Immunoblockade of PSGL-1 attenuates established experimental murine colitis by reduction of leukocyte rolling

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Immunoblockade of PSGL-1 attenuates established experimental murine colitis by reduction of leukocyte rolling. Am J Physiol Gastrointest Liver Physiol 287: G115–G124, 2004. First published March 4, 2004; 10.1152/ajpgi.00207.2003.—Recruitment of circulating leukocytes into the colonic tissue is a key feature of intestinal inflammation. P-selectin glycoprotein ligand-1 (PSGL-1) and very late antigen-4 (VLA-4) are expressed on leukocytes and play an important role in leukocyte-endothelial cell adhesive interactions. We examined the effects of immunoneutralization of PSGL-1 and VLA-4 on leukocyte recruitment in vivo in the development and treatment of experimental colitis. Chronic colitis was induced in balb/c mice by oral administration of dextran sodium sulfate (DSS). Monoclonal antibodies 2PH1 (anti-PSGL-1) and PS/2 (anti-VLA-4) or the combination of both were injected intravenously, and leukocyte adhesion was observed for 60 min in colonic submucosal venules by intravital microscopy (IVM) under isoflurane/N2 O anesthesia. In addition, mice with established colitis were treated by daily intraperitoneal injections of 2PH1, PS/2, or the combination of both over 5 days. Disease activity index (DAI), histology, and myeloperoxidase (MPO) levels were compared with sham-treated DSS controls. We found that 2PH1 reduced the number of rolling leukocytes (148.7 ± 29.8 vs. 36.9 ± 8.7/0.01 mm²/30 s, P < 0.05), whereas leukocyte velocity was increased (24.0 ± 3.6 vs. 127.8 ± 11.7 μm/s, P < 0.05). PS/2 reduced leukocyte rolling to a lesser extent. Leukocyte firm adhesion was not influenced by 2PH1 but was strongly reduced by PS/2 (24.1 ± 2 vs. 4.4 ± 0.9/0.01 mm²/30 s, P < 0.05). Combined application did not cause additional effects on leukocyte adhesion. Treatment of chronic colitis with 2PH1 or PS/2 reduced DAI, mucosal injury, and MPO levels significantly. Combined treatment led to a significantly better reduction of DAI (0.4 ± 0.1 vs. 2.1 ± 0.2 points) and histology (9.7 ± 0.9 vs. 21.4 ± 4.6 points). In conclusion, PSGL-1 and VLA-4 play an important role for leukocyte recruitment during intestinal inflammation. Therapeutic strategies designed to disrupt interactions mediated by PSGL-1 and/or VLA-4 may prove beneficial in treatment of chronic colitis.

P-selectin glycoprotein ligand-1; very late antigen-4; dextran sodium sulfate-colitis; selectins; inflammatory bowel disease
L-selectin in inflamed gut (28, 37). Targeting the adhesive mechanisms that underlie leukocyte recruitment has become an attractive approach to modulate the inflammatory response. Because PSGL-1 plays a crucial role for the initial steps of the adhesive process to inflammatory sites, blockade of PSGL-1 might elicit potent anti-inflammatory effects. PSGL-1 is expressed on all subsets of mucosal lymphocytes in patients with IBD (35), suggesting PSGL-1-P-selectin interactions might be also of importance in leukocyte recruitment in human IBD, such as ulcerative colitis and Crohn’s disease. In the present study, we examined the role of the selectin ligand PSGL-1 in a well-established animal model of murine colitis (8, 10, 13, 27, 43). To estimate the effect of immunoblockade of PSGL-1, we chose to compare it with an antibody directed against the α4-subunit of very late antigen-4 (VLA-4), which has functions in both leukocyte rolling and firm adhesion. VLA-4 is a heterodimer integrin and consists of a β7-subunit and an α4-subunit, binding to VCAM-1 on endothelial cells (30). VLA-4 is expressed on various leukocytes and is involved in the adhesion of lymphocytes, monocytes, and natural killer cells to cytokine-activated endothelial cells at sites of inflammation (3). α4-Integrins also exist in combination with a β7-subunit and interact predominantly with the endothelial ligand mucosal addressin cellular adhesion molecule (MAdCAM-1), which is almost exclusively expressed on mucosal vessels. We report for the first time the direct intravital microscopic observation of the inhibition of leukocyte rolling and leukocyte recruitment by mAbs against the NH2-terminal region of PSGL-1 in colonic venules in an experimental model of IBD. Furthermore, we were able to demonstrate that established chronic murine colitis can be ameliorated by repeated treatment with anti-PSGL-1 mAbs. This effect can be enhanced by combined treatment with mAbs directed against adhesion molecules involved in leukocyte firm adhesion, such as VLA-4.

