PECAM-1 (CD 31) mediates transendothelial leukocyte migration in experimental colitis

Emile Rijcken, Rudolf B. Mennigen, Sebastian D. Schaefer, Mike G. Laukoetter, Christoph Anthoni, Hans-Ulrich Spiegel, Matthias Bruwer, Norbert Senninger, and Christian F. Krieglstein

Department of General Surgery, Muenster University Hospital, Germany

Submitted 23 February 2007; accepted in final form 16 May 2007

RIJCKEN E, Mennigen RB, Schaefer SD, Laukoetter MG, Anthoni C, Spiegel H-U, Bruwer M, Senninger N, Krieglstein CF. PECAM-1 (CD 31) mediates transendothelial leukocyte migration in experimental colitis. Am J Physiol Gastrointest Liver Physiol 293: G446–G452, 2007. First published May 17, 2007; doi:10.1152/ajpgi.00097.2007.—Transendothelial migration of circulating leukocytes into the colonic wall is a key step in the development of the inflammatory infiltrate in inflammatory bowel disease (IBD). The platelet-endothelial cell adhesion molecule-1 PECAM-1 (CD31) is expressed in the tight junction area of endothelial cells, where it is supposed to support the transmigration process. The aim of this study was to determine the role of PECAM-1 in experimental IBD and to show whether blockade of PECAM-1 has therapeutic effects. Chronic colitis was induced in female BALB/c mice by cyclic oral administration of dextran sodium sulfate (DSS) 3% (wt/vol). Expression of PECAM-1 was visualized by immunohistochemistry. In the treatment group animals received 1 mg/kg anti-PECAM-1 (2H8) ip daily starting on day 26. On day 30 leukocyte adhesion and migration was measured during N2O-isoflurane anesthesia in the distal colon by intravital microscopy. Disease activity index (DAI), histology, and MPO levels were compared with healthy and diseased controls. PECAM-1 was expressed in colitic mice. Chronic DSS colitis was characterized by a marked increase in rolling, adherent, and transmigrated leukocytes compared with healthy controls. Immunoblockade of PECAM-1 reduced leukocyte transmigration significantly and also diminished leukocyte rolling and sticking in an indirect manner. It also resulted in a significantly diminished DAI and MPO levels, as well as an amelioration of the histological inflammation score. PECAM-1 plays an important role in transendothelial leukocyte migration in DSS colitis. PECAM-1 could be a novel target for antibody-based treatment in IBD.

platelet-endothelial cell adhesion molecule-1; leukocyte transmigration; dextran sodium sulfate colitis; intravital microscopy; inflammatory bowel disease

LEUKOCYTE RECRUITMENT FROM the blood circulation into the tissues is a crucial event in the generation and maintenance of the inflammatory infiltrate in inflammatory bowel disease (IBD) as in other inflammatory disorders (19, 25). The initial mechanisms of leukocyte margination, tethering, rolling, and firm adhesion to the vessel wall of postcapillary venules have been extensively studied in recent years, but particularly in inflammatory bowel disease relatively little is known about leukocyte migration through the endothelium and the underlying basal membrane into the mucosa and submucosa itself. There is growing evidence that activated leukocytes drift to the borders of the endothelial cells and then pass across them in an amoeboid fashion. Compared with the intestinal epithelium, the junctions between endothelial cells in intestinal postcapillary venules are leakier. In vitro, neutrophils accomplish this process in ~90 s.

