CLINICAL STUDY

Long-term (56-week) oral administration of probiotic Enterococcus faecium M-74 decreases the expression of sICAM-1 and monocyte CD54, and increases that of lymphocyte CD49d in humans

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Abstract

Objectives: **To study the effects of long-term probiotic [Enterococcus faecium (EF) M-74 strain] application in humans with respect to adhesion molecules, both soluble forms (sICAM-1, sPECAM-1) and their expression on leukocytes.**
Methods: **Double-blinded randomized and placebo controlled study lasting for 60 weeks. A single capsule containing either $2x10^9$ of bacteria EF M-74 with 50 μg of organically bound selenium (E-group) or placebo (P-group) was given to volunteers. Peripheral blood was analyzed for the expression of particular adhesive molecules.**
Results and conclusions: **We observed significant changes in CAMs expression in terms of a decrease in sICAM-1, CD54 on monocytes and CD11b on lymphocytes after one-year administration of Enterococcus faecium M-74 in humans. Anti-adhesion-aimed therapeutic modalities may provide the future approach to prevention and treatment of cardiovascular diseases. Application of probiotics may be part of such strategies.** ***(Tab. 2, Fig. 6, Ref. 41.)***
Key words: probiotic, cell adhesion molecules.

The adhesion of leukocytes to endothelium has been discovered only lately to be the key point in atherosclerosis. In the past decades, the attention has been dedicated especially to cellular surface proteins – adhesion molecules, which are suggested to play the control role in these interactions. During the inflammatory response, the adhesion molecules serve as enhancers of pairing many less avid receptors with their ligands and transmitters of signals regulating the specific effector functions. At least four superfamilies of adhesion molecules participate in these events: selectins, integrins, some members of the immunoglobuline superfamily, and cadherins. They play significant roles in nearly all stages of atherosclerosis, from monocytes movement to endothelial cells, through myocytes migration from media to intima, until later phases that are characterized by fibro/lipid plaque formation and potential complications due to thrombosis and/or plaque disruption and embolism.

Interactions between monocytes and endothelium belong to the earliest detectable changes in the complex process of atherosclerosis. When monocytes and endothelial cells are actived via direct cell-cell interaction, both types of cells express several biologically active molecules, including adhesion molecules, cytokines, coagulation and fibrinolytic factors, metaloproteinases and vasoactive substances. All of these molecules could contribute to atherogenesis and atherothrombosis.

Experimental and clinical studies have confirmed that serum concentrations of adhesive molecules correlate with the development of coronary artery disease (1) and its clinical presentations, i.e. myocardial infarction (2). Pathological studies have shown increased CAM expression in several components of the atherosclerotic plaque (3).

The progress in understanding the cellular and biochemical changes in atherosclerosis is a challenge in searching for new

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Tab. 1. Baseline subject characteristics.

	E-group	P-group	p-value
Number	20	18	
Gender (men/women)	3/17	4/14	
Age (years)	75.35±1.49	78.05±1.68	0.11
BMI (kg/m^2)	29.40±0.86	29.08±1.14	0.41
Waist-hip ratio (cm)	0.90±0.02	0.92±0.01	0.23
BP systolic (mmHg)	150.25±5.79	154.03±4.67	0.31
BP diastolic (mmHg)	85.25±2.60	84.58±1.94	0.42

Values are n (%) or mean±SD (standard error of mean), as indicated, BMI – represents body mass index, BP – blood pressure, p-value level of significance, significant if p<0.05.

Tab. 2. The presence of diseases in both groups at baseline.

Disorders/diseases	Group E		Group P	
	n	%	n	%
IHD	16	80	12	67
HLP/DYSLP	7	35	4	22
Hypertension	16	80	11	61
TIA/stroke	1	5	2	11
Diabetes	5	25	5	28
MUSD	13	65	10	56

Values are given in absolute numbers (%), IHD denotes ischemic heart disease, HLP/DYSLP – hyper/dyslipidaemia, hypertension – arterial hypertension, TIA – transient ischemic attack, Diabetes – diabetes mellitus, MUSD – musculoskeletal diseases.

therapeutic strategies. One of the possible links in the complex antiatherogenic therapy chain including antilipemic, antithrombotic, endothelprotective, could be the "anti-adhesion" treatment. With the exception of using monoclonal antibodies (4), our current options have been exhausted. This is the reason for searching for alternative methods.