MATERIALS AND METHODS

Induction of inflammation. The experimental protocol was reviewed and approved by the Animal Care Committee of the Regional Administration Muenster, Germany. Inbred female balb/c mice (Harlan-Winkelmann, 20–22 g) were housed in pairs in standard laboratory cages with free access to food and drinking water. Chronic colitis was induced by cyclic oral administration of dextran sodium sulfate [DSS; see Ref. 29, mol wt 40,000 (ICN Biomedicals) 3% (wt/vol) dissolved in Millipore water (Millipore, Schwabach, 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 (29). Starting from the first day of induction, disease activity index (DAI) was recorded daily. This score is specifically designed to evaluate the severity of DSS colitis. It includes weight loss, stool consistency, and the appearance of blood in stools (27).

Antibodies. 2PH1, a rat-anti-mouse IgG2a, mAb, vs. PSGL-1 recognizes an epitope within the first 19 amino acids at the NH2-terminus of the processed form of mouse PSGL-1 and was generated by Borges et al. (4). PS2, a rat IgG vs. mouse VLA-4, reacting with the α4-chain of the VLA-4 integrin heterodimer and the isotype control antibody LO-DNP-1 were purchased from Serotec. The antibodies were dissolved in PBS before injection. Peripheral neutrophil counts were not affected by any of the used antibodies at 1 and 2 h after intraperitoneal injection (Table 1).

Table 1. Peripheral neutrophil counts after antibody administration

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>2PH1</th>
<th>PS2</th>
<th>LO-DNP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils (× 10³/μl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h pi</td>
<td>4.9±1.5</td>
<td>4.2±0.5</td>
<td>5.2±1.3</td>
<td>4.9±0.6</td>
</tr>
<tr>
<td>2 h pi</td>
<td>4.2±1.1</td>
<td>5.0±1.1</td>
<td>6.8±2.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3 experiments for each group. pi, Postinjection. Shown as peripheral neutrophil counts in healthy mice after administration of 2PH1, PS2, and LO-DNP-1. None of the antibodies used affected peripheral neutrophil counts 1 and 2 h after ip injection.

Intravital microscopy. In isoflurane/N₂O inhalation anesthesia, the animals were placed in a supine position on a heating pad, and polyethylene catheters (Portex, Lythe, UK) were inserted in the right jugular vein and the left carotid artery for intravenous administration of erythrocytes, contrast dyes, and antibodies as well as for monitoring of arterial blood pressure and heart rate throughout the experiment (Servomed, Hellige, Germany). Homologous erythrocytes were stained in vitro with FITC (Sigma-Aldrich) before intravenous injection. Leukocytes were stained in vivo by intravenous injection of 17.5 µg/100 g Rhodamin 6G (Sigma-Aldrich) immediately before intravital microscopy (IVM), as described previously (33). With the epiluminescence technique, leukocyte-endothelial cell interaction was visualized in a randomly selected colonic submucosal venule (diameter 60–90 µm) after 15 min of equilibration (1). Final magnification of ×760 was achieved by ×160/0.5-mm water immersion objective (Plan-Neofluar, Zeiss, Germany) and a video camera using 0.5 zoom (FK 6990-IQ). Microscopic images were recorded for off-line quantitative assessment of microcirculatory parameters using a computer-assisted analysis system (analySIS; Soft Imaging System, Muenster, Germany). In each vessel, the mean value of five venular diameters (D) was calculated. Central line erythrocyte velocity (V) and leukocyte rolling velocity were determined by calculating the mean velocity of five single frame-to-frame tracked fluorescent cells. Flow rate (F) was calculated using the formula F = π × (D²/4) × V × t/Vt, where t is time. Leukocytes were defined as adherent when attached to the vessel wall for at least 30 s and as rolling when moving with a velocity < ½ of that of erythrocytes at the center line of the observed microvessel. Rolling and 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 (33).