The process of leukocyte transendothelial migration, also called diapedesis, involves several families of cell adhesion molecules, such as VE-cadherin, ICAM-2, CD99, JAM-A, JAM-B, JAM-C, and platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31) (19). In contrast to leukocyte rolling or sticking, leukocyte transmigration involves homophilic interactions between adhesion molecules such as PECAM-1 and CD99 both on leukocytes and endothelial cells (6). Other ligands of PECAM-1 are integrin αβ3, αβ1, or CD38. PECAM-1 is also involved in leukocyte migration through the second barrier, the subendothelial basal membrane (6, 19, 31). PECAM-1 is a 130-kDa glycoprotein that consists of 6 C2 immunoglobulin domains, a transmembrane region, and a small cytoplasmatic domain (25). Confocal microscopy studies demonstrated that PECAM-1 is densely expressed on the apical surface and the borders of endothelial cells (3, 19). Additionally, PECAM-1 is constitutively expressed on platelets, neutrophils, lymphocytes, natural killer cells, and monocytes. As a response to proinflammatory cytokines such as TNF-α, IL-1, or IFN-γ or to endotoxins, PECAM-1 expression is not upregulated transcriptionally (8), but PECAM-1 is redistributed away from the intercellular junction of endothelial cells, indicating that spatial allocation of PECAM-1 has a regulatory role in the processes mediated by PECAM-1 (28). PECAM-1 is suggested to guide leukocytes through the endothelium via enhanced expression of PECAM-1 at endothelial cell junctions during the transmigration process through recycling of PECAM-1-rich membrane invaginations below the plasma membrane at lateral junctions of endothelial cells (18). Ligation to PECAM-1 is also responsible for activation of β1, β2, and β3-integrins on monocytes, neutrophils, and natural killer cells (3, 30). Furthermore, homophilic interaction of PECAM-1 upregulates αβ1 integrins on transmigrated neutrophils in vivo (6). In summary, these data clearly demonstrate that PECAM-1 is critically involved in the transendothelial migration process. Various in vivo experiments could confirm the crucial role of PECAM-1 in different inflammatory conditions. Blockade of PECAM-1 by means of neutralizing antibodies ameliorated neutrophil extravasation in experimental peritonitis (4, 5), endotoxin-induced liver injury (5, 22), and ischemia-reperfusion injury (23, 32).

Despite extensive studies on leukocyte transmigration in other systems, the role of PECAM-1 in intestinal inflammation

Address for reprint requests and other correspondence: E. Rijcken, Dept. of General Surgery, Muenster Univ. Hospital, Waldeyerstrasse 1, D-48149 Muenster, Germany (e-mail: rijcken@uni-muenster.de).
has not been elucidated so far. However, PECAM-1 has been detected in colon tissue samples of patients with active Crohn’s disease or ulcerative colitis (2, 29, 33), suggesting that PECAM-1 interactions are also important in leukocyte transmigration in human IBD.

In this study we examined the role of the PECAM-1 in a well-established animal model of murine colitis. The dextran sodium sulfate (DSS) model of colitis was used to determine whether 1) PECAM-1 is expressed in colonic inflammation, 2) leukocyte transmigration in the distal colon can be inhibited by antibody blockade of PECAM-1, 3) PECAM-1 is involved in leukocyte rolling and firm adhesion, and 4) therapeutic blockade of PECAM-1 leads to a reduction of inflammatory activity in established disease. We were able to demonstrate that PECAM-1 mediates transendothelial migration in submucosal colonic venules in DSS colitis. PECAM-1 expression in DSS colitis could be visualized by immunocytochemistry in submucosal venules in the distal colon. Furthermore, we showed for the first time that monoclonal antibody blockade of PECAM-1 has therapeutic effects in this model of intestinal inflammation.

MATERIALS AND METHODS

Induction of inflammation. The experimental protocol was approved by the Animal Care Committee of the Regional Administration, Muenster, Germany. Inbred female BALB/c mice (Charles River, 20–22 g) were housed in pairs in standard laboratory cages with standard laboratory chow and drinking water ad libitum. Chronic colitis was induced by cyclic oral administration of DSS (24), molecular weight 40,000 (ICN Biomedicals) 3% (wt/vol) solved in MilliQ water (Millipore, Schwalbach, Germany) for 5 days interrupted by 5 days of Millipore water alone. A highly reproducible chronic colitis is established after completion of a total of three cycles. It is characterized by mucosal ulceration, crypt destruction, and infiltrating neutrophils and lymphocytes (24). Starting from the first day of induction, the disease activity index (DAI) was recorded daily. This well-established score is based on clinical symptoms and specifically designed to evaluate the severity of DSS colitis. It includes weight loss, stool consistency, and the appearance of blood in stools (21, 26).

