Monocytes That Have Ingested *Yersinia enterocolitica* Serotype O:3 Acquire Enhanced Capacity To Bind to Nonstimulated Vascular Endothelial Cells via P-Selectin

MAARIT WUORELA,1 SAMI TOHKA,2 KAISA GRANFORS,1 AND SIRPA JALKANEN1,2*

National Public Health Institute1 and MediCity Research Laboratory, University of Turku,2 Turku, Finland

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Reactive arthritis is usually a self-limiting polyarthritis which develops after certain gastrointestinal or urogenital infections. Microbial antigens found in the inflamed joints are thought to play a key role in the development of this disease. It is not known how antigens of the pathogenic organisms migrate from the mucosal tissues into the joints. The data presented here show that mononuclear phagocytes which mediate the dissemination of several intracellular pathogens acquire an enhanced capacity to bind to nonstimulated vascular endothelial cells after phagocytosis of *Yersinia enterocolitica* O:3, one of the causative organisms of reactive arthritis. The increased binding to previously nonstimulated endothelial cells was mediated by P-selectin, whose translocation to the endothelial cell surface was induced by monocytes with intracellular *Yersinia* bacteria. These results suggest that mononuclear phagocytes may be responsible for the dissemination of bacterial antigens and the initiation of the joint inflammation in reactive arthritis.

**MATERIALS AND METHODS**

**Bacteria.** The strain of *Y. enterocolitica* serotype O:3 used (4147/83) was a stool isolate from a patient developing reactive arthritis as a result of infection. The strain contains a virulence-associated 72-kb plasmid (9). The presence or absence of the virulence plasmid of *Yersinia* bacteria was verified by autoradiography (28). A plasmid-cured derivative of *Y. enterocolitica* O:3 was obtained by cultivating the bacteria on a magnesium-oxide agar (45). As a control bacterium we used *Streptococcus pyogenes* (strain 8184 from the American Type Culture Collection [ATCC]). *S. pyogenes* was chosen as a control bacterium because it can cause postinfection joint complications which are not linked to HLA-B27. *S. pyogenes* does not contain lipopolysaccharide (LPS). Stock cultures were maintained at 4°C in 20% (vol/vol) glycerol-Trypticase soy broth. The *Yersinia* bacteria were grown in RPMI 1640 medium mimicking the extracellular conditions or in Luria-Bertani broth. The resulting bacterial cultures were suspended in saline, harvested by centrifugation (20 min, 3,000 × g), and washed three times in saline. The bacteria were killed with heat (1 h, 100°C) and stored in phosphate-buffered saline (PBS) at 4°C. *S. pyogenes* was grown on blood agar plates for 2 days. Enteroinvasive *Escherichia coli* (strain RHE-3459 from the Central Public Health Laboratory, London, United Kingdom) was grown in Luria-Bertani broth like *Y. enterocolitica*. The resulting bacterial cultures were harvested, killed with heat, and suspended in saline.

**Preparation of LPS.** *Y. enterocolitica* O:3 was cultivated in nutrient broth at room temperature overnight. The LPS extraction with hot phenol-water was carried out by the method of Westphal et al. (44) as modified by Hurvell (19). After treatment with protease K (100 μg/ml) (Sigma, St. Louis, Mo.), RNase (100 μg/ml) (Sigma, St. Louis, Mo.), and DNase (100 μg/ml) (Boehringer), the LPS preparation was free of contaminating proteins and nucleic acids. *E. coli* LPS (O55:B5) was purchased from Difco Laboratories (Detroit, Mich.).

**Monocyte isolation.** Monocytes from healthy blood donors (Finnish Red Cross, Turku, Finland) were isolated as described previously (45). Briefly, human peripheral blood mononuclear cells were isolated by ficoll-Paque gradient centrifugation (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), and monocytes were allowed to adhere to plastic tissue culture chambers precoated with human AB serum (Finnish Red Cross) for 1 h. Thereafter, nonadherent cells were washed off. The purity of monocyte populations was ≥95% as analyzed by using morphological characteristics and, in several samples, by also using immunofluorescence staining of the monocyte-specific CD14.