Since immunologic and inflammatory mechanisms in atherosclerosis are emphasized in particular nowadays, modalities that are able to alter these processes are being considered. Probiotics are ones of those having such properties. They are living microorganisms, that when ingested in sufficient amount, beneficially influence the health of the host (5, 6). Their immunostimulatory effects have been demonstrated by some authors in different studies (7, 8, 9).

The aim of our study was to follow the effects of long-term probiotic (strain Enterococcus faecium M-74) application in humans with respect to adhesion molecules and their expression on monocytes, granulocytes and lymphocytes, as well as to their soluble forms (sICAM-1 and sPECAM-1).

Subjects and methods

The double-blinded randomized placebo controlled trial was carried out at a nursing home for elderly in Bratislava from April 2001 to May 2002. 43 volunteers were randomized into two groups. Participants in one group were given a single capsule containing 2x10^9 of bacteria EF M-74 and 50 μg of organically bound selenium (E-group) daily. At the same time, subjects in the second – placebo group (P-group) were given a placebo-containg capsule. All individuals have duly acknowledged the study protocol and gave their consent to the proposed study. The enrollment examination consisted of a complete scrutiny of case-histories especially focused on cardiovascular risk profile assessment, physical examination including antropometric measurement (to calculate BMI (body mass index) as person's weight in kilograms divided by height in meters squared – (BMI=kg/m^2), and WHR (waist/hip ratio)). We also took blood pressure at standard conditions.

Peripheral blood was analyzed during the enrollment examination, i.e. before intervention, after 56 weeks of capsule administration, and four weeks following the termination of administration. Blood samples were always drawn between 8–9.00 AM after overnight fasting and abstinence. Circulating soluble adhesive molecules sPECAM-1 (platelet endothelial cell adhesion molecule-1=CD31) and sICAM-1 (intercellular adhesion molecule-1=CD54) levels were determined by means of commercially available ELISA (enzyme-linked immunosorbent assay) (Bender MedSystems). We measured the expression of adhesive molecules on lymphocytes, monocytes, and granulocytes (CD11b, CD18, CD31, CD49d, CD54, CD62L) by flow cytometry.

Cells were stained for CD11b, CD18, CD31, CD49d, CD54, CD62L using monoclonal fluorescein isothiocyanate (FITC)-conjugated or phycoerythrin-conjugated anti-CD54, anti-CD11b, anti-CD18, anti-CD31, anti-CD49d, anti-CD54, or anti-CD62L antibodies, control IgG FITC and/or PE-labeled (Becton Dickinson), all at 1:5 dilution. Cells were analyzed using the Beckman Coulter EPICS XL (Becton Dickinson) flow cytometer. Granulocytes, monocytes and lymphocytes were separated on the basis of their forward- and side-scatter pattern. The control antibodies were used to set the background levels. The relative number of positive lymhocytes, monocytes or granulocytes for each antibody was measured (System II software). System II software calculated each reported lymhocytes (monocyte, granulocyte) subsets as a percentage of total lymphocytes (monocytes, granulocytes) in the lymhocyte (monocyte, granulocyte) acquisition gate.

Statistical analyses: Categorical variables are given as counts (expressed in %), continuous variables as means SEM, standard error of mean. Coefficients of kurtosis and skewness were used to test the data distribution. Data found to have normal (Gaussian) probability distribution were analyzed by parametric methods. For statistical analyses of quantitative variables, we used Student's t-test paired for comparisons within groups. Analyses were performed with SPSS (Statistical Package for the Social Sciences), version 8.0 for Windows and Microsoft Office 2000 (Microsoft Excel for Windows). All significance tests were 2-tailed at the level of significance α=0.05.

Results

Results are presented as changes between baseline values (week 0) and values obtained at the end of application of cap-

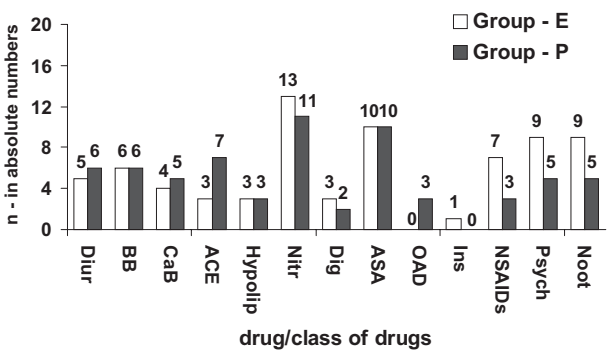

Fig. 1. Pharmacological treatment at baseline evaluation. Diur – diuretics, BB – betablockers, CaB – calcium channel blockers, ACE – Ace-inhibitors, Hypolip – hypolipidemics, Nitr – nitrates, peripheral vasodilators and rheologic agents, Dig – digoxin, ASA – acetyl salicylic acid, OAD – oral antidiabetics, Ins – insulin, NSAIDs – nonsteroidal antiinflammatory drugs (except ASA), Psych – psychopharmacologic agents (except nootropics), Noot – nootropics.

