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Antigenic proteins of *Lactobacillus acidophilus* that are recognised by serum IgG antibodies in children with type 1 diabetes and coeliac disease

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Immune responses to lactobacilli have been so far insufficiently investigated in patients with autoimmune diseases. We used whole-cell lysate of an indigenous *Lactobacillus acidophilus* strain isolated from an Estonian child to study serum IgG antibodies in children groups with type 1 diabetes [insulin dependent diabetes mellitus (IDDM)] (n = 21, age 4–18 yr) and with acute coeliac disease (CD) (n = 20, age 0.6–15 yr) and to compare the results with the controls (n = 24, age 2–17 yr). We found that our developed 1-D immunoblot assay readily enables to reveal antibodies against 28 *L. acidophilus* antigenic proteins in patients' and controls' sera. As verified by immunoproteomics analysis with 2-D and LC ESI-MS/MS the antigens of *L. acidophilus* were mainly common cytoplasmic proteins GroEL (HSP60), enolase, transcripton factor EF-Ts and EF-Tu. However, in addition we identified formyl-CoA transferase being target for antibodies in every tested IDDM patients' serum. We have characterized for the first time the antigenic profile of *L. acidophilus* whole-cell lysate using sera from children with IDDM, CD, and controls. The different prevalence of reactions against tested antigens in patients and controls sera may indicate significant differences in immune system and commensal bacteria cross-talk in these groups.

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Key words: antibodies; *Lactobacillus acidophilus;* immunoblot; immunoproteomics; oxalyl-CoA decarboxylase; formyl-CoA transferase

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The property of lactic acid bacteria (LAB) to stimulate immune system have attracted a number of researchers attention during recent years. The LAB influence has been described as enhancement of non-specific immunity as well as adjuvant and immunoregulatory effects in adaptive immune responses. Lactobacilli are well-known LAB which amongst various effects are known to be able to direct and maintain homeostasis of the immune system, especially through the induction of regulatory and Th1 cytokines (1). It has been demonstrated that when the acquisition of normal microbiota in a newborn child takes place, some lactobacilli strains are able to stimulate adaptive immune system more than others (2, 3). LAB probiotics influence on the adaptive immune system is still under investigation and certain probiotics are being tested to improve vaccine efficacy in newborns (4, 5).

However, the bacterial components responsible for these effects are often left unidentified. From previous studies (6–9) is known that the LAB membrane teichoic acids and cell wall peptidoglycans have strong immunomodulatory properties, whereas, proteins in cytoplasm may

elicit specific immune responses and development of LAB specific antibodies (10). Different species of lactobacilli show distinctive sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profiles that have proven reliable method for alternative identification (11). Identifying of LAB antigenic proteins that elicit adaptive immune responses using an immunoproteomic approach have gained important attention only recently (10). Therefore, it is understandable why the antigens of lactobacilli that are reacting with human IgG have been so far insufficiently investigated.

Previous studies have suggested that coeliac disease (CD) and type 1 diabetes (IDDM) might be accompanied with the changes in gastrointestinal microbial composition (12, 13). Differences in microbiota could also be reflected in variable CD and IDDM prevalence in several populations (14, 15). The human immune system is mostly hyporesponsive to the commensals, but some degree of innate immune recognition of these bacteria is essential for normal development and function of the mucosal and peripheral immune system (16). Immune responses to lactobacilli have an important role in regulation of the immune system and may be different in children with CD and IDDM from those seen in healthy individuals. Furthermore, when epithelium permeability is changed and mucosal immune response is altered, as it is proven in CD (17) and IDDM (18), much more commensal microbes can get contact with antigen presenting cells, which finally may cause increase of specific antibody level, i.e., IgG against LAB.

Based on these considerations, we aimed to analyze the prevalence and character of IgG antibodies against lactobacilli in children with IDDM and CD, and to compare these results with healthy children. Whereas the *Lactobacillus acidophilus* is a predominant *Lactobacillus* in human gut (19), we chose this strain as a model organism for our investigations. The particular bacterial strain that was explored in present work has been isolated from an Estonian child. Additionally, we aimed to perform a first known immunoproteomic analysis of *L. acidophilus* antigens recognized by patients' IgG.