**Incubation with bacteria, latex beads, or LPS.** Monocytes were allowed to phagocytose the bacteria or latex particles (Bacto latex 0.81; Difco) in RPMI medium supplemented with 10% AB serum for 1 h, and then extracellular bacteria were washed off. We used about 200 *Y. enterocolitica* O:3 or 20 *S. pyogenes* or *E. coli* bacteria per monocyte. Lower doses of *S. pyogenes* or *E. coli* were used because these bacteria were more toxic to monocytes and doses higher than 20 bacteria per monocyte affected the viability of the cells, especially after prolonged incubation periods. The number of heat-killed bacteria which were phagocytosed by the monocytes was studied by the indirect immunofluorescence technique. Briefly, cytocentrifuge preparations containing monocytes which had been phagocytosed were stained with fluorescein isothiocyanate-conjugated goat anti-*Y. enterocolitica* O:3 or goat anti-*S. pyogenes* or *E. coli* antibodies, and subjected to flow cytometry analysis. The number of heat-killed bacteria was determined by green laser fluorescence analysis.

**Incubation of monocytes with LPS.** Monocytes were allowed to incubate with LPS for 24 h. It has been shown that LPS binding to monocytes is increased after 18 h (3). The purity of monocyte populations was ≥95% as analyzed by using morphological characteristics and, in several samples, by also using immunofluorescence staining of the monocyte-specific CD14.

**Adhesion assays.** Adhesion assays were performed at 37°C using a 96-well plate. In some experiments, bacterial LPS was generated in vitro using chemical procedures (35) and purified by gel filtration (agarose) and dialysis. Monocytes were allowed to adhere to plastic tissue culture chambers precoated with human AB serum (Finnish Red Cross) for 20 min. The bound monocytes were detached by 5% (vol/vol) nonionic detergent (Triton X-100). The purity of monocyte populations was ≥95% as analyzed by using morphological characteristics and, in several samples, by also using immunofluorescence staining of the monocyte-specific CD14.
endothelial cells (HUVEC) were cultured on eight-well chamber slides (Nunc, Roskilde, Denmark). Isolated endothelial cells were overlaid with 2 mL of 0.1% gelatin (Sigma) to induce P-selectin induction and immunostaining. Human umbilical vein endothelial cells (HUVEC) were obtained by collagenase digestion of umbilical cord veins by the method of Jaffe et al. (21). Detached endothelial cells were cultured in RPMI 1640 medium (Gibco, Paisley, United Kingdom) supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine (Biological Industries, Kibbutz Beit Hерennek, Israel), 50 μg of gentamicin (G-mycin; Orion, Espoo, Finland) per mL, and 100 μg of streptomycin and 100 U of penicillin (Biological Industries) per mL in gelatin-coated plastic cell culture flasks at 37°C in 5% CO₂. The cells grew to confluence in 3 to 5 days, at which stage they were detached with trypsin-EDTA (Gibco).

**Endothelial cells.** Human umbilical vein endothelial cells (HUVEC) were cultured in 926 endothelial cells were grown on coverslips in the lower chambers of a Transwell system or directly on the endothelial cells. The monocytes were removed after 20 min of incubation. The endothelial cells were stained with an anti-P-selectin MAB (negative control MAB) and incubated on ice at 4°C for 20 min. After three washes with PBS-BSA, fluoresceinated second-stage immunoglobulin (Ig) (Sigma Chemical Co.) was added. After 20 min of incubation, the chambers were washed again three times with PBS-BSA and fixed with 1% paraformaldehyde (Merck) in PBS. The slides were mounted with PBS-glycerol (1:9 [vol/vol]) which contained 1 mg of 4',6-diamidino-2-phenylindole (Sigma) per mL and studied under a Dialux 20 fluorescence microscope (Leitz, Wetzlar, Germany).