Antibodies and reagents. 2H8, a hamster-anti-mouse IgG monoclonal antibody vs. PECAM-1, and LO-DNP-1, an unspecific control antibody, were purchased from Serotec, Oxford, UK. The antibodies were dissolved in PBS before injection.

For immunohistochemistry a rat monoclonal antibody against PECAM-1 (MEC 13.3) was obtained from BD Pharmingen (San Diego, CA). Secondary reagents were biotinylated goat anti-rat IgG (H+L) (VECTOR Laboratories, Burlingame, CA) and avidinylated Alexa Fluor 488 conjugate, Molecular Probes (Invitrogen, Karlsruhe, Germany).

IVM. In isoflurane/N₂O inhalation anesthesia, spontaneous breathing animals were placed in a supine position on a heating pad. Polyethylene catheters (Portex, Lythe, UK) were inserted into the ing animals were placed in a supine position on a heating pad. Polyethylene catheters (Portex, Lythe, UK) were inserted into the right jugular vein for intravenous administration of contrast dyes and right jugular vein for intravenous administration of contrast dyes and right jugular vein for intravenous administration of contrast dyes and blood samples (0.1 ml in EDTA) were taken before intravital microscopy. In each vessel the mean value of five venular diameters (D) was calculated. Central line leukocyte velocity (V) and leukocyte rolling velocity (Vrolling) were determined by calculating the mean velocity of five single frame-to-frame tracked fluorescent free flowing or rolling cells, respectively. Flow rate (Ft) was calculated by the formula Ft = \( \pi \times (D/2)^4 \times V \times t/10^6 \) where t = time. Leukocytes were defined as firm adherent (sticker) when attached to the vessel wall for at least 30 s and as rolling (roller) when moving with a velocity less than 2/5 of that of leukocytes at the centerline of the observed microvessel. Rolling and firm adherent cells were counted over a period of 30 s in a 100-μm section of the vessel and given as numbers per 0.01 mm² endothelial surface (26). Vrolling is a marker for the intensity of selectin interactions enabling rolling (17). For determination of leukocyte extravasation mucosal IVM was performed by opening the colon by a longitudinal incision along the antimesenteric border using microcurety (12, 36). In earlier studies we were able to show that the microcirculation in the studied area was not disturbed by surgery. After cleaning of feces and mucus the mucosal microcirculation was visualized in the mucosa in epiluminescence technique. The amount of extravasated leukocytes was assessed by counting the fluorescent cells outside the microvessels surrounding the crypts and averaged over five areas of 100 × 100 μm at the center of the opened bowel, given as numbers per square millimeter.

Tissue analysis. For histological studies the entire colon was excised. The colon was opened longitudinally and rinsed with saline. Each colon was divided in four parts, representing cecum with appendix, proximal, middle, and distal colon. Hematoxylin and eosin staining was performed on formalin-fixed, paraffin-embedded sections. From each region of the colon, four transverse slides were made, resulting in 16 slides for evaluation of colitis for each animal as described before (26). Histological inflammation was scored in a blinded fashion by a score introduced by Dieleman et al. (9), considering the degree of inflammation, the vertical extent of inflammation, and the crypt damage score, related to the percentage of involvement in each single slide (1, 9).

A segment of the distal colon in some healthy as well as some colitic animals was snap frozen in liquid nitrogen for immunohistochemical studies. Fluorescence staining of PECAM-1 was performed by the avidin-biotin technique in cryostat sections. Additional nuclear staining was achieved by using DAPI (Sigma Aldrich, Steinheim, Germany). Before antibody application, unspecific protein binding was blocked by incubation with BSA (Sigma Aldrich). Negative controls were prepared by omission of the specific antibody. The slides were given random numbers for blinding before examination.