Materials and methods

Human serum samples

Serum samples were collected from 65 children (age range 0.6–18 yr), seen at the Children's Clinic of Tartu University Hospital during 1999–2003. The control group (n = 24; age range 2–17 yr; 12 females) was compiled of hospital patients with health problems not related to the gastrointestinal tract (sent for investigations regarding developmental delay, headache, minor trauma, etc.). Twenty-one patients were with IDDM (age range 4–18; 10 females) and were seen at the onset of the disease. Twenty children with active CD (age range 0.6–15; 11 females) were diagnosed according to the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) criteria (20). From all patients, written informed consent was obtained.

Preparation of whole cell lysate of *Lactobacillus acidophilus*

Lactobacillus acidophilus strain 821-3 was originally isolated from the faecal sample of an Estonian child at the Department of Microbiology, University of Tartu. *L. acidophilus* was identified and characterized according to morphological and cultural properties using the catalase test (negative), and the API 50 CHL system (BioMerieux, Marcy l ' Etoile, France). Bacterial cells were grown in Man-Rogosa-sharpe broth (Oxoid, Hampshire, UK) for 24–48 h at 37°C in a microaerophilic environment (10% CO_2) (21). They were collected by centrifugation, washed twice with the phosphate-buffered saline (PBS) buffer and kept frozen at −20°C until use. The cells were thawed and suspended in PBS up to optical density 2.0 at 580 nm and disrupted with 0.1 mm glass beads (Biospec Products, Bartlesville, OK, USA). The total protein concentration in samples was determined using the Bio-Rad Protein Assay solution (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as a standard.

A 5–20% gradient SDS-PAGE and 1-D immunoblotting

Equal amount of bacterial cell proteins were mixed with 250–400 μl of the SDS-PAGE sample buffer (62.5 mM Tris (pH = 6.8), 2.3% SDS, 5% 2-mercaptoethanol, 10% glycerine, a few grains of bromophenol blue) and were heated for 15 min at 95°C. The gels were loaded with 75 μg of protein.

The proteins for the 1-D immunoblot were separated on a 5–20% gradient gel with a 5% concentrating gel with All Blue 10–250 kDa molecular weight (MW) markers (Bio-Rad, Hercules, CA, USA) as standards. Electrophoresis was carried out at current 40 mA and voltage 200 V for 5.5–6 h using the vertical electrophoresis system SE-600 (Hoefer, San

Francisco, CA, USA). The separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (0.45 μm pore size) using a semi-dry electroblotter (Hoefer, San Francisco, CA, USA) at a current density of 1 mA/cm^2 for 1.5 h with a Tris/glycine/acetone transfer buffer including 0.01% SDS according to Nilsson et al. (22). After transfer, the membranes were blocked twice: first 15 min with the solution containing 10 g/l polyvinyl pyrrolidone with the MW of 40 kDa and methanol (25%) in the ethanolamine/glycine buffer (pH = 9.6), containing 12.2 g/l ethanolamine (Merck and Co., Rahway, NJ, USA), 18 g/l glycine (Merck and Co., Rahway, NJ, USA) and then another 15 min with the ethanolamine/glycine buffer containing Tween-20 (0.14%) and gelatine hydrolysate (5 g/l).

