RAPID COMMUNICATION

Lectin-Like Cell Adhesion Molecule 1 Mediates Leukocyte Rolling in Mesentric Venules In Vivo

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During the inflammatory response, granulocytes and other leukocytes adhere to and emigrate from small venules. Before firm attachment, leukocytes are observed rolling slowly along the endothelium in venules of most tissues accessible to intravital microscopy. The molecular mechanism underlying this early type of leukocyte-endothelial interaction is unknown. Leukocyte rolling was investigated in venules (diameter, 40 μm) of the exposed rat mesentery. Micro-infusion of a recombinant soluble chimera (LEC-IgG) of the murine homing receptor lectin-like cell adhesion molecule 1 (LEC-CAM 1; gp90MEL) into individual venules reduced the number of rolling leukocytes by 89% ± 2% (mean ± SEM, n = 20 venules), while a similar CD4 chimera (CD4-IgG) had no effect (inhibition 14% ± 7%, n = 25). Rolling was also greatly reduced by a polyclonal serum against LEC-CAM 1 (inhibition 84% ± 3%, n = 35); preimmune serum was ineffective (11% ± 13% inhibition, n = 28). These findings indicate that LEC-CAM 1 mediates the adhesive interaction underlying leukocyte rolling and thus may play an important role in inflammation and in pathologic conditions involving leukocytes.

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MATERIALS AND METHODS

Female Sprague Dawley rats (approximately 300 g body weight) anesthetized with ketamine and pentobarbital were catheterized (carotid artery and jugular vein) and surgically prepared for microscopic observation of mesenteric microvessels. Blood pressure and heart rate remained constant, systemic leukocyte counts increased slightly (from 7,000 to 8,200 p.L/L) during the experimental period of approximately 90 minutes. Animal core temperature was thermostated to 37°C. The mesenteric preparation was superfused with warmed (37°C) bicarbonate-buffered physiologic saline solution equilibrated with 5% CO₂ in N₂. Glass micropipettes (tip diameter 7 to 10 μm) were filled through 0.45-μm filters (Millipore HV, Eschborn, Germany) and were introduced into side branches (approximate diameter 20 μm) of the investigated venules using a piezo-driven micromanipulator (PM 10; Märzhäuser, Wetzlar, Germany).

The following reagents were micro-infused for 1-minute periods by pressurizing an air-filled chamber connected with the micropipette (applied volume approximately 100 to 200 nL): (1) a recombinant chimera consisting of the extracellular domain of murine LEC-CAM 1 linked to human IgG Fc regions (LEC-IgG) at 100 μg/mL in phosphate-buffered saline (PBS); (2) a similar CD4 chimera (CD4-IgG) at 100 μg/mL in PBS; (3) a polyclonal serum against murine LEC-CAM 1, which cross-reacts with rat homing receptor (data not shown), diluted 1:10 in PBS; and (4) a matching preimmune serum, also diluted 1:10. The polyclonal serum was produced by injecting 10 μg of purified mouse LEC-CAM 1 in Freund's complete adjuvant into multiple subcutaneous sites of a rabbit, and obtaining serum after 1 month.

Observations were made on a modified Leitz intravital microscope with transillumination, using a 25×0.60 N.A. salt water immersion objective, and recorded on video tape (final magnification on monitor approximately 700×). With transillumination microscopy, rolling leukocytes are identified as diffractive, slowly moving objects. The number of rolling leukocytes passing per time (rolling leukocyte flux) was measured from the video recordings for each 2-second interval. Flow velocity was measured on line by temporal cross-correlation (auto tracking correlator 102B; Instrumentation for Physiology and Medicine, San Diego, CA) of signals.
from a photo-transistor pair on which the microscope image was projected.

RESULTS

Leukocyte rolling was investigated in 108 venules (diameter 40 ± 1 μm, mean ± SEM) of the exposed rat mesentery, several junctions downstream from the site of micro-infusion. Rolling leukocyte flux was reduced significantly from 83 ± 11 to 7 ± 1 cells/min (mean ± SEM, n = 20 venules) by micro-infusion of LEC-IgG (100 μg/mL), but was virtually unaffected (74 ± 10 during micro-infusion vs 64 ± 11 cells/min during control, n = 25) by CD4-IgG. Micro-infusion of anti-LEC-CAM 1 antiserum (diluted 1:10 in PBS) decreased leukocyte rolling from 90 ± 9 to 13 ± 3 cells/min (n = 35), while infusion of preimmune serum had no effect (69 ± 10 and 60 ± 12 cells/min, respectively; n = 28) (Fig 1). Micro-infusion of neither of the reagents had any effect on blood pressure, heart rate, or systemic leukocyte counts of the animals.

