Archivum Immunologiae et Therapiae Experimentalis, 1999, **47**, 169–178
PL ISSN 0004-069X

Adhesion Molecule Expression Stimulated by *Bacteroides thetaiotaomicron* Cell-Surface Antigens

ALICJA ROKOSZ[1], FELICJA MEISEL-MIKOŁAJCZYK[1]*, CEZARY MALCHAR[2], MARIA NOWACZYK[2] and ANDRZEJ GÓRSKI[2]

[1] Department of Medical Microbiology, Institute of Biostructure, University Medical School, Chałubińskiego 5, 02-004 Warsaw, Poland,
[2] Department of Clinical Immunology, Transplantation Institute, University Medical School, Nowogrodzka 59, 02-006 Warsaw, Poland

Abstract. *Bacteroides thetaiotaomicron*, a Gram-negative anaerobic rod belonging to the *Bacteroides fragilis* group (BFG), is involved in many systemic and local, most frequently suppurative infections in man. The cell envelope of these rods is composed of two carbohydrate-containing antigens: lipopolysaccharide (LPS) and capsular polysaccharide (CPS). Adhesion molecules ICAM-1, VCAM-1 and E-selectin (ELAM-1) are induced on the endothelial cells by mediators of inflammation. The aim of this study was to assay the ability of *B. thetaiotaomicron* surface antigens to induce adhesion molecule expression on the endothelial cells. The influence of LPS and CPS on the expression of adhesion molecules on HMEC-1 cell line was examined in an ELISA test. ELISA was performed with monoclonal mouse anti-human: ICAM-1, VCAM-1 and E-selectin antibodies of the IgG class. *B. thetaiotaomicron* lipopolysaccharides revealed the ability to induce ICAM-1, VCAM-1 and E-selectin expression on the endothelial cells. Their activities were similar, but lower than the activity of *Eschericha coli* LPS. ICAM-1 was the most stimulated adhesion molecule. The strongest activation by LPS was achieved at the concentrations of 10.0 and 1.0 μg/ml. The ability of capsular polysaccharide to induce the expression of adhesion molecules was considerably weaker.

Key words: adhesion molecules; endothelium; *Bacteroides thetaiotaomicron*; lipopolysaccharide; capsular polysaccharide.

Introduction

Bacteroides fragilis group (BFG) rods dominate among bacteria physiologically colonizing the human intestine. These Gram-negative rods are strictly anaerobic, non-sporeforming and capsule-producing. BFG strains are now recognized as the most frequently isolated and medically important anaerobic bacteria. Two species belonging to BFG, *B. fragilis* and *B. thetaiotaomicron*, are especially often isolated from clinical specimens and are also highly resistant to many antibacterial drugs. They are etiological agents of en-

* To whom all correspondence should be addressed.

Abbreviations used: BFG – *Bacteroides fragilis* group, LPS – lipopolysaccharide, CPS – capsular polysaccharide, ELISA – enzyme-linked immunosorbent assay, ICAM-1 – intercellular adhesion molecule-1, VCAM-1 – vascular cell adhesion molecule-1, ELAM-1 – endothelial leukocyte adhesion molecule-1, IL – interleukin, HMEC-1 – human dermal microvascular endothelial cells, LSR – local Shwartzman reaction, LAL – *Limulus* amoebocyte lysate, TNF-α – tumor necrosis factor α, HUVEC – human umbilical vein endothelial cells, LBP – LPS-binding protein.

dogenous, suppurative and septic infections in man. These bacteria primarily are isolated from abscesses, soft-tissue infections and blood in cases of bacteremia[8, 10, 30, 37, 43].

The virulence factors of *Bacteroides sp.* rods are quite well-known. The best characterized determinants of pathogenicity are the cell-surface antigens: lipopolysaccharide and capsular polysaccharide. Lipopolysaccharide (LPS; endotoxin) of these anaerobes is responsible for numerous biological activities such as: lethality for experimental animals, pyrogenicity, leukopenia and subsequent leukocytosis, positive local Shwartzman reaction (LSR), positive *Limulus* amoebocyte lysate (LAL) test, release of TNF-α, toxicity for chicken embryos and retardation of fetal growth[2, 3, 26, 28, 29, 34, 35]. Capsular polysaccharide of *Bacteroides sp.* rods is an antiphagocytic factor, contributes to adhesion and plays an important role in abscess formation[11, 17, 19, 25, 31].

The outer layers of *B. thetaiotaomicron* rod cell-wall are composed of two high-molecular weight, carbohydrate-containing antigens: LPS, which is a heteropolymer forming an integral part of the outer membrane of the cell-wall of Gram-negative bacteria and CPS, which forms a capsule localized on the outer surface of the cell-wall and composes the external layer of the cell[1, 24, 25, 39, 45]. The immunochemical properties (chemical composition and serological activity) and biological properties of purified *B. thetaiotaomicron* cell-surface antigens (LPS and CPS) were investigated by our group in 1987–1988 and their characterization was described previously[25, 33, 34, 36].