**Adhesion to endothelial cells.** Endothelial cells were cultured in 926 well-tissue culture plates and allowed to reach confluence. Results with HUVEC and EAHy 926 cells were comparable. In the studies of the role of β1-integrins, only HUVEC were used because only a small population of EAHy 926 cells expressed VCAM-1 after stimulation with TNF-α or IL-4. The increased expression of VCAM-1 and ICAM-1 on stimulated endothelial cells was confirmed by immunofluorescence. Monocytes incubated with Yersinia bacteria and control monocytes were labeled with 15 μg of ics-carboxybortec acidoxymuorescein (BCECF-

**Immunofluorescence staining and flow cytometry.** Cells for flow cytometry were stained by using a double immunofluorescence technique as described previously (45). The monoclonal antibodies (MABs) used in this study are listed in Table 1. Analyses were performed with a FACScan flow cytometer. Monocytes were gated according to their size and granularity. This correlated well with their expression of monocyte-specific antigen CD14. Routinely, 10,000 cells were analyzed per sample. During the incubation time, the monocytes matured. Monocytes incubated with bacteria were always compared to cells which had been incubated in the same way but without any exogenous stimuli.

**Adhesion of monocytes to cultured endothelial cells.** Results are expressed as mean relative adherence ratio ± standard errors of the means. Incubation time refers to the length of time that the monocytes were allowed to process the bacteria. Black bars and white bars indicate the binding of monocytes to nonstimulated endothelial cells and to endothelial cells which have been stimulated with TNF-α, respectively. The binding of adherent control monocytes not incubated with bacteria was given the value 1.0. An increase in relative binding from 1.0 to 1.5 means that the number of adhering monocytes increases by 50%. The increased binding by Yersinia-treated monocytes was studied with monocytes of 14 individuals in four separate experiments. * P < 0.05; ** P < 0.005.

![FIG. 1. Phagocytosis and processing of Y. enterocolitica (Ye) alters the binding of monocytes to cultured endothelial cells. Results are expressed as mean relative adherence ratio ± standard errors of the means.](http://iai.asm.org/).
Stained without fixation by using immunofluorescence. Cytes were washed away. P-selectin molecules on adherent endothelial cells were treated (Ba) or control (Bb) monocytes for 20 min, and then the monocytes with Yersinia but control monocytes do not. EAhy 926 endothelial cells were incubated with Yersinia bacteria induce the expression of P-selectin on cultured endothelial cells, after 1 day of incubation (Fig. 1). The correlation to the enhanced binding of monocytes to nonstimulated endothelial cells was 40% ± 15%. The results are expressed as relative binding ratios. The control binding was arbitrarily given the value 1.0.

Matrix adhesion assays. Ninety-six-well microtiter plates were incubated with 10 µg of human plasma fibronectin (Sigma) per ml or with the chymotryptic fragments FN-120 (cell binding domain) or FN-40 (heparin II binding domain) (Calbiochem, La Jolla, Calif.) in PBS at 37°C for 2 h and then with 1% BSA–PBS at 37°C for 1 h. Next, unbound cells were removed by washing the plates twice with RPMI 1640 medium, and the proportion of the fluorescence of the monocytes bound to each well was measured with the fluorometer. The percentage of monocytes binding to endothelial cells was calculated from the input fluorescence value of each well. In individual assays the percentage of monocytes binding to endothelial cells was 40% ± 22%. The results are expressed as relative binding ratios. The control binding to 1% gelatin was arbitrarily given the value 1.0.

Statistical analysis. Statistically significant differences between monocytes incubated with Yersinia bacteria and control monocytes were determined by using a two-tailed paired Student t test (42).

RESULTS

The role of P-selectin in adhesion of monocytes incubated with Yersinia bacteria. Numerous phagocytes containing antigens of arthritis-triggering microbes are found in inflamed joints of patients with reactive arthritis. We wanted to see whether phagocytosis and processing of one of the arthritis-triggering microbes, Y. enterocolitica (Ye) O:3 bacteria and incubated for 1 day. The cells were stained with two different MAbs against PSGL-1 (PL-1 and PL-2) or with a negative (neg.) control MAb. MFI, mean fluorescence intensity.