Detection of antigenic proteins and data analysis

From the membrane 3–4 mm strips were cut out and incubated with the human sera in dilution 1:50 in the incubating buffer containing 1.25 g/l gelatin hydrolysate, 0.25 g/l Tween-20, 6.1 g/l NaCl (Merck and Co., Rahway, NJ, USA) and 0.06 g/l Tris Base (Sigma, St. Louis, MO, USA). Rabbit anti-GroEL polyclonal antibodies (Stressgen Bioreagents, Victoria, BC, Canada) that recognize *L. acidophilus* HSP60 and rabbit serum *Candida albicans* anti-enolase polyclonal antibodies (Takara Bio Inc, Japan), both at dilution 1:1000 in the incubation buffer, were used to estimate the location of these immunogenic proteins on strips next to patient sera. Incubation with primary antibodies was done on a shaker overnight at 4°C. Before adding appropriate secondary antibodies labelled with horseradish peroxidase (HRP) (Dako, Copenhagen, Denmark) (diluted 1:500), the strips were washed three times for 10 min each with the incubating buffer. Incubation with HRP-conjugated secondary antibodies lasted for 2 h by shaking at room temperature. Before development with carbazole in the 50 mM sodium acetate buffer (pH = 5.0) in the presence of hydrogen peroxide during 30 min (0.04% 3-amino-9-ethylcarbazole and 0.015% hydrogen peroxide), the strips were washed three times 10 min each with the incubation buffer and finally briefly with the sodium acetate buffer. After 30 min, the reaction was terminated by washing the strips with distilled water.

The strips were scanned using a Bio-Rad GS-710 Imaging Densitometer (Bio-Rad, Hercules, CA, USA). The relative MW of the bands was estimated with the Bio-Rad Quantity One image analysis software (Bio-Rad, Hercules, CA, USA) according to the All Blue molecular markers (18). Statistical analysis was carried out using R 2.3.0 environment (Free Software Foundation, Boston, MA, USA). Fisher's exact test was applied for comparing the 1-D immunoblot positive antibody reactions between patient groups and controls. For statistical analysis a logistical regression model was used with to eliminate significant differences that had an effect of age or gender.

Two-dimensional polyacrylamide gel-electrophoresis

Sample preparation for 2-DE. Sample for 2-DE was prepared as follows: 36 μg of cell lysate proteins were mixed with two volumes of lysis solution containing 8 M urea, 4% CHAPS, 200 g/l 1,4-dithio-DL-threitol (DTT) and was kept 30 min at 22°C followed by centrifugation for 10 min at 15 700 × ***g***. The supernatant was collected and to the two parts of suspension one part of rehydration buffer (8 M urea, 2% (3-[3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS), 0.8 μl immobilized pH gradient (IPG) buffer, pH 3 to 10) was added to get a total volume of 125 μl for 7 cm strips.

In addition, PBS-soluble preparation was done for extracting mainly soluble cytoplasmic proteins. At first, the whole cell protein sample (75 μg of protein) was centrifuged for 10 min at 22°C at 15 700 × **g**. The obtained supernatant was treated with acetone at −20° C for 15 min and spun at 15 700 × **g** for 10 min. The precipitate was solubilized with 125 μl of rehydration buffer.

Isolectric focussing and SDS PAGE. Isolectric focussing (IEF) was carried out with IPGPhor system (Pharmacia Biotech, Uppsala, Sweden) using 7 cm strips with linear pH gradient 3–10 (GE Healthcare Bio-Sciences, NJ, USA). After 12 h of rehydration, the following focussing parameters were applied: 50 μA per strip, voltage increase over 8 h from 100 V to 8000 V. After IEF up to 35 kVh, IPG strips were equilibrated according to manufacturers recommendation in equilibration buffer [50 mM Tris–HCl (pH = 8.8), 6 M urea, 30% glycerol, 2% SDS] containing 10 g/l DTT for 15 min and another 15 min in equilibrating buffer containing 25 g/l iodoacetamide. Electrophoresis was carried out on 10% SDS-polyacrylamide gel using Bio-Rad Mini-Protean 3 system for 1.5 h at 150 V and 35 mA, followed by staining with GE Healthcare PlusOne Coomassie tablets Phastgel Blue R-350 (Piscataway, NJ, USA) or semi-dry protein transfer to PVDF membrane.