Appearance and upregulation of the adhesion molecules on endothelium are significant for leukocyte extravasation and migration towards inflammatory sites. Adhesion molecules ICAM-1 (CD54), VCAM-1 (CD106) and E-selectin (CD62E) are induced on the endothelial cells by mediators of inflammation[5, 9, 38]. Intercellular adhesion molecule-1 (ICAM-1) belongs to the endothelial Ig-proteins (Ig gene superfamily). Five members of this family, expressed on the endothelial cells, are involved in leukocyte adhesion and transmigration. ICAM-1 is expressed on leukocytes, fibroblasts, epithelial cells and endothelial cells. ICAM-1 binds to leukocytes via integrins CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1). Mediators of inflammation such as IL-1, TNF-α as well as LPS, enhance production of this molecule[5, 9, 15]. Vascular cell adhesion molecule-1 (VCAM-1), another member of the Ig gene superfamily (Ig SF), serves as an endothelial adhesion molecule for leukocytes. The counter-receptor for this molecule is leukocyte integrin VLA-4 (CD49d/CD29)[5, 9, 15]. The selectin family comprises 3 proteins. One of them is E-selectin (CD62E). This adhesion molecule is expressed by the endothelial cells and is mainly involved in the adhesion of neutrophils. Selectins recognize and bind the carbohydrate ligands: sialyl Lewis X and closely related structures. These carbohydrate determinants are heavily expressed on neutrophils and monocytes, and are also found on natural killer cells. E-selectin appears on the endothelial cells after their stimulation. Production of this molecule is induced by LPS, TNF-α, IL-1 and thrombin. E-selectin mediates a rolling interaction of neutrophils with endothelium[5, 9, 15].

It is known that endotoxic activity of *B. fragilis* group rods is lower than biological activity of LPS extracted from the *Enterobacteriaceae* family rods[17, 18, 22, 23, 40]. In 1993 stimulation of the human T cell adhesion to endothelium by the components (LPS and CPS) extracted from *B. vulgatus* strains was examined by our group. The degree of T cell adhesion to human umbilical vein endothelial cells (HUVEC) stimulated by *B. vulgatus* antigens was markedly lower than the adhesion caused by *E. coli* 055: B5 LPS. There were no differences in stimulatory activity between *B. vulgatus* LPS and CPS[27].

The aim of this study was to determine the ability of *B. thetaiotaomicron* LPS and CPS to induce adhesion molecule expression on the vascular endothelial cells. For this purpose a direct method of adhesion molecule estimation (ELISA with monoclonal antibodies) was applied.

Materials and Methods

Bacterial strains. B. thetaiotaomicron NCTC 10582, a reference strain of the species, representing serotype A of the *B. fragilis* group according to BEERENS et al.[4] was kindly supplied by Dr. H. Beerens (Faculté de Pharmacie, Lille, France). *B. thetaiotaomicron* 312/85 was isolated from a clinical specimen (pancreatic fistula drain). *B. thetaiotaomicron* 9/18 was isolated from a normal human intestinal microflora. The two latter strains were cultured and identified at the Department of Medical Microbiology of the University Medical School in Warsaw.

Media and growth conditions. The bacterial bulk for LPS extraction was grown at 37°C for 48 h in 150 l of liquid VL (yeast-broth medium) supplemented with hemin and vitamin K_1[6]. The bacterial mass for capsular polysaccharide isolation was washed off the surface of a solid VL medium containing blood, hemin and vitamin K_1[6]. The capsular material was obtained from a strain cultured at 37°C for 72 h in the system "Gas generating box H_2+CO_2" (bioMérieux, France).

Extraction and purification of lipopolysaccharides. Three analyzed strains were used for the extraction of endotoxin. Pelleted bacteria were extracted by the hot phenol-water method according to WESTPHAL and JANN[44]. The water phase after extraction was ultracentrifuged (105 000 × g for 3 h) and finally lyophilized. Further purification was achieved with nuclease treatment according to GMEINER[7]. This treatment was done in 10 mM Tris – HCl buffer (pH 7.6) for 18 h at room temperature with RNAse and DNAse (Merck, Germany), each at the concentration of 2 mg/g LPS, with subsequent ultracentrifugation (105 000 × g for 3 h).

Extraction and purification of capsular polysaccharide. The capsular antigen of clinical strain 312/85 was prepared according to the procedure described by POXTON and IP in 1981[32].

Expression of adhesion molecules. Cell of line human dermal microvascular endothelial cells (HMEC-1), obtained from the Department of Dermatology, Emory University School of Medicine in Atlanta (Georgia, USA), was used in each experiment. HMEC-1 cells were cultured in plastic bottles (Sigma, USA) coated with 0.2% gelatin (Sigma, USA) for 24 h and transferred by means of 0.5% trypsin – 0.2% EDTA solution (Sigma, USA) to the flat-bottom 96-microwell plates (Nunc, Denmark) also coated with 0.2% gelatin. Confluent HMEC-1 monolayers were stimulated with LPS and CPS. The following concentrations of antigens were applied: 0.01, 0.1, 1.0 and 10.0 μg/ml. LPS of *E. coli* O55: B5 (Sigma, USA) and resting HMEC-1 cells were used in each experiment as controls. Determinations of ICAM-1 and VCAM-1 expression were performed after 24 h of cell activation, and E-selectin after 4 h. After stimulation of the cells, the plate was rinsed 3 times with PBS and fixed with 0.025% glutardialdehyde (Merck, Germany) for 15 min. Then the plate was rinsed 3 times with PBS and blocked with 1% BSA (Sigma, USA) in PBS for 1 h.

The immunoenzymatic ELISA test was used to examine the influence of *B. thetaiotaomicron* cell-surface antigens on the expression of adhesion molecules: ICAM-1, VCAM-1 and E-selectin on the endothelial cells. ELISA was performed using the modified methods described by LASSALLE et al.[20], SWERLICK et al.[41] and LEE et al.[21]. Monoclonal mouse anti-human: ICAM-1, VCAM-1 and E-selectin antibodies of IgG class (Genzyme, USA) at the concentration of 1 μg/ml were applied. Antibody solution (100 μl) was added to each well containing cells for 90 min (37ºC, 5% CO_2). Then the plate was rinsed thrice with PBS and 100 μl of peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dako A/S, Denmark) diluted 1:1500 was supplied to each well for further 90 min (37ºC, 5% CO_2). The conjugate was diluted with 1% BSA in PBS. Thereafter the plate was washed 3 times with PBS. 100 μl of o-phenylenediamine dihydrochloride (Sigma, USA) at the concentration of 1 mg/ml in 0.1 M phosphate-citrate buffer (pH 5.0) containing H_2O_2, was added to each well. The plate was incubated for 10–15 min at room temperature in darkness. The enzymatic reaction was stopped with 100 μl of 6 N H_2SO_4. The colored reaction product was measured by reading the absorbance at 492 nm in SPECTRA II reader (SLT, Austria), Each determination was carried out 3 times.