AM (Lambda Fluorezenztechnologie, Graz, Austria) per ml at 37°C for 25 min. Thereafter, the monocytes were washed two times and incubated with human gamma globulin (20 µg/ml; Finnish Red Cross, Helsinki, Finland) for 20 min to block the Fc receptors and washed again two times. The monocytes were then incubated with saturating concentrations of function-blocking or negative control MAbs for 20 min. A total of 2 × 10⁶ monocytes were added to each well in 200 µl of 10% fetal calf serum–RPMI 1640 and allowed to adhere at 37°C for 20 min. Blocking antibodies were present during the adhesion. The fluorescence of cells added to each well was measured with a Fluoroskan II fluorometer (Labsystems, Helsinki, Finland). Next, unbound cells were removed by washing the plates twice with RPMI 1640 medium, and the proportion of the fluorescence of the monocytes bound to each well was measured with the fluorometer. The percentage of monocytes binding to endothelial cells was calculated from the input fluorescence value of each well. In individual assays the percentage of monocytes binding to endothelial cells was 40% ± 15%. The results are expressed as relative binding ratios. The control binding was arbitrarily given the value 1.0.

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Statistical analysis. Statistically significant differences between monocytes incubated with Yersinia bacteria and control monocytes were determined by using a two-tailed paired Student t test (42).
MAb against P-selectin blocks the binding of monocytes incubated with \textit{Yersinia} bacteria to endothelial cells. The ability of a MAb against a functional epitope of P-selectin to inhibit the adhesion of monocytes incubated with \textit{Yersinia} bacteria was demonstrated. Furthermore, monocytes which had processed \textit{Y. enterocolitica} O:3 for 1 day in vitro also mobilized P-selectin from the intracellular storage granules to the surfaces of endothelial cells (Fig. 2). The effect was dose dependent, since the inhibition of adhesion by the MAb against P-selectin and the intensity of the staining of endothelial cells increased gradually when more monocytes incubated with \textit{Yersinia} bacteria were added to the endothelial cell cultures (not shown). P-selectin upregulation was seen with both virulence plasmid-positive and -negative \textit{Y. enterocolitica} O:3 bacteria, indicating that the factors responsible for the adhesion were chromosomally encoded. P-selectin upregulation was most probably mediated by short-lived soluble products. The participation of long-lived mediators was excluded by the fact that conditioned medium of the monocytes did not induce the expression of P-selectin. Direct cell-cell contacts were not necessary, because P-selectin expression on endothelial cells was induced when \textit{Yersinia}-treated monocytes were incubated on grids above the endothelial cells growing in the lower wells of the Transwell cell culture system (not shown). Monocytes incubated with inert latex particles and enteroinvasive \textit{E. coli} did not have the capacity to induce P-selectin-mediated adhesion (data not shown). We also studied the expression of P-selectin glycoprotein ligand-1 (PSGL-1) on monocytes incubated with \textit{Y. enterocolitica} O:3 bacteria by using two different MAbs. The PSGL-1 expression on monocytes was not modified by \textit{Yersinia} bacteria.

**FIG. 4.** Changes in the expression of adhesion molecules on monocytes incubated with \textit{Y. enterocolitica} O:3, \textit{S. pyogenes}, or LPS of \textit{Y. enterocolitica} O:3. (A) \textit{Y. enterocolitica} O:3 downregulates the expression of CD11a but not the expression of \(\beta_1\)-integrins VLA-\(\alpha_4\) and VLA-\(\alpha_5\). (B) The effect of \textit{Y. enterocolitica} on the expression of \(\beta_2\)-integrins seemed to be at least partially mediated by LPS. (C) \textit{S. pyogenes} was able to slightly reduce the expression of \(\alpha_4\) and \(\alpha_5\) integrins. Incubation time refers to the length of time that the monocytes were allowed to process the bacteria. Results are counted as net mean fluorescence intensity (MFI) values (negative control value subtracted from the experimental value) ± standard errors of the means and expressed as relative mean fluorescence intensities, in which the mean fluorescence intensity of the control monocytes was given the value 1.0. *, \(P \leq 0.05\); **, \(P < 0.005\).
of maximal binding to bacteria. Results after each incubation time are expressed as a mean percentage of maximal binding.