Tissue analysis. For histological studies, the entire colon was excised. The colon was opened longitudinally and washed with saline. Each colon was divided in four parts, representing cecum with appendix and 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. Histological inflammation was scored in a blinded fashion using a modification of a score introduced by Dieleman et al. (8), which reflects the degree of inflammation, the vertical extent of inflammation, and the crypt damage score, related to the percentage of involvement in each single slide (8). Myeloperoxidase (MPO) is contained in cytoplasmatic granules of neutrophils and monocytes (20). MPO activity correlates with the presence of neutrophils in intestinal inflammation (21). Tissue MPO activity was measured using the o-dianisidine assay, as previously described (22).

Experimental protocol. In the first series of experiments, we examined the effects of mAbs against PSGL-1 (2PH1) and VLA-4 (PS2) on leukocyte-endothelial interactions in colonic submucosal venules in inflamed animals by IVM. After baseline leukocyte adhesion was recorded for 10 min, the antibody (2 mg/kg each) was injected intravenously under direct IVM vision. This dose was based...
Table 2. Hemodynamic parameters in submucosal venules of the distal colon

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diameter, µm</th>
<th>Flow, µl/min</th>
<th>Shear Rate, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls + PBS</td>
<td>51.4 ± 1.5</td>
<td>198.5 ± 25.7</td>
<td>231.6 ± 14.3</td>
</tr>
<tr>
<td>0 min</td>
<td>52.5 ± 2.3</td>
<td>200.5 ± 21.3</td>
<td>209.6 ± 22.7</td>
</tr>
<tr>
<td>45 min</td>
<td>54.2 ± 2.9</td>
<td>219.7 ± 36.2</td>
<td>228.2 ± 12.1</td>
</tr>
<tr>
<td>DSS + LO-DNP-1</td>
<td>54.9 ± 0.6</td>
<td>246.6 ± 11.7</td>
<td>253.7 ± 16.1</td>
</tr>
<tr>
<td>0 min</td>
<td>55.3 ± 1.0</td>
<td>219.5 ± 12.0</td>
<td>221.9 ± 14.7</td>
</tr>
<tr>
<td>45 min</td>
<td>55.5 ± 1.5</td>
<td>237.1 ± 19.4</td>
<td>234.8 ± 14.9</td>
</tr>
<tr>
<td>DSS + 2PH1</td>
<td>48.4 ± 2.9</td>
<td>169.4 ± 23.9</td>
<td>259.2 ± 23.7</td>
</tr>
<tr>
<td>0 min</td>
<td>48.1 ± 2.4</td>
<td>155.9 ± 15.5</td>
<td>245.3 ± 27.9</td>
</tr>
<tr>
<td>45 min</td>
<td>46.9 ± 1.5</td>
<td>152.3 ± 13.2</td>
<td>256.3 ± 27.7</td>
</tr>
<tr>
<td>DSS + PS/2</td>
<td>57.7 ± 2.7</td>
<td>215.3 ± 16.5</td>
<td>192.4 ± 11.3</td>
</tr>
<tr>
<td>0 min</td>
<td>56.7 ± 2.2</td>
<td>215.1 ± 18.5</td>
<td>197.3 ± 7.4</td>
</tr>
<tr>
<td>45 min</td>
<td>58.4 ± 2.5</td>
<td>231.5 ± 22.8</td>
<td>195.6 ± 7.5</td>
</tr>
<tr>
<td>DSS + 2PH1 + PS/2</td>
<td>58.8 ± 4.3</td>
<td>281.6 ± 56.9</td>
<td>250.1 ± 15.5</td>
</tr>
<tr>
<td>0 min</td>
<td>58.1 ± 3.2</td>
<td>285.9 ± 46.1</td>
<td>244.8 ± 7.2</td>
</tr>
<tr>
<td>45 min</td>
<td>56.5 ± 3.4</td>
<td>283.7 ± 44.6</td>
<td>257.7 ± 11.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 experiments in each group. DSS, dextran sodium sulfate. Flow was calculated by off-line analysis of the recorded images. During the entire observation period, no changes in venule diameters, blood flow, or shear rates occurred. Measurements were taken in a stable hemodynamic system. In chronic DDS colitis, blood flow in the distal colon is not impaired.

on previous studies (4). All standard parameters of IVM that define leukocyte/endothelial adhesive interactions were measured 1, 5, 10, 20, 30, 45, and 60 min after antibody administration. Control animals received 2 mg/kg of an isotype-matched unspecific antibody. Also, the combination of 2PH1 and PS/2 was tested. Each group included seven animals (n = 7).