Statistical analysis. Statistical significance was determined by the Student's *t*-test and the differences were regarded as significant for $p<0.05$.

Results

The obtained results are presented in Fig. 1–4. Each figure illustrates the influence of one bacterial antigen (in 4 concentrations) on the expression of 3 examined adhesion molecules. The data are presented as a mean ± SEM. The results marked with an asterisk are statistically significant ($p<0.05$) in comparison to the values obtained for resting cells. Values obtained for resting HMEC-1 cells indicate the level of expression of respective adhesion molecules on non-stimulated endothelial cells. *E. coli* O55:B5 LPS was used as a positive control.

B. thetaiotaomicron NCTC 10582 LPS as the stimulator of adhesion molecules

The results presented in Fig. 1 demonstrate the influence of *B. thetaiotaomicron* NCTC 10582 LPS on adhesion molecule expression on the HMEC-1 cell line. Almost all obtained results are statistically significant except for the result obtained for 0.01 μg/ml dose of LPS in the case of VCAM-1 expression. The most stimulated adhesion molecule is ICAM-1. *B. thetaiotaomicron* NCTC 10582 LPS ability to upregulate ICAM-1 is slightly lower than *E. coli* O55:B5 LPS activity. Doses of 1.0 and 10.0 μg/ml of NCTC 10582 LPS are the most effective in the induction of the tested adhesion molecules. Additionally, this LPS at the concentration of 0.01 μg/ml shows similar activity to *E. coli* LPS in stimulation of E-selectin.

B. thetaiotaomicron 312/85 LPS as the stimulator of adhesion molecules

The results obtained for *B. thetaiotaomicron* 312/85 LPS are shown in Fig. 2. All concentrations of antigen

