Soluble ICAM-1 reduces leukocyte adhesion to vascular endothelium in ischemia-reperfusion injury in mice

KLAUS KUSTERER,1 JÖRG BOJUNGA,1 MICHAEL ENGHOFER,1 EDMUND HEIDENTHAL,2 KLAUS H. USADEL,1 HUBERT KOLB,2 AND STEPHAN MARTIN2

1Department of Medicine I, Johann Wolfgang Goethe University, D-60590 Frankfurt; and 2Diabetes Research Institute, Heinrich-Heine University, D-40225 Düsseldorf, Germany

A GROWING BODY OF EVIDENCE indicates that infiltrating leukocytes are key mediators in ischemia-reperfusion injury (4, 6, 7). This phenomenon plays an important role in the clinical course of a variety of different clinical disorders, including myocardial infarction, stroke, organ transplantation, burns, and circulatory shock. The migration of leukocytes from the bloodstream into inflamed tissue requires a cascade of events in the microcirculation (24). The sequence of binding events starts with rolling of leukocytes along the endothelium. This first step is mediated by selectins (9, 10, 12, 15, 17, 25). On activation by inflammatory signals, such as chemoattractants, rolling progresses to firmer adhesion because of the interaction of the integrins on leukocytes with adhesion molecules of the immunoglobulin family, such as intercellular adhesion molecule-1 (ICAM-1), on the endothelium. Monoclonal antibodies that interfere with different steps of the cell-binding cascade have been shown to have a beneficial effect on ischemia-reperfusion injury (7).

Soluble adhesion molecules are detectable only in small quantities in serum of healthy individuals (21, 22). However, increased serum levels of soluble adhesion molecules have been described in different pathological situations, including in ischemia-reperfusion injuries (3, 5).

In the present study, we demonstrate that the recombinant form of soluble murine ICAM-1 (rICAM-1) reduces leukocyte adhesion during ischemia-reperfusion injury.

METHODS

C57BL/6 mice aged 6–10 wk were obtained from Charles River (Sulzfeld, Germany). The experiments were approved by the coverture of Darmstadt (Hessen, Germany). The animals had free access to food and water.

In vivo microscopy. After anesthesia (5 g/kg Urethane im; Sigma, Deideshofen, Germany), a catheter was inserted into the inferior vena cava after laparotomy. A loop of the small intestine was carefully fixed on a metal plate that was kept at 37°C constantly. Physiological saline with a temperature of 37°C was continuously rinsed over the intestinal loop. Leukocytes were stained in vivo by an intravenous injection of 0.1 ml acridine red solution (1% Chroma, Köngen, Germany). The microcirculation of a mesenteric vein was observed with an epi-illumination microscope (objective: CF Fluor 10/0.3 W; Nikon, Düsseldorf, Germany; filter set: excitation filter 515–560 nm; barrier filter: >580 nm; final magnification: ×1,333). A mesenteric vein was occluded with a clamp for 45 min. During the reperfusion period, the microcirculation was recorded for 30 min on videotape (5-VHS video recorder Panasonic AG 7355; charge-coupled device video camera FK 6990-IQ, Pieper, Schwerte, Germany). The analog video signal was digitized with an image-analyzing system (Datacube, Peabody, MA). The software (TEBA, Gießen, Germany) allows the measurements of vascular diameters and cross sections. A leukocyte was considered to be adherent if the cell did not move along the endothelium over an observation period of 10.220.33.1 on June 24, 2017 http://ajpgi.physiology.org/ Downloaded from
period of >30 s. The number of adherent leukocytes was measured at 10-min intervals and expressed as cells per square millimeter of vein cross section. A rolling leukocyte was defined as a cell that clearly moved along the endothelium of the venule more slowly than the blood flow. Rolling leukocytes were expressed as the number of leukocytes per millimeter of vein diameter per minute.

The diameter of veins was ~100 µm (Table 1). C57BL/6 mice did not have smaller mesenteric veins accessible for in vivo microscopy. The velocities of five freely flowing fluorescent-labeled leukocytes were determined by measurement of the distance traveled between two or more successive video frames. The highest cell velocity (Vmax) was used to estimate mean blood flow velocity, using the equation

\[
V_b = V_{max}(2 - e^2),
\]

where \(e\) represents the ratio of the diameter of leukocytes (8 µm) divided by the respective venular diameter (11, 13).

The leukocyte count was measured in a sample of venous blood drawn before and after ischemia-reperfusion using a Neubauer cell counting chamber.

Production of rICAM-1. To generate rICAM-1, the cDNA encoding the extracellular portion of the murine ICAM-1 protein, including the codon for N-460 followed by a translational stop codon, was generated by PCR. The cDNA was cloned in the pBlueBac2 transfer vector (Invitrogen, San Diego, CA), and recombinant baculovirus was generated using a transfection module (AcMNPV linear DNA transfection module, Invitrogen). After the virus was cloned, rICAM-1 was expressed by infecting Sf9 cells at a multiplicity of 10. Cells were cultured in Grace’s insect medium (Life Technologies, Eggenstein, Germany) supplemented with 10% FCS, gentamicin, and amphotericin B in spinner culture. Supernatants were harvested, and recombinant proteins were purified by immunoaffinity chromatography on murine ICAM-1 monoclonal antibody YN1/1.7.4-Sepharose (Pharmacia, Uppsala, Sweden). Extinction coefficients of 0.80 were estimated by adding known concentrations of purified proteins to SDS-PAGE gels or 2.5 or 5 µg rICAM-1 in 0.1 ml iv of either saline or solvent (250 mM Tris, 150 mM NaCl, and 70 mM triethylamine, pH 7.5) or 2.5 or 5 µg rICAM-1 in 0.1 ml iv or 0.1 ml fucoidin (25 mg/kg body wt iv) 10 min before reperfusion. Fucoidin was obtained from Sigma.