Two-dimensional immunoblotting. Immunoblot was conducted according to the method of Nilsson et al. (22). Briefly, proteins on 2-D gels were transferred to PVDF membranes and blocked as previously described. To identify immunogenic proteins the membrane was placed in a plastic envelope, soaked with 4 ml incubation buffer with CD patients' serum in dilution 1:100. After 8–10 h of incubation at 4°C, the PVDF membranes were washed twice and incubated with anti-human IgG HRP-labelled secondary antibodies diluted 1:500. Carbazole in sodium acetate buffer was used as a substrate for visualizing antigenic reactions. All-Blue precision Plus standards (Bio-Rad, Hercules, CA, USA) were used during electrophoresis as MW standards. 1-D and 2-D experiments were performed in duplicates and triplicates.

Two-DE image analysis. Gels and immunoblots were scanned with a GS-710 Imaging Densitometer (BioRad). For estimation of pI, MW and matching between gels and 2-D immunoblots the 2-DE images were analyzed with the MELANIE 3 software (Bio-Rad, Hercules, CA, USA).

Protein identification by mass-spectrometry

In-gel trypsination. For LC ESI-MS/MS analysis Coomassie stained spots, corresponding to serum antibodies recognized spots, were manually excised from preparative 2-DE gels. Spots for identification were obtained from the 2-D PAGE with the whole cell lysate and PBS-soluble fraction lysate of *L. acidophilus* proteins. The digestion of protein with trypsin within the gel was performed as previously described by Wilm et al. (23). Briefly, gel plugs were washed with 100 mM ammonium hydrogen carbonate solution and acetonitrile. Gel piece was dried at room temperature, swelled in the digestion buffer containing 50–500 ng trypsin in 30 µl 50 mM ammonium hydrogen carbonate and incubated overnight at 37°C.

Peptide fragmentation and sequencing by LC-ESI-MS/MS

The tryptic peptide mixtures (7–15 µl) were separated on 2.1 × 150 mm RESTEK (Restek Co, Bellefonte, PA, USA) Ultra C18 column (Buffer A 0.1% formic acid in H_2O, buffer B: 0.1% formic acid in acetonitrile) with Thermo Electron Accela LC instrument. Peptides carrying charge +2 and +3 were selected for further fragmentation and MS/MS spectrum was recorded with Thermo Electron LTQ Orbitrap (Thermo Finnigan, Ringoes, NJ, USA) instrument.

Database searching

Obtained peptide fragmentation spectra were submitted to Thermo Electron Bioworks 3.3 software with algorithm SEQUEST (Thermo Electron, San Jose, CA, USA) to search against *L. acidophilus* NCFM genome. For positive identification, following criteria were used: for peptides $z = +2$ XCorr was >2 and for peptides $z = +3$ XCorr was >2.5. Theoretical pI and MW for identified polypeptides were calculated with the ExPASy Compute pI/Mw webtool (http://www.expasy.org/tools/pi_tool.html).

Results

Antibody reactions against *L. acidophilus* antigens on 1-D immunoblot

Immunoglobulin G antibody reactions were seen in CD patients against 24, in IDDM patients and controls against 28 clearly distinguishable *L. acidophilus* antigens of 11–86 kDa range. Average reactivity per person was 16 for a CD patient and 17 for a IDDM patient and a healthy control. Very high prevalence of reactions was demonstrated amongst all groups against 58 kDa (94%), 50 kDa (88%), 37 kDa (99%) and 32 kDa (97%) antigens, whereas 100% reactivity was seen against 65 kDa, 45 kDa and 40 kDa antigens. This high reactivity gave suspicion about possible cross-reactions to common immunogenic proteins and was tested with antibodies against *C. albicans* enolase (47% identity with *L. acidophilus* enolase) and against *E. coli* GroEL which bound to 45 kDa and 58 kDa bands, respectively.