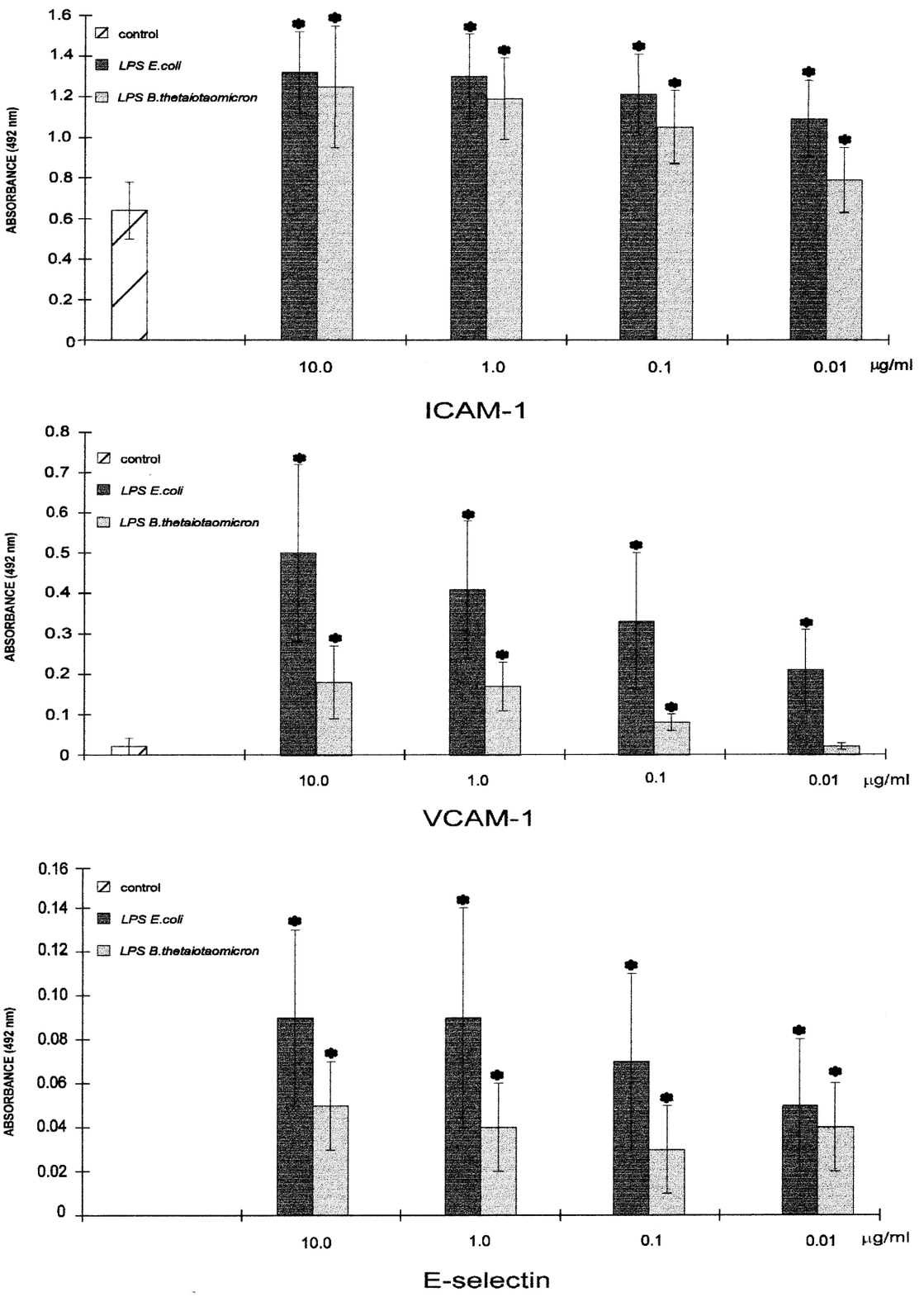

Fig. 1. The influence of *B. thetaiotaomicron* NCTC 10582 LPS on the adhesion molecule expression on HMEC-1. The influence of *B. thetaiotaomicron* LPSs and CPS on the adhesion molecule expression on endothelial cells (HMEC-1) was examined in ELISA test. Mouse monoclonal antibodies anti-human: ICAM-1, VCAM-1 and E-selectin were applied. Resting HMEC-1 and *E. coli* O55: B5 LPS were used as control. * $p<0.05$ (statistically significant result)

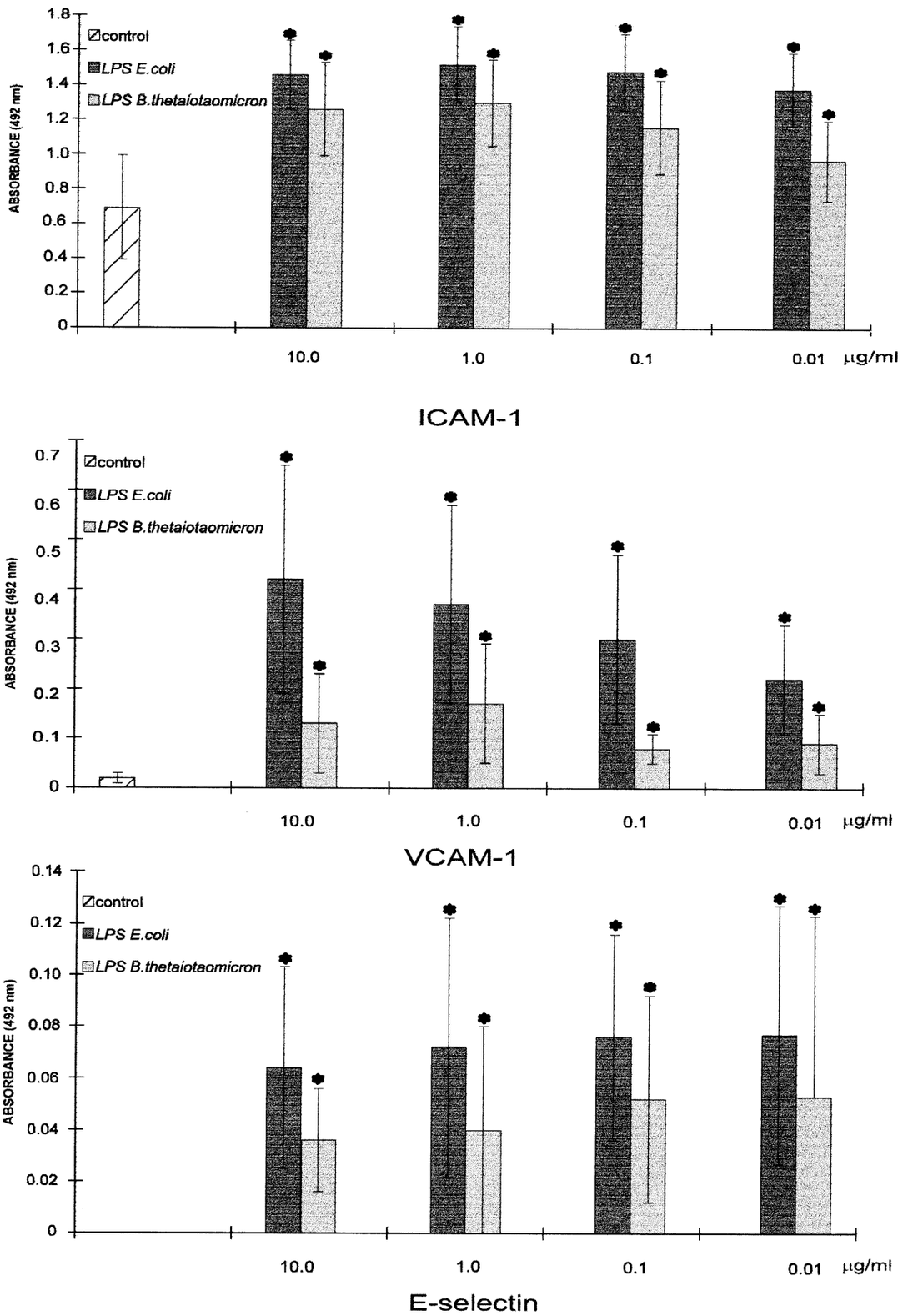

Fig. 2. The influence of *B. thetaiotaomicron* 312/85 LPS on the adhesion molecule expression on HMEC-1. Further information see under Fig. 1