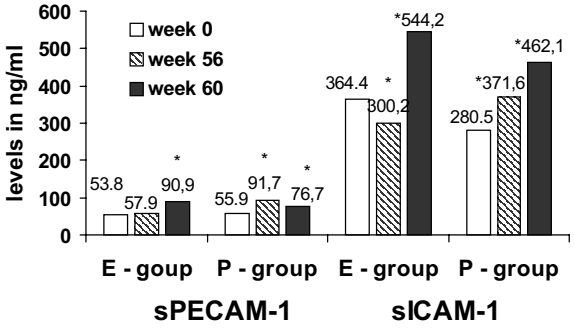

Fig. 2. Effect of probiotic on soluble cell adhesion molecules serum levels (E-group). CAM – cells adhesion molecules, sICAM-1 – soluble intercellular adhesion molecule-1, asterisk – p<0.05.

sules (i.e. after 56 weeks) and one month after discontinuing the administration (i.e. after 60 weeks). All of these data are given for both of the two groups, E-group and P-group. 20 subjects in group E (17 women and 3 men, mean age 75.35±1.49 year) and 18 subjects in group P (14 women and 4 men, mean age 78.05±1.68 year) completed the study. The basic subject characteristics are summarized in Table 1. No significant differences were found between the two groups. Morbidity of participants was verified according to the history; medical records and physical examinations are shown in Table 2. Fundamental medical treatment is displayed in Figure 1.

Adhesion molecules

Inividuals given *E. faecium* M-74 expressed a decrease in sICAM-1 after 56 weeks (364.38±26.40 ng/ml at week 0 vs 300.19±17.27 ng/ml at week 56, p=0.019) (Fig. 2). Four weeks after capsule cessation, serum ICAM-1 concentrations increased significantly (544.24±49.73 ng/ml, p<0.001). As far as sPECAM-1 is concerned in E-group, no significant changes were found after 56 weeks of probiotics consumption. However, one month after stopping the administration a statistically important increase in sPECAM-1 appeared (90.89±6.08 ng/ml, p<0.001) (Fig. 2). In the *placebo group*, both soluble adhesion molecules (sICAM-1 and sPECAM-1) rose up. The latter increase became statistically significant after 56 weeks of administration and also 4 weeks after its termination (Fig. 2).

Out of all of surface adhesion molecules we measured the expression of CD11b, CD18, CD31, CD49d, CD54, and CD62L on lymphocytes, monocytes, and granulocytes. The results are given as percentage of total lymphocytes (granulocytes, monocytes) in the lymphocyte (granulocyte, monocyte) acquisition gate±SEM, standard error of mean. Oral administration of *E. faecium M-74* has resulted in decreased expression of CD11b on lymphocytes (25.54±3.24 at week 0 vs 11.04±0.74 after 56 weeks, p<0.001). Expression of CD18 (67.47±2.63 vs 74.44±2.34, p<0.001), CD49d (9.11±1.61 vs 17.78±2.26, p<0.005), and CD62 (67.47±2.63 vs 28.12±2.92, p<0.001) increased notably. Four weeks after application discontinuation, the expression decrease in CD11b became statistically significant (p=0.022). In addition to the latter, a decline in CD31 and CD54 (both p<0.001) expression was observed. An elevated expression of CD62 on lymphycytes continued to be important in the respective period (p<0.001) (Figs 3 and 4).

On monocytes, we noticed a statistically significant decrease in the expression of CD54 after 56 weeks (8.23±1.05 at week 0 vs 3.29±0.45 after 56 weeks, p<0.001), which persisted for one month after stopping the capsule application (p<0.001). Non-significant changes in the expression of other CAMs after 56 weeks were followed by a significant decrease in the expression of CD31 and CD49d and an increase in CD11b, CD18, and CD62 four weeks later (Figs 3 and 4).