Higher prevalence of serum-IgG against two *L. acidophilus* antigens in CD group and three in IDDM group were revealed (Table 1, Fig. 1). The CD patients had significantly more antibodies against 42 kDa antigen ($p < 0.001$) where also a tendency of higher reactivity against 46 kDa antigen ($p = 0.05$) was seen compared with the control group. Similarly, more IgG reactions were against 46 kDa in the IDDM group ($p = 0.05$) where also reactivities against 47 and 30 kDa antigens ($p = 0.05$ and $p < 0.05$, respectively) were highly represented. Interestingly some antigenic reactions were more frequent in the control group: 86 kDa ($p < 0.05$), 77 kDa ($p < 0.05$) and 15 kDa ($p < 0.01$) antigens showed more reactions compared with the CD group, and the

Table 1. Differences between patient and control groups in anti-*Lactobacillus acidophilus* serum IgG prevalence on 1-D immunoblot

Antigen MW (kDa)	IgG antibodies against *L. acidophilus* antigenic proteins in: Controls no. (%)	CD group	IDDM group	p-Value
86	22 (92)	11 (55)*	15 (71)	0.01208
77	23 (96)	14 (70)*	16 (76)	0.03533
47	19 (79)	18 (90)	21 (100)*	0.05144
46	16 (67)	19 (95)*	20 (95)*	0.05441/0.05102†
42	9 (38)	18 (90)*	4 (19)	7.047e-06
34	14 (58)	15 (75)	3 (14)*	0.004816
30	11 (46)	13 (65)	17 (81)*	0.02968
15	9 (38)	0 (0)*	5 (24)	0.002081

IDDM, insulin dependent diabetes mellitus; CD, coeliac disease.
p-Values are obtained using the Fisher`s exact test featuring age and gender adjusted logistical regression analysis.
*Represents statistically significant difference compared with the control group.
†Anti-46 kDa IgG prevalence was significantly high in CD and IDDM groups.

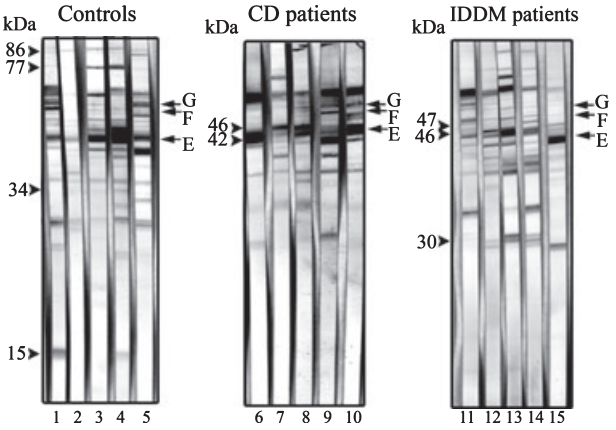

Fig. 1. Representative sera reactions of controls and patient groups against predominant antigens of *Lactobacillus acidophilus* on 1-D immunoblot. Sera are reactive with HSP60 (G) in the 58 kDa region and with enolase (E) in the 45 kDa region. F stands for the 50 kDa identified FRC protein. In the control group, 86 kDa positive are sera on lanes 1–5, 77 kDa positive are sera on lanes 2–5, 34 kDa positive are sera on lanes 2–4 and 15 kDa positive are sera on lanes 1 and 4. In the CD group, 46 kDa and 42 positive are sera on lanes 6–10. In IDDM group, 47 kDa and 46 kDa positive are sera on lanes 11–15 and 30 kDa positive are sera on lanes 12–15. The bands on different immunoblots were found according to standard serum reactivity patterns.

34 kDa antigen ($p < 0.01$) compared with the IDDM group.

Identification of antigenic proteins and matching between 1-D and 2-D results

The results from 1-D immunoblot analysis was subsequently applied for identification of *L. acidophilus* antigenic proteins using 2-DE. Twelve polypeptides were identified out of eight spots (Table 2, Fig. 2) including Glyceraldehyde-3-p-dehydrogenase (GAPDH), Elongation factor Tu (EF-Tu), Enolase (ENO), Formyl-CoA-transferase (FRC), Pyruvate kinase (PYK), Elongation factor Ts (EF-Ts), Phosphoglycerate kinase (PGK), 60 kDa chaperonin (GroEL protein), hypothetical protein LBA0396 and Fructose-bisphosphate aldolase (FBA). Spot number 1' was firstly identified as hypothetical protein LBA0396. A Uniprot BLAST (http://www.expasy.org/tools/blast/) was performed with the LBA0396 alignment which manifested in 92% identity (positives 92%) of Oxalyl-CoA decarboxylase (OXC).