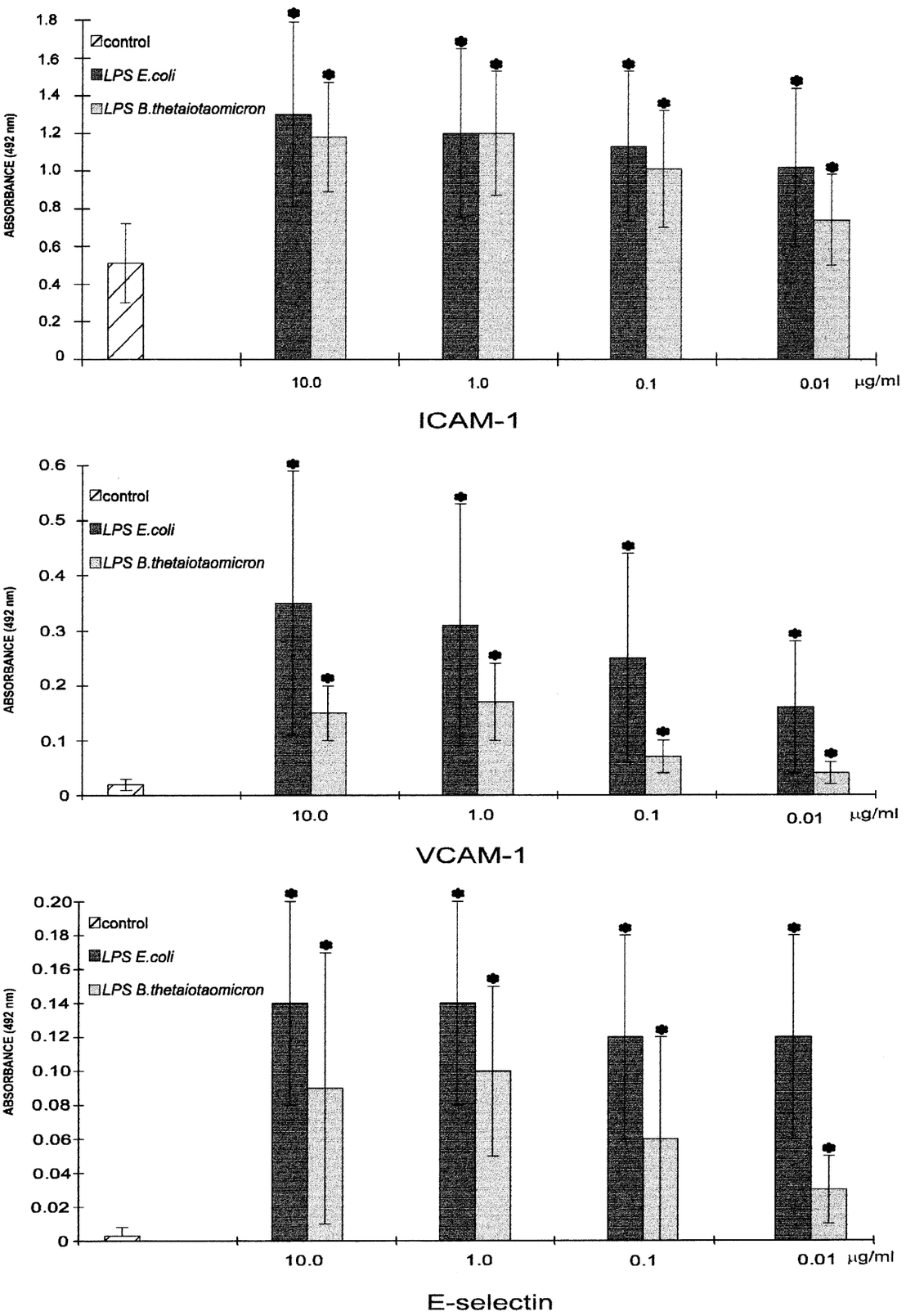

Fig. 3. The influence of *B. thetaiotaomicron* 9/18 LPS on the adhesion molecule expression on HMEC-1. Further information see under Fig. 1