Because the increase of blood flow velocity induced locally by micro-infusion may affect rolling leukocyte flux, velocities were measured in each group and found to be increased during micro-infusion to a similar extent: by 131% ± 26% above control velocity in the LEC-IgG group, by 200% ± 24% in the CD4-IgG group, by 159% ± 19% in the anti-LEC-CAM 1 group, and by 187% ± 30% in the preimmune serum group. On termination of micro-infusion of both LEC-IgG and anti-LEC-CAM 1, leukocyte rolling reached its original value within about 15 seconds (Fig 2).

DISCUSSION

Anti-LEC-CAM 1 and LEC-IgG markedly reduced the number of rolling leukocytes while the respective control reagents did not, with similar hemodynamic conditions prevailing in the investigated venules. LEC-IgG has recently been shown to inhibit PMN recruitment to an inflammatory site.4 However, it remained unclear which of the different steps involved in the process (leukocyte rolling, firm adhesion, emigration) was blocked by LEC-IgG. The present intravital microscopic data indicate that LEC-IgG interferes with the earliest form of leukocyte-endothelial interaction, ie, leukocyte rolling. A preliminary report suggests that leukocyte rolling is a prerequisite for firm adhesion and emigration.5 Hence, it appears likely that LEC-IgG precludes PMN recruitment by blocking their rolling.

On termination of micro-infusion of anti-LEC-CAM 1, leukocyte rolling resumed within about 15 seconds, which is in accordance with earlier observations with application of sulfated glycosaminoglycans.6 This behavior was to be expected, because leukocytes exposed to micro-infused anti-LEC-CAM 1 were swept away into the systemic circulation and replaced by fresh leukocytes. The similar lag-time seen after infusion of LEC-IgG was surprising, because LEC-IgG interacts with the endothelial ligand of LEC-CAM 1 and, hence, should remain attached to the endothelium of the investigated venule. LEC-IgG has previously been shown to specifically bind to lymph node high endothelial venules and block lymphocyte attachment.23 The transitory effect of LEC-IgG infusion on

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Fig. 1. Flux of rolling leukocytes during micro-infusion. The percent of control flux, mean ± SEM, and number of applications are indicated. LEC-IgG, recombinant chimera of murine LEC-CAM 1; CD4-IgG, similar chimera of CD4, both 100 μg/mL; PolyMel, rabbit antimouse LEC-CAM 1 serum (1:10); preimmune, matching preimmune serum.

Fig. 2. Flux of rolling leukocytes during and following micro-infusion. Top, anti-LEC-CAM 1 antiserum (35 applications, solid line) and preimmune serum (28 applications, broken line). Bottom, LEC-IgG (average of 20 applications, solid line) and CD4-IgG (25 applications, broken line). Micro-infusion was terminated at 60 seconds.
LEC-CAM 1 mediates leukocyte rolling

Leukocyte rolling indicates that LEC-IgG binding to and release from its ligand on extra-lymphoid venular endothelium may have a short time constant of the order of several seconds. This concept is consistent with the nature of the bond established between the rolling leukocyte and the endothelium. In the process of rolling, attachment and detachment of individual bonds probably alternate very frequently.

The conclusion that LEC-CAM 1 plays a major role in mediating leukocyte rolling in vivo is supported by several other observations. Both leukocyte rolling\(^5,6\) and LEC-CAM 1-mediated lymphocyte adhesion to high endothelial venules of lymph node\(^7,8\) are inhibited by sulfated polysaccharides, among them the sulfated fucose polymer fucoidin. LEC-CAM 1 has been shown to be downregulated upon chemotactic stimulation of PMNs,\(^9,10\) which correlates with the inability of stimulated PMNs to "home" to an inflammatory site.\(^21\) Likewise, leukocyte rolling in venules of the hamster cheek pouch has been reported to be reduced upon chemotactic stimulation.\(^26\)

It is concluded that the inhibitory effects of LEC-IgG and anti-LEC-CAM 1 antisera on leukocyte rolling seen in the present study are caused by interference with the interaction between LEC-CAM 1 and its endothelial ligand. The rapid onset (1 to 3 minutes) of leukocyte rolling upon surgical trauma\(^6\) suggests that a pre-existing endothelial ligand is exposed at the luminal surface of venules, or modified to allow leukocyte adhesion via LEC-CAM 1.

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REFERENCES


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