Myeloperoxidase (MPO) is contained in cytoplasmatic granules of neutrophils and monocytes (13), and MPO activity correlates with the presence of neutrophils in intestinal inflammation (15). Tissue MPO activity was measured in units per gram by the o-dianisidine assay. In brief, samples of the distal colon were snap frozen in liquid nitrogen and stored at −80°C until thawed for MPO activity determination. The samples were homogenized and sonicated in hexadecyltrimethylammonium bromide 0.5% (Sigma Aldrich) while kept on ice and finally centrifuged at 12,000 rpm at 4°C. The supernatant was transferred into 3-ml glass tubes containing 50 mM KPi, pH 6, 20 mM H₂O₂, and 30 μl of o-dianisidine 20 mg/ml to start the reaction. The reaction was stopped after 10 min by adding sodium azide 2% (Fischer Scientific, Fair Lawn, NJ). The change in absorbance was read at 460 nm in a spectrophotometer (Biochrom Libra S11, Cambridge, UK). MPO activity was expressed as the amount of enzyme...
necessary to evoke a change in absorbance of 1.0 per minute per wet weight gram of tissue.

**Experimental protocol.** In the first experiment, the effect of monoclonal antibodies against PECAM-1 (2H8) on leukocyte adhesion and transmigration and their effect on intestinal inflammation were studied. After completing the third DSS cycle, 2H8 (1 mg/kg body wt) was administered in the treatment group by daily intraperitoneal injection starting on day 25 over a period of 5 days. The dosage was based on previous studies in ischemia-reperfusion injury (32). DAI scores were recorded daily. On day 30 leukocyte-endothelial interactions in colonic submucosal venules were studied by use of IVM. After opening of the distal colon, leukocyte extravasation was visualized in the mucosa. The treatment group was compared with a healthy control group receiving Millipore water alone and a diseased control group, which received the antibody-carrier PBS alone (n = 10 for each group). At the end of the IVM experiments the animals were killed and colon was prepared for histology, immunohistochemistry, and MPO measurements.

The second experiment was designed to evaluate a direct effect of the antibody 2H8 on leukocyte rolling and sticking. IVM was performed in chronic inflamed animals. A submucosal venule in the distal colon was randomly selected, and leukocyte rolling and firm adhesion were visualized after in vivo staining by repeated intravenous injections of acridine orange (Sigma Aldrich). After the baseline leukocyte adhesion was recorded for 10 min after an equilibration period, the antibody 2H8 (1 mg/kg body wt) was injected intravenously under direct IVM vision. All standard parameters of IVM that define leukocyte-endothelial adhesive interactions were measured in submucosal venules in the distal colon 1, 5, 10, 20, 30, 45, and 60 min after antibody administration. Control animals received 1 mg/kg body wt of the unspecific antibody LO-DNP-1. Each group included seven animals (n = 7).

**Statistics.** Results are given as mean values ± SE. Statistical evaluations were performed by using the Kruskal-Wallis ranking test for unpaired samples supplemented by Dunn’s method. Mann-Whitney-Wilcoxon signed-rank test was applied for nonparametric paired samples. P values of <0.05 were considered significant.

**RESULTS**

**Expression of PECAM-1 in DSS colitis.** Immunofluorescence was used to evaluate PECAM-1 expression in the distal colon during chronic DSS colitis. Cryosections were prepared from the distal colon of healthy and chronically inflamed mice. The segment of the distal colon corresponded to the segment that was used for IVM. Immunohistochemically, marked PECAM-1 expression was detected in submucosal venules in healthy animals (Fig. 1A). Also, strong staining of PECAM-1 was found in submucosal venules in animals with DSS-induced colitis (Fig. 1B). This indicated that PECAM-1 expression in colitic mice was not upregulated compared with healthy controls but rather expressed constitutively in marked levels independent from the inflammatory stimulus.