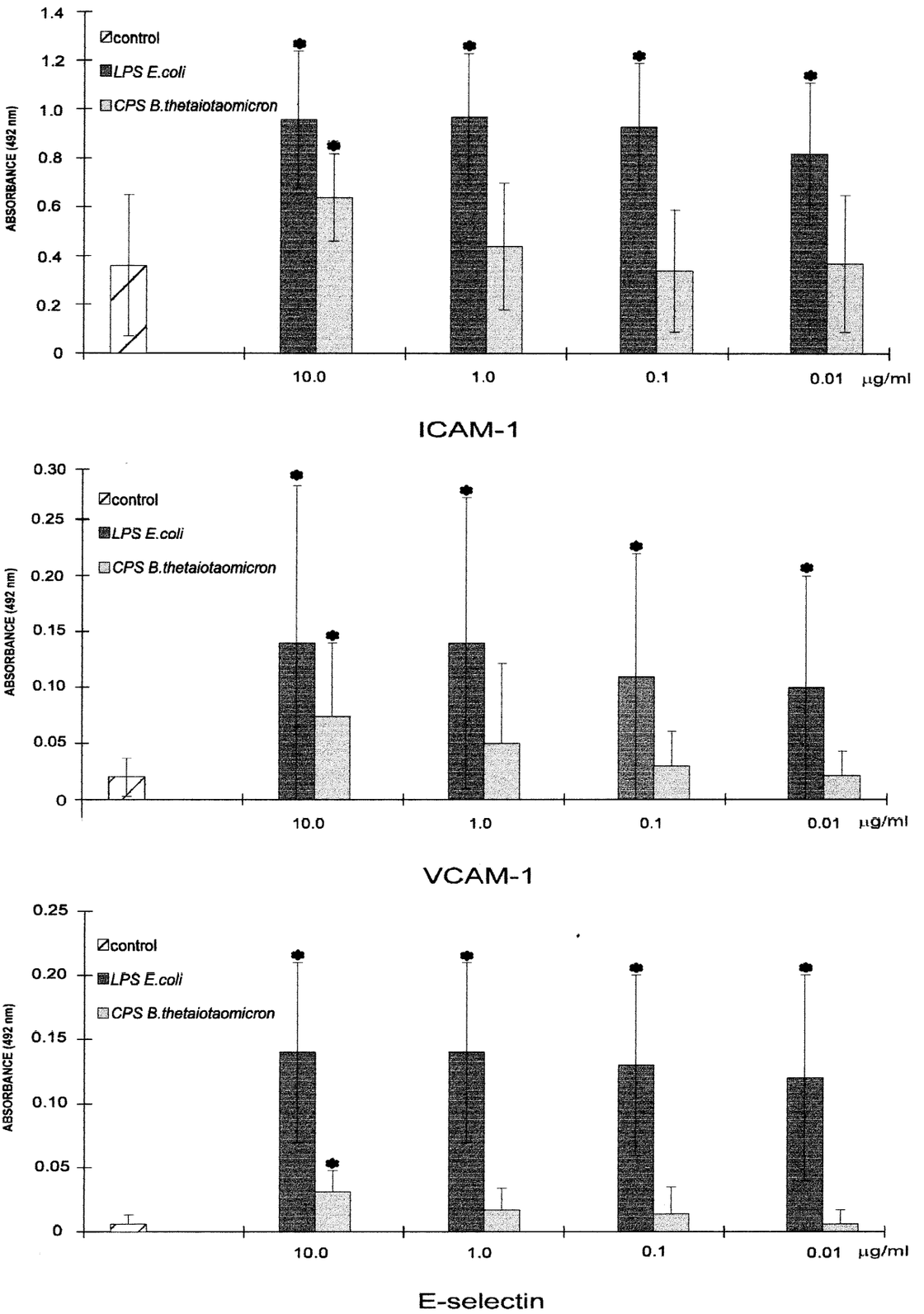

Fig. 4. The influence of *B. thetaiotaomicron* 312/85 CPS on the adhesion molecule expression on HMEC-1. Further information see under Fig. 1

caused a statistically significant induction of adhesion molecules. The highest upregulation of ICAM-1 is observed the concentration of 1.0 μg/ml and is slightly weaker than in the case of standard *E. coli* LPS. VCAM-1 is most efficiently stimulated by 1.0 μg/ml dose of LPS and E-selection by lower doses 0.1 and 0.01 μg/ml.

B. thetaiotaomicron 9/18 LPS as the stimulator of adhesion molecules

Figure 3 illustrates the ability of *B. thetaiotaomicron* 9/18 LPS to induce adhesion molecules on the endothelial cells. All results obtained with this antigen are statistically significant. The ICAM-1 upregulation ability of this LPS is comparable with that of *E. coli* LPS and even identical results are received for these two lipopolysaccharides at the concentration of 1.0 μg/ml. The strongest stimulation of VCAM-1 and E-selectin is obtained by 1.0 μg/ml dose of 9/18 LPS.

B. thetaiotaomicron 312/85 CPS as the stimulator of adhesion molecules

The results obtained for *B. thetaiotaomicron* 312/85 CPS are presented in Fig. 4. They are statistically significant only for the dose of 10.0 μg/ml of this antigen. CPS induces the expression of 3 examined adhesion molecules on the endothelial cells, but its stimulatory ability is considerably weaker than in the case of *E. coli* LPS and *B. thetaiotaomicron* LPS.

Discussion

Bacteroides fragilis group rods exist in a normal intestinal microflora of humans and animals. These Gram-negative anaerobic rods are most commonly isolated from clinical specimens. The gastrointestinal tract is often the source from which endogenous infection begins. BFG rods first of all cause abscesses, soft-tissue infections and bacteremia. Leukocytic infiltration, associated with infection and inflammation, depends on cell extravasation and trafficking towards the site of antigen. This process is regulated by adhesion molecules expressed on the endothelial cells, which are able to recognize endotoxin and respond to it. The vascular endothelial cells can be activated during infection and produce cytokines (IL-1, IL-6, IL-8) and other mediators of inflammation. LPS alone has a stimulatory activity, but activation by cytokines (IL-1, TNF) is most effective. Serum proteins that mediate stimulation of cells by LPS are: LPS-binding protein (LBP) and sCD14. LPS forms complexes with LBP and sCD14, LBP also enhances the binding of LPS to sCD14. The endothelial cells probably have a surface receptor for LPS/sCD14 complexes[14].

The ability of *B. thetaiotaomicron* LPS and CPS to induce adhesion molecule expression on the endothelial cells was investigated in our report. *B. thetaiotaomicron* lipopolysaccharides revealed the capacity to stimulate ICAM-1, VCAM-1 and E-selectin on the cells of endothelium. The activities of lipopolysaccharides obtained from 3 strains of different origin were similar, but lower than in the case of *E. coli* LPS. The lowered ability of endothelial ICAM-1, VCAM-1 and E-selectin stimulation by *B. thetaiotaomicron* products was proved with the direct method of adhesion molecule determination. The strongest stimulation was noted for the ICAM-1 adhesion molecule. In general, the strongest activations by *B. thetaiotaomicron* LPS were achieved at the concentrations of 10.0 and 1.0 μg/ml. The ability of CPS to induce expression of the adhesion molecules was weak. Statistically significant results were obtained only for 10.0 μg/ml dose of CPS, whereas in the case of LPS – for much lower dose 0.01 μg/ml.

We can conclude that following *B. thetaiotaomicron* LPS stimulation, the constitutive endothelial ICAM-1 expression is upregulated and expression of VCAM-1 and E-selectin is induced on HMEC-1. *B. thetaiotaomicron* CPS was found to induce the appearance of adhesion molecules markedly weaker than the LPS of this species. Both cell-surface antigens caused the strongest stimulation of the ICAM-1 adhesion molecule. *B. thetaiotaomicron* LPS had lower and CPS had much lower ability to upregulate endothelial ICAM-1 expression and to induce VCAM-1 and E-selectin expression in comparison to *E. coli* LPS. This lower endotoxic activity of *Bacteroides sp.* LPS depends probably upon the differences in lipid A structure. LPS of the genus *Bacteroides* rods contains fatty acids with longer carbon chains and its lipid component differs also in the phosphorylation and acylation patterns[12, 13, 42].