Incubation time refers to the length of time that the monocytes were allowed to process the bacteria. Results after each incubation time are expressed as a mean percentage of maximal binding ± standard error of the mean, in which the number of adherent monocytes in the presence of the negative control antibody defines 100% binding. *, P < 0.05; **, P < 0.005; ***, P < 0.0005.

(Fig. 3). It is, however, possible that phagocytosis and processing of Y. enterocolitica may have affected the functionally active population of PSGL-1 molecules.

Expression and function of other adhesion molecules known to contribute to the monocyte binding in arthritis. The binding of monocytes incubated with Y. enterocolitica O:3 to cytokine-activated endothelial cells was less than the binding of control monocytes for 3 days after Yersinia bacteria were phagocytosed (Fig. 1). In searching for reasons for this unexpected finding, we studied the expression of adhesion molecules which have been shown to be important in the binding of monocytes to cytokine-activated endothelial cells. We found that the expression of the CD11a molecule, which is important in mediating cell-cell and cell-matrix interactions, was increased after the induction of P-selectin expression increased after the incubation time refers to the length of time that the monocytes were allowed to process the bacteria. Results after each incubation time are expressed as a mean percentage of maximal binding ± standard error of the mean, in which the number of adherent monocytes in the presence of the negative control antibody defines 100% binding. *, P < 0.05; **, P < 0.005; ***, P < 0.0005.

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S. pyogenes was able to reduce to some extent the expression of the α4 and α5 integrins. Differences in the capacities of Y. enterocolitica O:3 and S. pyogenes to change the expression of adhesion molecules led us to treat monocytes with isolated LPS of Y. enterocolitica O:3. We wanted to see whether LPS, which is an important component of the outer surface of Yersinia bacteria but is not present in S. pyogenes, was the cause of the observed difference. The effects of Y. enterocolitica O:3 could also be obtained with isolated LPS (Fig. 4). Interestingly, isolated LPS also effectively inhibited the expression of α4 and α5.

Cell-matrix interactions are important in regulating recruitment and migration of cells in tissues. We studied the binding of monocytes incubated with Yersinia bacteria to the extracellular matrix molecule fibronectin, which is a ligand for VLA-4 and VLA-5. Monocytes which had phagocytosed Yersinia bacteria bound more avidly than the control monocytes to fibronectin after 1 h of incubation (P < 0.05). This was the time of minimal endothelial cell binding (Fig. 1). Statistically, the fibronectin binding was mediated mainly by the heparin II binding domain of the molecule (Fig. 6).

Role of HLA-B27. Thirty-two percent of the samples studied (18 of 75) were from HLA-B27-positive individuals, but they did not differ from the HLA-B27-negative ones. Therefore, the data from the HLA-B27-positive and the HLA-B27-negative samples are combined. The growth conditions of the bacteria also did not have any effect on the results (data not shown).

DISCUSSION

We observed that monocytes incubated with Yersinia bacteria acquired the capacity to bind to nonactivated vascular endothelial cells via P-selectin. The inhibition observed with a MAB against P-selectin was usually 30 to 40%. Greater inhibition would not be expected, because we used human monocytes which adhere to endothelial cells by using several different adhesion molecules. This inhibition means that one-third of the monocytes which had phagocytosed Y. enterocolitica O:3 used P-selectin in binding to previously nonstimulated endothelial cells. Moreover, in general the sum of the inhibition percentages of individual antibodies may well exceed 100% due to the multistep nature of leukocyte-endothelial cell interactions. The role of P-selectin in binding of Yersinia-treated monocytes is further strengthened by our unpublished observations showing that monocytes which have phagocytosed Y. enterocolitica O:3 but not control monocytes roll on nonstimulated endothelial cells.

