to a 2:1 ratio (active = 32; placebo = 16)
✓ The study was conducted from April to September 2010
✓ Patients were visited at weeks 0, 12, and 20.
✓ The clinical severity of AD was evaluated using the SCORAD index and the DLQ questionnaire.

Patients in the active group took 2 sachets/die of a mixture of *Lactobacillus salivarius* LS01 (DSM 22775) and *Bifidobacterium breve* BR03 (DSM 16604) (1x10^9 CFU/strain/dose) for 12 weeks.

Recruited = 60 pts

Excluded = 12 pts

Assessed for eligibility = 48 pts

Randomized 2:1 = 48 pts

Active group = 32 pts

Lost to follow up for low compliance = 1 pt

Analysed = 31 pts

Follow up after 2 months = 31 pts

Placebo group = 16 pts

Lost to follow up for inefficacy = 1 pt

Analysed = 15 pts

Follow up after 2 months = 15 pts
SCORAD and DLQ index

<table>
<thead>
<tr>
<th></th>
<th>Probiotic Pts (n = 31)</th>
<th></th>
<th>Placebo Pts (n = 15)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 3-mo Treatment</td>
<td>2 mo After Suspension</td>
<td>Baseline</td>
</tr>
<tr>
<td>SCORAD score</td>
<td>46.25 ± 3.70</td>
<td>29.45 ± 2.01</td>
<td>22.63 ± 2.81</td>
<td>45.00 ± 2.60</td>
</tr>
<tr>
<td>DLQ index</td>
<td>9.16 ± 0.80</td>
<td>6.58 ± 1.25</td>
<td>4.73 ± 0.75</td>
<td>6.52 ± 0.40</td>
</tr>
</tbody>
</table>

*Significant differences in probiotic patients.†SCORAD score and DLQ index in probiotic and placebo patients analyzed at baseline, after 3 months of treatment, and 2 months after suspension. Mean values and SE are shown. DLQ indicates Dermatology Life Quality; pts, patients.

Patients in the active group showed a significant reduction of SCORAD at the end of treatment (p=0.001). This persisted after the suspension (p=0.006) and an improvement in DLQI (baseline vs. 3 mo after treatment: p=0.024; baseline vs. 2 mo after suspension: p=0.001).
LPS and activated T cells

Plasma LPS concentration (A) and percentage of CD38-CD45RO-CD8-expressing T cells (B) in active and placebo groups analysed at baseline, at the end of treatment, and 2 months after suspension of treatment. Mean values, SE, and P-values are shown.
Th1, Th2, and Th17 cells: IFN-γ secreting Tbet-expressing Th1 cells in unstimulated (A) and LPS-stimulated conditions (B); IL-4 secreting GATA3-expressing Th2 cells in unstimulated (C) and LPS-stimulated conditions (D).
Th1, Th2 and Th17 subpopulations

Th1, Th2, and Th17 cells: IL-17 secreting RORγt-expressing Th17 cells in unstimulated (E) and LPS-stimulated conditions (F) in active and placebo groups analyzed at baseline, at the end of treatment, and 2 months after suspension of treatment. Mean values, SE, and P-values are shown.
Microbiota composition

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 3-mo Treatment</th>
<th>2 mo After Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probiotic patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total aerobes</td>
<td>7.7 ± 1.0</td>
<td>7.9 ± 0.9</td>
<td>8.2 ± 0.7</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>6.8 ± 1.3</td>
<td>7.3 ± 1.2</td>
<td>6.7 ± 2.6</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>4.0 ± 1.6</td>
<td>2.8 ± 1.5*,**</td>
<td>2.3 ± 1.6*,**</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>5.4 ± 2.0</td>
<td>5.1 ± 1.8</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>9.1 ± 0.6</td>
<td>8.8 ± 0.5</td>
<td>8.4 ± 0.6</td>
</tr>
<tr>
<td><strong>Placebo patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total aerobes</td>
<td>7.1 ± 1.5</td>
<td>7.5 ± 1.2</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>5.8 ± 1.7</td>
<td>6.7 ± 1.6</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>4.1 ± 0.9</td>
<td>3.9 ± 1.1</td>
<td>4.0 ± 1.1</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>5.4 ± 1.9</td>
<td>5.2 ± 1.6</td>
<td>5.4 ± 1.8</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>8.5 ± 1.1</td>
<td>8.6 ± 0.6</td>
<td>8.6 ± 0.9</td>
</tr>
</tbody>
</table>

* Significant decrease (p<0.05) compared with baseline.
** Significant difference (p<0.05) compared with placebo patients at the same time.

Bacterial counts are expressed as mean ± SD of log_{10} per gram of wet feces.
The positive implications of immunomodulation

- Lowering the incidence and severity of **ATOPIC DISEASES** and beneficial changes in response to allergens
- Protection against harmful or pathogenic bacteria
- Reduction of the incidence, duration and severity of Acute Respiratory infections (ARI)
- Usefulness in people who play sports and in competitive athletes
- Relief of troublesome symptoms related to inflammatory gut conditions and diseases
Probiotics, immunity and pathogens

Production of adhesins and exopolysaccharides (EPS)

Transmigration from the gut to vagina

Synthesis of antimicrobial substances

Stimulation of non specific and specific immune responses to pathogens

Competitive inhibition of pathogens adhesion
Conclusions (1)

- Probiotic bacteria can **modulate the immune system** in different ways, thus delivering a selected beneficial effect to the host.

- The biotherapeutic bacteria employed in the studies are effective in **reducing the severity of AD symptoms** in subjects diagnosed with a moderate to severe intensity of the disease.

- The restoration of the **physiological barrier of the gut** is a key point in order to reduce microbial translocation through the gut wall.

- The beneficial effects recorded in children with DA persisted even 1 month after cessation of supplementation. This could be attributed to **long-term modification of gut microbiota composition**.
Conclusions (2)

The exact mechanism by which *L. salivarius* LS01 can improve the symptoms of atopic dermatitis is not fully understood yet, but it is possible that its action is mediated by *articulated immunological effects started in the gastrointestinal mucosa*.

A significant decrease of *Staphylococcus aureus* in the gut has been recorded in the first study. In patients with AD high fecal counts of staphylococci were detected compared to healthy subjects, and these lead to the hypothesis that intestinal *S. aureus* can influence the cutaneous symptoms of AD.

Since the activity of bacteria is *strain-specific*, a careful assessment of their effects should be performed prior to marketing of any preparations containing at least one microorganism with biotherapeutic value.
Thank you very much for your attention!!