During the study period, the expression of CD31 (9.02±0.94 at baseline vs 3.34±0.72 after 56 weeks, p<0.001) and that of CD54 (4.14±0.52 vs 1.02±0.32, p<0.001) on granulocytes decreased. This decrease remained significant for four weeks after capsule cessation. After 56 and 60 weeks, the marked increases in expressions of CD11b, CD18 and CD62 were significant (Figs 3 and 4).

After 56 weeks, the *placebo group*, yielded a significant increase in the expression of CD11b on lymphocytes (19.90±1.84 at week 0 vs 27.32±1.92 at week 56, p<0.001) and granulocytes (47.57±3.55 vs 59.04±1.72, p=0.006), CD18 on granulocytes (68.96±4.72 vs 90.28±1.89, p<0.001), CD49d on lymphocytes (9.30±1.85 vs 16.29±1.83, p=0.002), monocytes (6.81±0.96 vs 16.29±1.27, p<0.001), and CD62L both on lymphocytes (14.21±1.84 vs 30.10±2.59, p<0.001) and granulocytes (18.28±3.14 vs 39.94±3.58, p<0.001). The statistically significant increases in the expressions of all of the above listed CAMs were still present after 4 weeks following the discontinuation of the capsule administration. Subtle elevation in CD62L expression on monocytes after 56 weeks became significant as late as

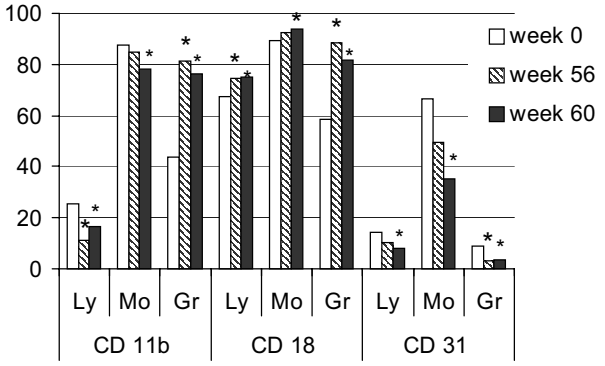

Fig. 3. Expression levels of adhesion molecules in group E (1). Values are expressed as percentage of total lymphocytes (granulocytes, monocytes) in the lymphocyte (granulocyte, monocyte) acquisition gate, asterisk – p<0.05, Ly – lymphocyte. Mo – monocyte, Gr – granulocyte (see also discussion).

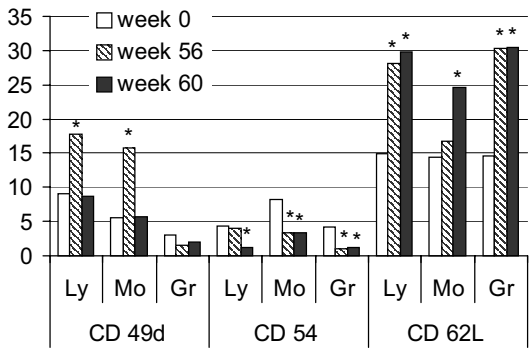

Fig. 4. Expression levels of adhesion molecules in group E (2). Legend as in the Figure 3.

one month later (p<0.001) (Figs 5 and 6). In this group of individuals, a decrease in CD31 expression on lymphocytes, monocytes and granulocytes was recorded, while the decrease in two former expressions was significant. Another statistically important change to be observed was the decreased expression of CD11b on monocytes and CD54 on lymphocytes. In both cases the expression decreased as late as after 4 weeks following the discontinuation of administration of capsules (Figs 5 and 6).