Upregulation and induction of adhesion molecule expression on the endothelial cells should be considered as the novel immunobiological activities of *B. thetaiotaomicron* cell-surface antigens LPS and CPS. Out of these two surface *B. thetaiotaomicron* antigens, LPS is considerably more active as the inducer of adhesion molecules on the endothelial cells.

Acknowledgment. This work was supported by grant 4-P05B 012 11 from the State Committee for Scientific Research (KBN). Authors are grateful to Prof. Wiesław Kaca (Łódź, Poland) for purification of lipopolysaccharides.

References

1. BABB J. L. and CUMMINS C. S. (1978): Encapsulation of *Bacteroides* species. Infect. Immun., **19**, 1088–1091.
2. BECKMANN I., MEISEL-MIKOŁAJCZYK F., LESZCZYŃSKI P., BROOIJMANS M. and WALLENBURG H. C. S. (1993): Endotoxin-induced fetal growth retardation in the pregnant guinea pig. Am. J. Obstet. Gynecol., **168**, 714–718.
3. BECKMANN I., MEISEL-MIKOŁAJCZYK F., LOTGERING F. K. and WALLENBURG H. C. S. (1996): Tumor necrosis factor-alpha in response to endotoxin administration in the pregnant guinea pig. Am. J. Obstet. Gynecol., **175**, 218–221.
4. BEERENS H., WATTRE P., SHINJO T. and ROMOND C. (1971): Premiers resultats d'un essai de classification sérologique de 131 souches de *Bacteroides* du groupe *fragilis* (*Eggerthella*). Ann. Inst. Pasteur, **121**, 187–198.
5. CARLOS T. M. and HARLAN J. M. (1994): Leukocyte-endothelial adhesion molecules. Blood, **84**, 2068–2101.
6. DOWELL V. R. JR. and HAWKINS T. M. (1974): Laboratory methods in anaerobic bacteriology. CDC Lab. Manual, D. E. H. W. Atlanta.
7. GMEINER J. (1975): The isolation of two different lipopolysaccharide fractions from various *Proteus mirabilis* strains. Eur. J. Biochem., **58**, 621–626.
8. GORBACH S. L. and BARTLETT J. G. (1974): Anaerobic infections. N. Engl. J. Med., **290**, 1177–1184.
9. GÓRSKI A. (1994): The role of cell adhesion molecules in immunopathology. Immunol. Today, **15**, 251–255.
10. HÖFFLER U. (1987): Anaerobic bacteria in human medicine. Labmedica, **4**, 21–26.
11. HOFSTAD T. (1984): Pathogenicity of anaerobic Gram-negative rods: possible mechanisms. Rev. Infect. Dis., **6**, 189–199.
12. HOFSTAD T. (1992): Virulence factors in anaerobic bacteria. Eur. J. Clin. Microbiol. Infect. Dis., **11**, 1044–1048.
13. HOFSTAD T., SKAUG N. and SVEEN K. (1993): Stimulation of B lymphocytes by lipopolysaccharides from anaerobic bacteria. Clin. Infect. Dis., **16** (suppl. 4), S200–202.
14. HOLST O., ULMER A. J., BRADE H., FLAD H.-D. and RIETSCHEL E. T. (1996): Biochemistry and cell biology of bacterial endotoxins. FEMS Immunol. Med. Microbiol., **16**, 83–104.
15. JANIAK M. K. (1996): Role of cell adhesion receptors in tumor metastasis. Centr. Eur. J. Immunol., **21** (suppl. 1), S63–S83.
16. KASPER D. L. (1976): The polysaccharide capsule of *Bacteroides fragilis* subspecies *fragilis*. Immunochemical and morphologic definition. J. Infect. Dis., **133**, 79–87.
17. KASPER D. L. (1976): Chemical and biological characterization of the lipopolysaccharide of *Bacteroides fragilis* subspecies *fragilis*. J. Infect. Dis., **134**, 59–66.
18. KASPER D. L. (1979): Virulence factors of anaerobic bacteria: an overview. Rev. Infect. Dis., **1**, 246–247.
19. KASPER D. L., HAYES M. E., REINAP B. G., CRAFT F. O., ONDERDONK A. B. and POLK B. F. (1977): Isolation and identification of encapsulated strains of *Bacteroides fragilis*. J. Infect. Dis., **136**, 75–81.
20. LASSALLE P., GOSSET P., DELNESTE Y., TSICOPOULOS A., CAPRON A., JOSEPH M. and TONNEL A. B. (1993): Modulation of adhesion molecule expression on endothelial cells during the late asthmatic reaction: role of macrophage-derived tumour necrosis factor-alpha. Clin. Exp. Immunol., **94**, 105–110.
21. LEE K. H., LAWLEY T. J., XU Y. and SWERLICK R. A. (1992): VCAM-1-, ELAM-1-, and ICAM-1-independent adhesion of melanoma cells to cultured human dermal microvascular endothelial cells. J. Invest. Dermatol., **98**, 79–85.
22. LINDBERG A. A., WEINTRAUB A., ZÄHRINGER U. and RIETSCHEL E. T. (1990): Structure-activity relationship in lipopolysaccharides of *B. fragilis*. Rev. Infect. Dis., **12** (suppl. 21), 133–141.
23. LOPPNOW H., BRADE H. and DURRBAUM I. (1988): Interleukin-1 induction-capacity of defined lipopolysaccharide partial structures. J. Immunol., **142**, 3229–3238.
24. MEISEL-MIKOŁAJCZYK F. and GAŁKOWSKA H. (1976): The immunochemical investigations of six *Bacteroides* serotypes. Acta Microbiol. Pol., **25**, 389–399.
25. MEISEL-MIKOŁAJCZYK F., ROKOSZ A. and KACA W. (1989): The cell-surface antigens of *Bacteroides thetaiotaomicron*. Eur. J. Epidemiol., **5**, 486–496.
26. MEISEL-MIKOŁAJCZYK F., ROKOSZ A. and ROUYAN G. S. (1997): Influence of clindamycin on endotoxin and enterotoxin release from *Bacteroides fragilis*. Alpe Adria Microbiol. J., **6**, 113–117.
27. MEISEL-MIKOŁAJCZYK F., ROUYAN G. S., DYBOWSKA B. and GÓRSKI A. (1993): Human T cell adhesion to endothelium stimulated by membrane components extracted from strains of *Bacteroides vulgatus* (member of *B. fragilis* group). Arch. Immunol. Ther. Exp., **41**, 129–131.
28. MEISEL-MIKOŁAJCZYK F., SAWICKA A. and GAŁKOWSKA H. (1977): Local Shwartzman reaction (LSR) evoked by endotoxin of various *Bacteroides fragilis* subspecies. Bull. Pol. Acad. Sci. (Biol.), **25**, 577–582.
29. MIKOŁAJCZYK E., ROKOSZ A., SAWICKA-GRZELAK A. and MEISEL-MIKOŁAJCZYK F. (1981): The toxicity of *Bacteroides fragilis* endotoxins before and after removal of nucleic compounds. Acta Microbiol. Pol., **30**, 97–100.
30. MOORE W. E. C., CATO E. P. and HOLDEMAN L. V. (1978): Some current topics in intestinal bacteriology. Am. J. Clin. Nutr., **31**, S33–S42.
31. ONDERDONK A. B., KASPER D. L., CISNEROS R. L. and BARTLETT J. G. (1977): The capsular polysaccharide of *Bacteroides fragilis* as a virulence factor. Comparison of the pathogenic potential of encapsulated and unencapsulated strains. J. Infect. Dis., **136**, 82–89.
32. POXTON I. R. and IP M. K. Y. (1981): The cell-surface antigens of *Bacteroides vulgatus*. J. Gen. Microbiol., **126**, 103–109.
33. ROKOSZ A., GRZYBOWSKI J., KACA W. and MEISEL-MIKOŁAJCZYK F. (1989): The cell-surface components of *Bacteroides thetaiotaomicron* as coating antigens in ELISA test. Acta Microbiol. Pol., **38**, 285–291.
34. ROKOSZ A., KRUSZEWSKA D., RUMIN W. and MEISEL-MIKOŁAJCZYK F. (1990): The toxicity of *Bacteroides thetaiotaomicron* cell-surface antigens to chicken embryos. Acta Microbiol. Pol., **39**, 211–214.
35. ROKOSZ A., MARCINIAK-RUSEK A., ALEKSANDROWICZ J. and MEISEL-MIKOŁAJCZYK F. (1997): LAL test for quantitative determination of *Bacteroides fragilis* endotoxin. Med. Dośw. Mikrobiol., **49**, 157–164.
36. ROKOSZ A. and MEISEL-MIKOŁAJCZYK F. (1995): Application of the cell-surface antigens of *Bacteroides thetaiotaomicron* in the passive hemagglutination test and in the inhibition of passive hemagglutination. Med. Dośw. Mikrobiol., **47**, 189–195.
37. SIMON G. L. and GORBACH S. L. (1984): Intestinal health and disease. Gastroenterology, **86**, 174–193.
38. SPRINGER T. A. (1990): Adhesion receptors of the immune system. Nature, **346**, 425–434.