**IVM.** Chronic DSS colitis was characterized by a marked increase in rolling leukocytes (116.4 ± 19.8 vs. 51.1 ± 8.6 per 0.01 mm²/30 s, P < 0.05) and adherent leukocytes (28.0 ± 3.6 vs. 4.3 ± 1.2 per 0.01 mm²/30 s, P < 0.05) in submucosal venules of the distal colon compared with healthy controls. Accordingly, leukocyte rolling velocity was markedly decreased (23.9 ± 2.3 vs. 48.8 ± 4.7 μm/s, P < 0.05). The number of transmigrated leukocytes was significantly increased in chronic DSS colitis compared with healthy control animals (404 ± 40.8 vs. 38.8 ± 8.4 per mm²/30 s, P < 0.05) (Fig. 2). In the first set of experiments, treatment with anti PECAM-1 antibodies resulted in a significant reduction of leukocyte transmigration by ~84% in the distal colon (65.8 ± 6.8 mm²/30 s, P < 0.05). After 5 days of treatment, also the number of rolling (59.3 ± 14.2 vs. 116.4 ± 19.8 per 0.01 mm²/30 s, P < 0.05) and firm adherent leukocytes (5.6 ± 0.6 vs. 28.0 ± 3.6 per 0.01 mm²/30 s, P < 0.05) was significantly reduced compared with diseased controls. However, rolling velocity was not decreased in 2H8-treated animals compared...
with diseased animals (27.9 ± 3.0 μm/s, P = not significant). The microcirculatory parameters central line leukocyte velocity, vessel diameter, blood flow, or shear rate were not different in healthy controls, DSS colitis, or 2H8-treated colitic animals (Table 1), showing stable microcirculatory conditions in all IVM experiments. There were no complications related to intraperitoneal administration of the antibodies. Peripheral leukocyte counts were not affected by 2H8 (Table 2). Hemoglobin levels in chronic colitic animals tended to be lower than in healthy mice as a sign of fecal blood loss, but this did not reach statistical significance.

In the second set of experiments, we intended to study whether the reduction of leukocyte rolling and sticking as observed in long-term-treated animals was caused by an immediate blockade of PECAM-1, implicating a direct involvement of PECAM-1 in leukocyte rolling and firm adherence in the distal colon. Therefore we administered 2H8 intravenously in chronically diseased animals under direct IVM vision in submucosal venules. Neither 2H8 nor the unspecific control antibody LO-DNP significantly reduced leukocyte rolling, rolling velocity, or leukocyte firm adherence during constant observation over a period of 60 min (data not shown).

**Effects of blockade of PECAM-1 on intestinal inflammation.**

All mice fed with DSS survived the colitis-induction protocol and were killed on day 30 after the IVM experiments. Mice receiving DSS developed symptoms of colitis after 3–4 days, when weight loss and diarrhea became apparent. Bloody stools were frequent after 5–6 days of DSS 3%. All colitic animals exhibited a similar DAI course until day 26, when treatment with 2H8 was initiated (Fig. 3). On day 30, treatment with anti-PECAM-1 significantly reduced DAI compared with DSS controls (2.8 ± 0.3 vs. 5 ± 0.4 points, P < 0.05). This effect could already be observed after 2 days of treatment (Fig. 3). In chronic DSS colitis MPO levels in the distal colon were significantly increased compared with healthy controls (46.1 ± 4.2 vs. 12.9 ± 1.3 U/g, P < 0.05). Accordingly, MPO levels were significantly decreased in 2H8-treated animals (25.8 ± 4.7 U/g, P < 0.05), although still being significantly higher as compared with healthy controls.

![Image](http://ajpgi.physiology.org/)

**Fig. 2.** Epiluminescence mucosal intravital microscopy. Crypts are clearly recognizable as pale gray rings surrounded by dark-colored intramucosal microvessels. The extravasated leukocytes, stained with acridine orange, are the bright white spots. In chronic DSS colitis there is a marked increase of microvessels. The extravasated leukocytes, stained with acridine orange, are recognizable as pale gray rings surrounded by dark-colored intramucosal microvessels.

**Table 1. Microcirculatory and hemodynamic parameters in the distal colon**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Controls</th>
<th>DSS Colitis</th>
<th>DSS + Anti-PECAM-1 (2H8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central line velocity, μm/s</td>
<td>591.7±47.5</td>
<td>843.5±119.5</td>
<td>932.0±142.8</td>
</tr>
<tr>
<td>Vessel diameter, μm</td>
<td>79.0±8.4</td>
<td>74.8±8.0</td>
<td>88.4±7.1</td>
</tr>
<tr>
<td>Bloodflow, μl/min</td>
<td>21.4±3.7</td>
<td>30.6±8.2</td>
<td>44.4±9.7</td>
</tr>
<tr>
<td>Shear rate, s⁻¹</td>
<td>70.5±11.1</td>
<td>94.8±12.1</td>
<td>87.6±12.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. PECAM-1, platelet-endothelial cell adhesion molecule-1. All differences are not significant.