Discussion

The present study was conducted to examine the effect of probiotics on the expression of adhesive molecules. The cell membrane consists of a huge number of different molecules that participate in intercellular communication, adhesive reactions or determine the cell's presence in particular biological species, tissue, organ or its evolution stage. They are named systematically by assigning their cluster of differentiation (CD) antigen number (10). The selectin family consists of three members named according to the cells in which they had been originally discovered. L-selectin (CD62L) is constutively expressed on leukocytes and its target cells are activated endothelial cells. E-selectin (CD62E) is produced exclusively by endothelial cells after cytokine activation and its counter-receptors are located on neutrophils, monocytes, eosinophils, lymphocyte subsets, and some tumour cells. P-selectin (CD62P) is pre-formed and stored for rapid release in platelets or endothelial cells. Integrins are a large family of heterodimeric glycoproteins that can be subdivided according to their particular ß subunit, which is shared by all members of the group. The integrins are expressed particularly by leukocytes. The leukocyte integrins are represented by three heterodimeric molecules: LFA-1 (CD11a/CD18), CR3 (CD11b/CD18), and CR4 (CD11c/CD18). The intact CD11a/CD18 molecule is the lymphocyte function-associated antigen-1, LFA-1, and is expressed by lymphocytes (including T-cells), myeloid cells (monocytes, macrophages, and granulocytes), and a variety of other cell types. CD11b/CD18 is the complement receptor type 3, CR3, and CD11c/CD18 is CR4 (also referred to as p150.95). Both CR3 and CR4 are expressed by myeloid cells. There are two or more ligands for LFA-1 currently referred to as ICAM-1 (CD54) and ICAM-2 (CD102) and defined as members of the immunoglobulin superfamily. Alongside with intercellular adhesion molecules ICAM-1 and ICAM-2, there is an additional member of the diverse immunoglobulin superfamily – platelet-endothelial cell adhesion molecule, PECAM-1 (CD31). Both ICAM-1 and ICAM-2 are expressed by endothelial cells. PECAM-1 has been identified on neutrophils, monocytes, and platelets, and is present in large amounts on endothelial cells where it is concentrated in sites of cell-cell junctions (11).

Cell adhesion molecules (CAMs) belong to molecules that contribute to atherosclerosis and its clinical sequelae (12). An increased CAMs expression on endothelial cells has been observed even in preclinical stages of atherosclerosis. CAMs are membrane-binding glycoproteins, which allow intercellular interactions. They permit and facilitate adhesion and subsequent transendothelial migration of immunocompetent cells including leukocytes. It is suggested to be an imortant step in pathogenesis of many vascular diseases including atherosclerosis (13, 14). These molecules can be expressed on the endothelial membrane in response to several inflammatory cytokines, e.g., interleukin-1 (IL-1), tumor necrosis factor (TNF), and interferon gamma (IFN-gamma). Increased CAMs expression in atherosclerotic plaques (15) indicates their important role in acute atherothrombotic syndromes (16).

The first stage of atherogenesis, which is nowadays believed to be the endothelial damage, is followed by a cascade of reactions, generally known as the "inflammatory response to injury". In response to infection or other tissue damage, leukocytes move from the bloodstream to tissues. The essential moment in this process is that of adhesion of leukocytes to endothelial cells. When the regulation is appropriate, the inflammatory response is physiological and homeostatic. However, when the stimulus is inappropriate as to space, time, or quantity, adhesion and activation of leukocytes can have pathological effects.

In case of immediate inflammatory response of polymorphonuclear neutrophils to endothelial cells, the initial adhesion is me-

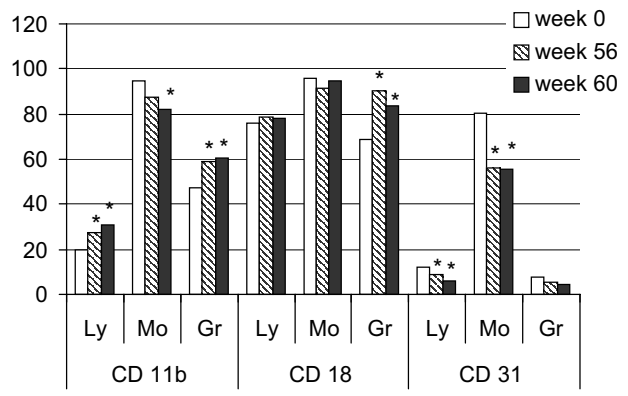

Fig. 5. Expression levels of adhesion molecules in group P (2). Legend as in the Figure 3.

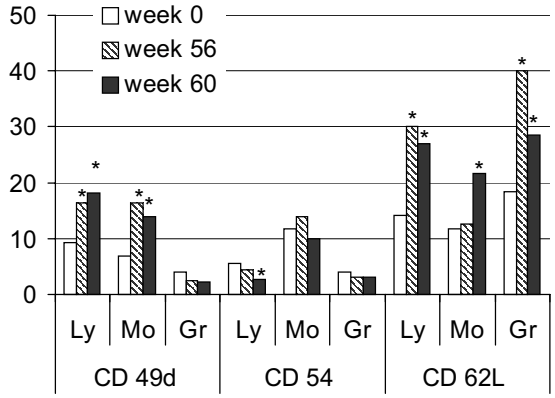

Fig. 6. Expression levels of adhesion molecules in group P (2). Legend as in the Figure 3.