Statistics. Data are presented as means ± SE. The significance of differences between the groups was evaluated using one-way ANOVA with Bonferroni correction. Student’s t-test for paired samples was used for statistical analysis between 0 min and other time points.

RESULTS

Hemodynamic parameters. The mean blood flow velocity before and after ischemia was not statistically different. Treatment with rICAM-1 or fucoidin had no influence on mean blood flow velocity (Table 1).

Leukocyte rolling. The number of leukocytes rolling along the endothelial surface increased more than twofold during postischemic perfusion (P < 0.05; Fig. 1). Fucoidin is a selectin-binding polysaccharide and served as a positive control. As expected, rolling was inhibited by fucoidin (P < 0.01; Fig. 1). rICAM-1 did not have any influence on the number of rolling leukocytes in ischemia-reperfusion injury of mesenteric veins in C57BL/6 mice (Fig. 1). There was no difference in leukocyte count in the peripheral blood in the different treatment groups.

Adherent leukocytes. The integrins bind to the immunoglobulin superfamily members, increasing adhesiveness and resulting in arrest of the rolling leukocyte. Firm adherence of leukocytes to venular endothelium increased ~10-fold (P < 0.001) in response to prior ischemia (Fig. 2). This pathological increase of leukocyte adherence was almost completely suppressed in the presence of circulating rICAM-1 (P < 0.01). Because fucoidin interferes with rolling of leukocytes, it also leads to suppressed adhesion of leukocytes to the endothelium of mesenteric venules during ischemia-reperfusion (P < 0.01; Fig. 2).

DISCUSSION

This study demonstrates for the first time that leukocyte adherence to vascular endothelium in ischemia-reperfusion injury can be reduced by rICAM-1. Soluble ICAM-1 is a naturally occurring circulating adhesion molecule (21, 22) and binds mainly to its ligands lymphocyte function-associated antigen-1 (LFA-1) and Mac-1 (CD11b/CD18) expressed on leukocytes (16, 26). Interestingly, in a recent report (5) elevated levels of soluble ICAM-1 were found in patients with ischemic heart disease. Therefore, reduction of increased leukocyte adhesion by soluble ICAM-1 as shown here might be a physiological response to reduce ischemia-reperfusion injury.

Monoclonal antibodies to ICAM-1, CD18, or Mac-1 could reduce the increased leukocyte adhesion and the increased vascular permeability during ischemia-reperfusion injury (6, 18). However, repeated application of an antibody provokes an immune response directed against the antibody and eliminates its capacity to suppress leukocyte emigration. The clinical applicability of monoclonal antibodies is therefore limited. rICAM-1 consists of all five extracellular immunoglobulin-like domains of naturally occurring circulating ICAM-1, which is elevated in various clinical disorders (3, 8, 19, 22). In this study, we demonstrated that...
rICAM-1 dose dependently reduces the increased leukocyte adhesion during ischemia-reperfusion injury. Our study demonstrates that the therapeutic application of rICAM-1 may be a novel approach to prevent the injury caused by postischemic reperfusion. Therapeutic administration of soluble adhesion molecules can be repeated without loss of effectiveness.

The mechanism by which rICAM-1 reduces leukocyte adhesion seems to be multifactorial; binding of rICAM-1 to LFA-1 or Mac-1 (16, 26) might sterically interfere with the binding to membrane ICAM-1. Direct binding experiments indicate that monomeric rICAM-1/LFA-1 interaction may involve only a moderate binding affinity (26). Nevertheless, rICAM-1 can bind to LFA-1 and completely inhibit ICAM-1/LFA-1-mediated cell-cell interaction (16). Cell aggregation in tissue culture devoid of shearing forces occurring in circulation is inhibited at rICAM-1 concentrations of 20–40 µM, and LFA-1-dependent binding of micelles to surface-bound ICAM-1 is inhibited at 1–2 µM rICAM-1 (16). In the current study, the calculated initial concentration of intravenously administered rICAM-1 is ~100 nM in plasma. Endogenous levels of soluble ICAM-1 measured in the same ELISA as described above were found to be ~1 nM (100 ng/ml). Therefore, the amount of injected rICAM-1 is ~100 times higher than the endogenous level of soluble ICAM-1 and may be sufficient to interfere with the initial phase of leukocyte adhesion, which is still labile because of shear stress. In previous studies with freshly isolated peripheral blood cells (19), rICAM-1 was observed to interfere with antigen-specific T cell stimulation at concentrations as low as 2 nM. The latter is well below that required for steric hindrance, and we therefore have proposed that soluble ICAM-1 mediates a negative signal by binding to LFA-1. At present, it cannot be excluded that negative signaling induced by soluble rICAM-1 contributes to the inhibition of stable adhesion between leukocytes and the vessel wall.

Ischemia-reperfusion injury is associated with many clinical disorders, especially vascular diseases such as myocardial infarction and stroke. The prognosis for these patients is critically dependent on the extent of the ischemia-reperfusion injury. rICAM-1 may provide a novel approach to prevent ischemia-reperfusion injury, possibly mimicking endogenous protective responses.

This work was supported by Deutsche Forschungsgemeinschaft Grants KU 622/4-1 and MA 1260/4, by the Minister für Wissenschaft.
REFERENCES