The induction of P-selectin expression increased after the
monocytes incubated with *Yersinia* bacteria had been in contact with the endothelial cells for only 20 min. This suggests that the increase of P-selectin expression could not depend on de novo protein synthesis. Rather, P-selectin was rapidly translocated to the cell surface from intracellular storage granules, the Wei- bEL-PaLADE bodies, of endothelial cells (3, 29). Such a rapid P-selectin expression is induced by, for example, histamine, thrombin, and oxygen radicals (17, 31, 33). The results of this study suggest that in our system certain short-lived soluble mediators which were produced by monocytes incubated with *Yersinia* bacteria were responsible for the rapid mobilization of P-selectin. LPS could not have any major role, because entero- invasive *E. coli* bacteria or LPS isolated from *Y. enterocolitica* O:3 or *E. coli* could not increase binding of monocytes to non- stimulated endothelial cells. Experiments clarifying the mechan- isms of induction of P-selectin expression are in progress.

Numerous phagocytes with antigens of the arthritis-trigger- ing organisms have been found in the peripheral blood of patients with reactive arthritis (12). This shows that in reactive arthritis phagocytes are able to leave the gut and enter the peripheral circulation via lymphatics or retrogradely through the vascular wall (25, 35). Other factors, like the specific prop- erties of synovial vessels (40) and the adhesion molecules re- sponsible for synovium-specific homing (24, 36), will contribute to the guiding of the monocytes from the mucosal areas into the joints. We observed that the change in the adhesion ca- pacity appeared relatively soon after monocytes had phagocy- tosed the bacteria. This is well in line with animal models of *Yersinia*-induced arthritis, where antigens of the triggering mi- crobes can be found in the joints already on the third day after the primary infection (14). In patients with reactive arthritis, bacterial antigens have been found in the first synovial fluid samples taken from the inflamed joints, which means that bacterial fragments are in the joints in large quantities already 1 week after the onset of the disease (11). A small number of phagocytes probably have entered the joints long before that.

The capacity of bacteria to induce the binding of monocytes to endothelial cells seems to be at least to some extent microbe specific, because monocytes incubated with *Y. enterocolitica* showed increased binding to nonstimulated endothelial cells but monocytes incubated with enteroinvasive *E. coli* did not. On the other hand, the expression of β2-integrins and conse- quently the adhesion of monocytes incubated with *Yersinia* bacteria to cytokine-stimulated endothelial cells expressing ICAM-1 was reduced. This suggests that different microbes can induce the binding of cells by different adhesion molecules. Previous studies have shown that *S. pneumoniae* can induce a CD18-independent emigration of polymorphonuclear leuko- cytes (PMN) into the peritoneum and lungs but that *E. coli* does not do this. This non-CD18-dependent mechanism of PMN emigration is augmented by the presence of macro- phages (30). Mediators secreted by the macrophages in certain organs were suggested to induce PMN adherence by a CD18- independent mechanism (7), but the adhesion molecules in- volved in this CD18-independent pathway have not been char- acterized.

We monitored the usage of adhesion molecules for 1 week, and the situation may change thereafter. Most of the studies concerning involvement of adhesion molecules mediating leuko- cyte migration into the joints in humans have been con- ducted with already chronically inflamed synovium (15) and are not illustrative of the primary events. On the other hand, all molecules mediating later interactions may not participate in our experimental system. MAbs against P-selectin have been shown to block almost completely the binding of human gut- derived (37) and peripheral blood monocytes (15) to chroni- cally inflamed synovium. This shows that the crucial role of P-selectin in mediating adhesion of monocytes to specialized synovial endothelial cells is even more important in vivo than in our experimental in vitro system. In addition to monocytes, P-selectin is also used by Th1 cells, the main T-lymphocyte subtype present in inflamed joints of patients with reactive arthritis, in binding to synovium (2, 27).

Our results show for the first time that bacteria can change the adhesion of human monocytes to vascular endothelial cells via a certain adhesion molecule, P-selectin. This may have an essential role in initiating and maintaining the arthritis and may even open new possibilities for prevention and treatment of both acute and chronic forms of joint inflammation. Further studies will reveal whether similar changes in the adhesion also operate in other diseases, such as atherosclerosis, in which the recruitment of mononuclear phagocytes has a role in the pathogenesis and a microbial etiology has been suggested.

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REFERENCES


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