diated by P-selectin (17). After adequate stimulation by agonists, P-selectin stored in endothelial cells is quickly translocated to the cellular surface. Subsequently, non-adhesive and non-thrombotic surface of endothelium slowly becomes pro-adhesive. P-selectin binds to its counterreceptors, such as P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes. The next step consists of tethered leucocyte activation on the basis of signals that come out from activated endothelial cells. There exists a difference between immediate and delayed (requiring more then one hour to become apparent) inflammatory response. However, the general principles stay the same. In either case, endothelial cells are stimulated to express cell adhesion molecules, members of the selectin family. Then they bind with the constitutively expressed counterreceptors on circulating leukocytes. In delayed reactions, E-selectin is used by endothelial cells as a tether, and chemokines are activated some time later (e.g., interleukin-8). Surface expression of E-selectin reaches its maximum within 12 hours and typically returns to its initial levels in 24 hours (17). Endothelial cells express P-selectin only temporarily during various physiological conditions. As opposed to the latter, it is expressed by platelets only under specific circumstances and in a relatively stable manner.

Cellular interactions between monocytes and the endothelium are critical events in vascular pathology, such as atherosclerosis and acute coronary syndromes. During the past few years, preexisting mononuclear cell infiltrations in healthy arteries in children and teenagers have been described. These arterial accumulations in regions known as the predilection sites of later development of atherosclerosis consist mostly of activated T-cells, macrophages, and dendritic cells, with only a few mast cells and virtually no B- or natural killer cells. In analogy to the mucosa-associated lymphoid tissue, Millonig et al refer to these accumulations as “vascular-associated lymphoid tissue” (18). They assume that its function is similar to that of the local immunosurveillance system that monitors the bloodstream for potentially harmful endogenous or exogenous antigens. Apart from the remarkable accumulation of mononuclear cells, the vascular-associated lymphoid tissue regions are characterized by a typical distribution of extracellular matrix proteins: collagens of types I and III, fibronectin, and tenascin, whereas collagens of types IV, V and VI and laminin show homogenous distribution throughout all regions of the intima (18).

The adhesion to P-selectin activates the cytokine gene expression in monocytes. One of the mediators of this process seems to be the nuclear factor $_{\kappa}$B (NF-$_{\kappa}$B). In their experimental studies, Prescott et al have found that the adhesion of monocytes to purified immobilized P-selectin causes a small but consistent translocation of NF-$_{\kappa}$B to the nucleus. It was dramatically enhanced when the cells were stimulated with PAF. This suggests the mechanism by which adhesion to P-selectin amplifies MCP-1 (monocyte chemotactic protein-1) and TNF (17).

It is necessary to take into account some other factors when interpreting the obtained P-selectin values, namely e.g. the existence of gene polymorphisms for P-selectin, or age-dependent relation between P-selectine levels in, for instance, coronary artery disease. It underlines the likelihood of different roles of this molecule in the atherothrombotic process (19).

In our study, from the long-term point of view we observed increased expressions of L-selectin (CD62L) on granulocytes, lymphocytes and monocytes, paradoxically in both groups. L-selectin can be involved in three types of cell-cell adhesion interactions: rolling of polymorphonuclear leukocytes (PMN) and lymphocytes along the vessel wall, migration towards the sites of inflammation, and homing of blood-borne lymphocytes to peripheral lymph nodes. It mediates leukocyte adhesion to vascular addressins of specific tissue (e.g., MAdCAM-1 (mucosal addressin cellular adhesion molecule-1) expressed on endothelial cells of intestine tissues) (11). This is the way, how the leukocytes precisely migrate to specific organs, tissues or a particular area in a certain tissue. This is also the mechanism by which lymphocytes could be targeted to regions of antigen concentration within lymph nodes or other areas of inflammation (20). Upon lymphocytes activation by proinflammatory cytokines, CD62L expression is downregulated, and integrin (e.g., VLA-4) expression and binding affinity are rapidly increased inducing tight adhesion conductive to extravasation of lymphocytes to sites of inflammation (21). On the other hand, soluble L-selectin inhibits leukocyte adhesion to cytokine-activated endothelial cells, and the increased L-selectin expres-

sion in our study could have been the result of compensatory effect against the increase in CD11a and ICAM-1-induced adherence. This is in concordance with other studies (22).