**Table 2. Peripheral blood counts after intraperitoneal anti-PECAM-1 (2H8) antibody administration**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Controls</th>
<th>DSS Colitis</th>
<th>DSS + Anti-PECAM-1 (2H8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, 1,000/μl</td>
<td>5.2±0.9</td>
<td>3.1±0.6</td>
<td>4.9±0.8</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>13.2±1.8</td>
<td>10.8±1.3</td>
<td>14.9±0.3</td>
</tr>
<tr>
<td>Hct, %</td>
<td>32.6±2.9</td>
<td>30.3±3.8</td>
<td>37.6±2.8</td>
</tr>
<tr>
<td>Thrombocytes, 1,000/μl</td>
<td>288±28</td>
<td>408±14</td>
<td>352±14</td>
</tr>
</tbody>
</table>

Values are means ± SE. All differences are not significant.

**Fig. 3.** Disease activity index (DAI) scores of all mice during the last 10 days of the experiment. Treatment by daily intraperitoneal injections of anti-PECAM-1 was initiated on day 26. There was a significant reduction of clinical inflammation as early as 2 days after the beginning of the therapy. However, DAI of 2H8-treated animals did not reach the values of the healthy control group (values given as means ± SE).
in healthy control animals (Fig. 4). Upon histological examination, healthy animals exhibited a normal microscopic anatomy of the colon, with infrequent areas of mild inflammation, leading to a slightly elevated histological inflammation score. After three cycles of DSS, colonic tissue showed a discontinuous chronic inflammation, including a mixed inflammatory infiltrate containing mononuclear cells and neutrophils and a loss of epithelium and crypt shortening, as described previously. In chronic DSS colitis there was an increase of the severity of histological inflammation toward the distal colon (Fig. 5). Administration of 2H8 blunted histological alterations significantly in all parts of the colon. In the distal colon blockade of PECAM-1 lead to significant decrease of the inflammation score from 23.9 ± 1.2 points (DSS colitis) to 15.4 ± 1.5 points (treatment group) (P < 0.05).

**DISCUSSION**

The present study provides new insights in the mechanisms involved in leukocyte transmigration in intestinal inflammation. We examined the role of PECAM-1 in the recruitment of leukocytes in experimental inflammatory bowel disease. By immunoblockade of PECAM-1 using the highly specific anti-PECAM-1 monoclonal antibody 2H8, leukocyte transmigration was reduced by ~80%, and furthermore the inflammation-associated tissue injury was significantly ameliorated. This implicates that the generation of the inflammatory infiltrate in DSS colitis is largely dependent on the presence of PECAM-1 on leukocytes and endothelial cells.

The concept of neutralizing adhesion molecules to control intestinal inflammation has been successfully applied both in experimental and, in part, in clinical studies. However, PECAM-1 has not recognized much attention in this regard. In contrast to other adhesion molecules such as ICAM-1 or P-selectin that show elevated levels in tissue and plasma samples of patients with active IBD, PECAM-1 seems not to be upregulated on endothelial cells (33). Quantification of basal expression of PECAM-1 in the vascular beds of the murine distal colon under unstimulated conditions was demonstrated by the dual radiolabeled monoclonal antibody technique (34). By means of immunohistochemistry we could demonstrate that there was no enhanced expression of PECAM-1 in submucosal venules of the distal colon of inflamed animals compared with healthy controls. Because of its marked constitutive expression on endothelial cells, PECAM-1 has been used as an immunohistochemical marker for endothelium by many groups (34). Nonetheless it becomes apparent that upregulation is not required to play a significant role in the process of leukocyte recruitment in an inflammatory setting.