39. STROHM H., PAYNE C. M. and RYAN K. J. (1983): Demonstration of *Bacteroides* capsules by light microscopy and ultrastructural cytochemistry. Am. J. Clin. Pathol., **79**, 591–597.
40. SVEEN K. (1977): The capacity of lipopolysaccharides from *Bacteroides, Fusobacterium* and *Veillonella* to produce skin inflammation and the local and generalized Shwartzman reaction in rabbits. J. Periodontal Res., **12**, 340–350.
41. SWERLICK R. A., LEE K. H., LI L.-J., SEPP N. T., CAUGHMAN S. W. and LAWLEY T. J. (1992): Regulation of vascular cell adhesion molecule 1 on human dermal microvascular endothelial cells. J. Immunol., **149**, 698–705.
42. WEINTRAUB A., ZÄHRINGER U., WOLLENWEBER H. W., SEYDEL U. and RIETSCHEL E. T. (1989): Structural characterization of the lipid A component of *Bacteroides fragilis* strain NCTC 9343 lipopolysaccharide. Eur. J. Biochem., **183**, 425–431.
43. WERNER H. (1974): Differentiation and medical importance of saccharolytic intestinal *Bacteroides.* Drug Res., **24**, 340–343.
44. WESTPHAL O. and JANN K. (1965): Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem., **5**, 83–91.
45. WOLLENWEBER H. W., RIETSCHEL E. T., HOFSTAD T., WEINTRAUB A. and LINDBERG A. A. (1980): Nature, type of linkage, quantity and absolute configuration of (3-hydroxy) fatty acids in lipopolysaccharides from *Bacteroides fragilis* NCTC 9343 and related strains. J. Bacteriol., **144**, 898–903.

Received in July 1998
Accepted in November 1998