One-D immunoreactivity pattern of one CD patients' serum with the representative IgG recognition profile was taken for a reference to match 2-D analysis (Fig. 2b), with 1-D immunoblot according to MW. The 2-D immunoreactive proteins in the 33 kDa to 71 kDa range were comparable to the 32 to 65 kDa region of 1-D analysis results. The 65 kDa antigen matched with PYK and the 58 kDa antigen with HSP60 as determined with anti Gro-EL antibodies. The location of the OXC representing band in 58 to 65 remains kDa region unclear because of the insufficient separation on 1-D SDS-PAGE.

All antigens on 1-D immunoblot with an estimated MW of 40 to 50 kDa could be found in 2-D spots number 3, 4, 5 and 6 (Fig. 2). Taking into account that the serum was strongly reactive only with the 45 and 50 kDa antigens and ENO location was determined by polyclonal antibodies as a predominant reactive 45 kDa band, we suggest that the 50 kDa band

Table 2. *L. acidophilus* antigenic proteins identified by LC-ESI-MS/MS

2-DE Spot No	Uniprot access No	Theor. MW/pI	Exp. MW/pI	Sequence coverage (%)	Matching peptides (no.)	Protein identification
1	Q5FKG5	63/5.1	71/5.6	51.3	29	PYK
2`		61/4.9	65/5.3	41.3	18	LBA0396*
2`	Q5FLY7	61/5.0	65/5.3	41.3	18	OXC
2	Q93G07	58/4.8	65/5.3	50.6	26	GroEL
3	Q5FLY8	50/5.2	49/5.1	34.2	12	FRC
3	Q5FKM6	47/4.6	49/5.1	57.7	35	ENO
4	Q5FKR8	44/4.8	52/5.7	47.7	18	EF-Tu
5	Q5FL50	43/5.1	46/5.6	17.9	6	PGK
6	Q5FJM4	38/4.8	44/5.2	34.3	9	EF-Ts
7	Q5FL51	37/5.9	39/6.4	69.2	29	GAPDH
8	Q5FIR0	33/4.8	31/5.2	11.9	3	FBA

PYK, pyruvate kinase; OXC, oxalyl-CoA decarboxylase; FRC, formyl-CoA-transferase; ENO, enolase; EF-Tu, elongation factor Tu; EF-Ts, elongation factor Ts; GAPDH, glyceraldehyde-3-P-dehydrogenase; FBA, fructose-bisphosphate aldolase.
*LBA0396 is a hypothetical protein without a Uniprot accession number.

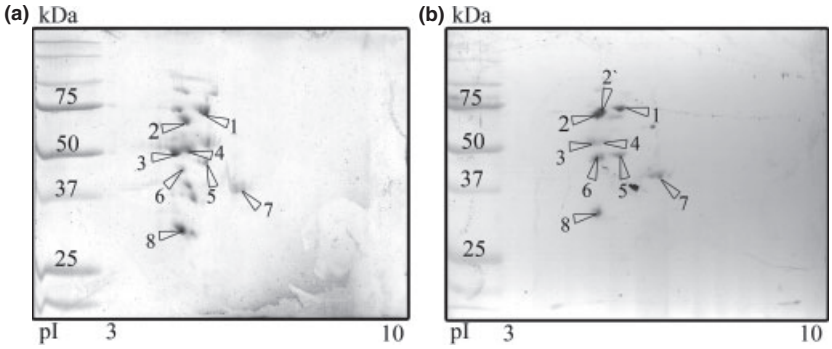

Fig. 2. Coomassie R-350 stained 2-DE gel (a) and 2-D immunoblot (b) of *L. acidophilus* proteins in the pI range of 3–10. On the immunoblot (b) is presented 2-D immunoreactivity pattern found with active CD patient serum. Arrows show the area where the gel plugs were excised for identification (further information can be found in Table 2). On the left side of the images are All Blue Precision Plus MW markers in kDa.

corresponds to FRC which having a larger MW was identified from the same spot as ENO for the insufficient separation. According to MW, the 44 kDa band corresponds to EF-Tu, the 43 kDa band corresponds to PGK and the 40 kDa band to EF-Ts.