In our model, PECAM-1 was mainly involved in leukocyte transmigration across the endothelial layer and basal membrane as the amount of leukocytes in the crypts was significantly reduced after treatment with 2H8. After 5 days of treatment with 2H8 also leukocyte rolling and firm adhesion appeared to be significantly diminished. We interpreted this observation as an indirect effect due to the general downregulation of inflammatory activity. To test this hypothesis we performed additional IVM experiments in which 2H8 was injected intravenously under direct intravital microscopic observation of submucosal venules in colitic animals. Although there was a large number of adherent leukocytes present in the vessel, in this experiment no significant alteration in leukocyte-endothelial cell-to-cell adhesive interactions was observed. This indicates that PECAM-1 is not participating in the early stages of leukocyte recruitment such as rolling or firm adhesion. This is consistent with the observation that anti-PECAM-1 antibodies or soluble recombinant PECAM-1 administered to leukocytes or endothelial cells could specifically inhibit trans-endothelial migration in vitro while leukocytes remain firm adherent to the apical surface of the endothelial cells (20), demonstrating that PECAM-1 does not influence leukocyte firm adhesion.

Platelets have been shown to mediate leukocyte adhesive interactions with endothelial cells in many physiological and pathophysiological conditions. This process has been demonstrated to be largely regulated by CD40-CD40 ligand interactions in the inflamed colon (35). Since platelets express high levels of PECAM-1, too, it might be suggested that 2H8 may also interfere with PECAM-1 homophilic interactions in platelet-leukocyte interactions in the distal colon. This is consistent with the observation that anti-PECAM-1 antibodies or soluble recombinant PECAM-1 administered to leukocytes or endothelial cells could specifically inhibit trans-endothelial migration in vitro while leukocytes remain firm adherent to the apical surface of the endothelial cells (20), demonstrating that PECAM-1 does not influence leukocyte firm adhesion.

Platelets have been shown to mediate leukocyte adhesive interactions with endothelial cells in many physiological and pathophysiological conditions. This process has been demonstrated to be largely regulated by CD40-CD40 ligand interactions in the inflamed colon (35). Since platelets express high levels of PECAM-1, too, it might be suggested that 2H8 may also interfere with PECAM-1 homophilic interactions in platelets.
lets as well as in platelet leukocyte interactions and thereby limit leukocyte extravasation. This might be another explanation for the observed reduction of leukocyte rolling and firm adhesion in this study. However, a direct involvement of CD40 in the extravasation process has not been demonstrated yet (35).

In similarity to other adhesion molecules such as CD40, there is increasing evidence that PECAM-1 has important signaling properties, too. For example, CD31 is an important amplifier of β₁-integrin (CD29)-mediated T cell adhesion to endothelial cells (30). β₁-Integrin as a compound of VLA-4 is a major ligand for VCAM-1, which is involved in the firm adhesion of eosinophils, lymphocytes, monocytes, and natural killer cells to cytokine-activated endothelium. CD31 also activates β₂-integrins (CD18) on the surface of monocytes, neutrophils, and natural killer cells (3). β₂-Integrin is a compound of LFA-1 and Mac-1, both serving as a ligand for ICAM-1, which also plays an important role for leukocyte firm adhesion (27). Thus an inhibition of integrin activation must also be considered to play a role in the observed reduction of rolling and firm adherence of leukocytes as seen in the 5-day treatment experiments as well as in the general reduction of inflammatory activity.

Therapeutic blockade of PECAM-1 did not entirely cure intestinal inflammation since DAI, histological score, and MPO levels were still significantly increased compared with healthy controls. Also, different in vivo studies using antibody blockade of PECAM-1 in various inflammatory settings only achieved a significant reduction but not a complete blockade of leukocyte transmigration (4, 5, 23, 32). Possible explanations are 1) the compensation of PECAM-1 function by other adhesion molecules involved in leukocyte transmigration such as CD99 or ICAM-2 (10, 19), an increased rate of migration of leukocytes, or 3) nonsaturating doses of 2H8. Studies using PECAM-1-deficient mice showed that these CD31 knockout mice had no impaired leukocyte migration in the models of IL-1β- or thioglycollate-induced peritonitis and showed a normal cutaneous hypersensitivity response (10). These results suggest that there are CD31-independent pathways, although they might be quantitatively less important.