After probiotic administration, we observed changes in the expression of some adhesion molecules belonging to integrins. In persons who had been given E. faecium M-74, the expression of CD11b on lymhocytes decreased after one year ($p<0.001$). The decrease was still significant four weeks after the cessation of capsule administration. An interesting finding was an important increase in CD11b and CD18 on monocytes one month after stopping the probiotic application. This could have important clinical consequences because some studies have proven that patients with the diagnosis of coronary artery disease have increased levels of CD11 (as well as those of ICAM-1) expression on monocytes (22).

In general, adhesion molecules can be detected either as cellular surface molecules or as soluble forms found in the circulation. While the function of the surface forms is more or less clear, the role of the soluble ones remains uncertain. They can originate in vessel wall components, i.e. in endothelial cells, smooth muscle cells (23), activated lymphocytes, dendritic cells, fibroblasts and hepatocytes (24, 25). Elevated plasmatic levels of soluble form of CAMs have been observed in various pathological conditions: in patients with chronic inflammatory disorders (26), diabetes mellitus and dyslipidaemia (27, 28), even in the absence of evident clinical cardiovascular disease (27), etc.

The relation between the soluble form of ICAM-1 and cardiovascular mortality in patients with a proved diagnosis of coronary artery disease has been described (29), its increased levels have also been found in patients with stable angina pectoris as well as in those with acute coronary syndromes (30). The relation of CAM to atherosclerosis is supported by the direct finding of this molecule in atherosclerotic lesions (31). The increased concentration of soluble CAMs in patients with atherosclerosis (32, 33) and its sequelae, e.g., acute coronary syndromes (16, 34) or peripheral artery disease (35) has been mentioned hereinabove. However, there still exists a possibility of their increased levels in apparently healthy persons who are at risk of future cardiovascular events. In the study conducted by Ridker et al, baseline sICAM-1 concentrations have been raised among apparently healthy men being at risk of future myocardial infarction (36). A significant relationship of circulating ICAM-1 with coronary heart disease, as measured by the number of incident patients, has also been defined in the ARIC (Atherosclerosis Risk in Communities) study (37).

One of our aims was to find out whether consumption of probiotic strain E. faecium M-74 could have some effect on soluble adhesion molecules. After 56 weeks of administration of capsules containing the probiotic culture, we recorded a decrease in sICAM-1. It was also notable to find out that plasma sICAM-1 levels increased no sooner than four weeks after probiotic administration discontinuation. In the placebo group, serum sICAM-1 concentrations rose in up to one year and another increase followed four weeks after stopping the use of capsules ($p<0.001$). In the context of the above facts we consider these results to be favourable. Non-pharmacological approach seems to represent an alternative to pharmacological treatment in this respect (e.g., patients treated with statins exhibited a deacrease in the expression of adhesion molecules (38)). On the other hand, despite the accumulated data it is still unclear whether increased levels of adhesion molecules documented in atherosclerosis may be regarded as causal, or it is just a finding that is concomitant to a simultaneously occurring variety of conditions, e.g., smoking (39). Ridker et al found no correlation between sICAM-1 and total cholesterol, and there was no evidence that lipid concentrations had modified the effect of sICAM-1 on the risk of future myocardial infarction. However, smokers had significantly higher concentrations of sICAM-1 than non-smokers (36).

The biological properties and functions of soluble forms of CAMs remain ambiguous. These molecules sustain the ability to bind their ligands, and thus may inhibit interactions between leukocytes and the cellular surface forms of molecules. Conversely, it is possible that circulating adhesion molecules, by occupying their binding sites on leukocytes, may activate them, increasing their integrin expression (40), even before they interact with the endothelium (41).

In conclusion it is possible to state that the research in the field of atherosclerosis at the molecular and genetic level during the past decade has given us some indications of how to state the diagnosis of atherosclerosis most appropriately and as early as possible. Recently, the role of specific adhesive molecules in this process has been explored. Therapeutic modalities aimed at antiadhesion may provide the future approach to prevention and treatment of cardiovascular diseases. The application of probiotics may belong to such strategies. In our study, we observed a significant decrease in soluble ICAM-1 and expression of CD54 on monocytes and CD11b on lymphocytes after one-year administration of Enterococcus faecium M-74 in humans.

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Received December 12, 2004.
Accepted March 15, 2005.