Spots number 7 and 8 were identified as single polypeptides and the corresponding bands were clearly distinctive on 1-D immunoblot. GAPDH was identified from spot number 7 with the theoretical MW of 37 kDa that is in good accordance with the obtained 1-D immunoblot analysis where a strong reaction was seen in the 37 kDa region. Spot number 8 was identified as FBA. The tested serum had a single reactive band in the 32 kDa region and was in good accordance with the theoretical MW of identified 33 kDa FBA.

Discussion

This study was undertaken to develop an immunoblot assay for determination of IgG type serum antibodies against antigens of a common indigenous LAB, *L. acidophilus*. For the first time, we described an immunoblot method to investigate humoral immune reactions of sera from IDDM, CD patients and controls against *L. acidophilus* proteins.

The immunoblot assay compared with the enzyme-linked immunosorbent assays enables comparatively and efficiently to analyze antibodies against different antigenic proteins of a LAB. In our study a 5–20% gradient gel was used for better antigenic protein separation. With this method we found numerous antigens that were reacting with IgG antibodies at high frequency in both disease groups. This might be associated with the alterations of the integrity of gastrointestinal mucosa in patients with CD and IDDM (24, 25), enabling selected antigens pass through mucosa more easily and hereby effectively come to contact with the immune system. However, one should not rule out the differences that exist in mucosal immune system of CD and IDDM patients, and controls (26).

According to immunoreactivity pattern of the serum obtained from a patient with active CD we identified *L. acidophilus* antigens as follows: 37 kDa as GAPDH, 40 kDa as EF-Ts and PGK, 45 kDa as EF-Tu, 58 kDa as HSP60 (GroEL), 65 kDa as PYK. All these are common immunogenic proteins that are present in several microorganisms and could well contribute to the known immunomodulatory effects of lactobacilli.

We found high serum reactivity towards previously considered very immunogenic FBA (32 kDA), GAPDH and ENO in all groups (27, 28) which might reflect the fact that the proteins are widely distributed in microbial kingdom and readily available for immune system activation and immune system counter-reaction.

Indeed, for further studies we need more information about the specific proteins of this particular *L. acidophilus* strain. As a first step we have identified FRC and OXC as *L. acidophilus* antigenic proteins. FRC and OXC are obligatory enzymes for oxalate degradation in the gastrointestinal tract. The genes for these enzymes are encoded by a single operon present in *L. acidophilus*, *L. gasseri* and *Bifidobacterium lactis*, but not in *L. plantarum* or *L. johnssonii* (29). Other oxalate degrading bacteria isolated from the human gastrointestinal tract are *Oxalobacterium formigenes* (30) and *Eubacterium lentum* (31). These data suggests that OXC and FRC are not common enzymes among microorganisms and could be therefore used for characterization of particular groups of microorganisms and evaluation of the host immune system reactions. Our

study demonstrated for the first time that *L. acidophilus* FRC and OXC are immunoreactive with human sera. Most interestingly, the IgG reactivity against the 50 kDa FRC was demonstrated in all patients with IDDM compared with lower reactions prevalence in other groups. To evaluate significance of this promising finding additional studies on larger IDDM patients population are needed.

The different pattern of immunoreactivities in children with CD and IDDM from controls, suggests that LAB antigenic proteins may play an important role in the gastrointestinal tract regulated immunology. An immunoblot analysis used in this study can be used in further studies to identify analogous effects. Such studies may detect specificities in immune responses against commensal microbiota that may be involved in the development of CD and IDDM.

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