Recent studies have revealed that there is a cytokine-specific effect for PECAM-1-mediated leukocyte transmigration (7, 23, 31). Leukocyte migration through venular walls provoked by injection of IL-1β was found to be PECAM-1 dependent (31), whereas PECAM-1 was not required for leukocyte transmigration induced by TNF-α (7, 23, 31). PECAM-1 seems to be crucial for induction of integrin αβ₁ on the surface of neutrophils (6). This PECAM-1-mediated activation is unnecessary following TNF-α stimulation, because TNF-α in contrast to IL-1β is able to directly activate neutrophils (3, 7, 23). Taken together, PECAM-1 is suggested to be more involved in IL-1β-triggered inflammation than in TNF-α-mediated inflammation. Chronic DSS colitis is a much more complex state of inflammation than direct stimulation of endothelium by addition of purified cytokines and it is consensus that it is not dominated by a certain cytokine. Egger et al. (11) reported a progressive upregulation of Th1 cytokines such as IL-12, IFN-γ, IL-1, and TNF-α during the induction of chronic DSS colitis, whereas in that study the expression of Th2 cytokines (e.g., IL-4) was only modest. On the other hand, Dieleman et al. (9) found that DSS colitis is mediated by both Th1 and Th2 cells, since high mucosal levels of IL-4 and IFN-γ were detected especially in the chronic phase of DSS colitis. DSS colitis can be ameliorated by both TNF-α- and IFN-γ-neutralizing antibodies, indicating that these cytokines are both important for maintenance of inflammation in DSS colitis. In contrast, antibody-blockade of IL-1 failed to reduce DSS colitis, referring to a subordinated role of IL-1 in this model (14). Interestingly, in our study blockade of PECAM-1 reduced leukocyte transmigration and inflammatory activity in DSS colitis, which cannot be attributed only to IL-1-mediated and TNF-α-independent mechanisms. Therefore pathways other than IL-1β might be important for leukocyte transmigration in colitis, too. Furthermore, in other models of inflammation driven by both TNF-α and IL-1β, for example murine endotoxemia, antibody blockade of PECAM-1 was shown to reduce MPO levels in the tissue as an index of leukocyte transmigration (22). In chronic DSS colitis as in active human IBD integrins on leukocytes and endothelial cells are already activated by many proinflammatory stimuli. The reduced activation of β₁ and β₂ integrins might be important in our model, but also direct inhibition of PECAM-1-mediated transmigration should be considered.

By blocking the step of leukocyte transmigration, the repetitive influx of inflammatory cells into the bowel wall is interrupted, giving opportunity for tissue regeneration. Furthermore, the engagement of αβ₁ integrins on the surface of monocytes and leukocytes has been shown to play a major role in binding to extracellular matrix structures such as collagen I and thereby stabilizing the inflammatory infiltrate (16). This might lead to a reduced shedding of proinflammatory cytokines and a reduced cytokine gradient guiding the inflammatory cells into the tissue, resulting in decreased leukocyte activation in the microcirculation.

In conclusion, the present study shows that PECAM-1 plays a major role in this experimental model of chronic colitis and should therefore be considered as a valuable target for future anti-PECAM-1-based strategies in human IBD, too. The fact that we could successfully treat established chronic DSS colitis by anti-PECAM-1 and not work in a prophylactic manner as in the acute DSS colitis model further supports our recommendation. However, whether therapeutic blockade of PECAM-1 alone will have clinical implications in human IBD remains speculative. So far many adhesion molecules have been tested for treatment of IBD in clinical studies without achieving a final breakthrough. This might be due to the diversity of overlapping functions of single adhesion molecules. Therefore a combined therapeutic blockade with several monoclonal antibodies, each directed at a different level of the leukocyte recruitment cascade, for example PSGL-1 for leukocyte rolling and PECAM-1 for leukocyte transmigration, might be a more promising strategy than the blockade of a single adhesion molecule alone.

GRANTS

This work was supported by a grant of the Deutsche Morbus Crohn/Colitis ulcerosa Vereinigung DCCV e.V. (Crohn and Colitis Foundation of Germany).

REFERENCES