In a separate series of experiments, we investigated whether an established chronic colitis can be influenced by immunoblockade of PSGL-1 or VLA-4. Colitic animals were treated by daily intraperitoneal injections of either 2PH1 or PS/2 or the combination of both, each 2 mg/kg, over 5 days after completing the third DSS cycle. The treatment groups were compared with a healthy control group receiving Millipore water alone and a diseased control group, which received the antibody carrier PBS alone (n = 7 for each group). DAI scores were recorded daily. After 5 days of antibody treatment, the animals were killed, and the colon was prepared for histology and MPO measurements, as described above.

Statistics. Results are given as mean values ± SE. Statistical evaluations were performed using the Kruskal-Wallis ranking test for unpaired samples. Wilcoxon signed-rank test was applied for paired samples. P values < 0.05 were considered significant.

RESULTS

IVM. Hematological investigations of peripheral blood in colitic mice showed 18.5% neutrophils, 78.2% lymphocytes, 1.5% monocytes, and 1.4% eosinophils after completion of three DSS cycles. In each group, the hemodynamic parameters vessel diameter, blood flow, and shear rate remained unchanged during the entire observation period (Table 2). Intra-venous injection of 2PH1 led almost immediately to a dramatic drop in the numbers of rolling leukocytes. Baseline number of rolling leukocytes in colitic animals was 148.7 ± 29.8/0.01 mm²/30 s, whereas 1 min after administration of 2PH1 rolling leukocytes were reduced to 36.9 ± 8.7/0.01 mm²/30 s (P < 0.05). This effect lasted throughout the entire 60 min (Fig. 1). Mean leukocyte rolling velocity is a parameter for the tightness of selectin and selectin-ligand binding. Administration of 2PH1 resulted in an increase of leukocyte velocity from 24.0 ± 3.6 to 127.8 ± 11.7 µm/s (P < 0.05). The maximal increase in rolling velocity was reached 10 min after the antibody application and was maintained for the entire observation time (Fig. 2). In contrast, 2PH1 did not influence leukocyte firm adhesion. During the 60 min of observation, the number of adherent leukocytes maintained at ~25/0.01 mm²/30 s. PS/2 mainly mediated firm adherence (Fig. 3). After antibody application (20 min), adherent leukocytes were significantly reduced from 23.5 to 5.9/0.01 mm²/30 s (P < 0.05). After 60 min, this was further reduced to 3.7 firm adherent leukocytes/0.01 mm²/30 s. However, PS/2 also reduced leukocyte rolling (from 154.2 to 109.4/0.01 mm²/30 s, P < 0.05), but clearly less than 2PH1. Inhibition of rolling was delayed compared with 2PH1, and the maximal suppression was reached after 20 min (Fig. 1) but was

Fig. 1. Effects of 2PH1 and PS/2 on leukocyte rolling in submucosal venules of the chronically inflamed distal colon. Arrow indicates the time of antibody application. Blockade of P-selectin glycoprotein-ligand-1 (PSGL-1) almost immediately decreased the number of rolling leukocytes by ~80%. Anti-very late antigen-4 (VLA-4) also influenced leukocyte rolling, but to a lesser extent. The combination of both antibodies did not cause any additional effects.
maintained for the rest of the observation period. Rolling velocity was slightly increased, but not statistically significant (Fig. 2). The combination of 2PH1 and PS/2 showed no additional effects, although rolling velocity was slightly increased. The time course of reduction of adherent leukocyte in the combination group was similar to the group that received PS/2 alone. The isotype control antibody LO-DNP-1 caused no changes in leukocyte rolling, in rolling velocity, or firm adherence.

Treatment trials. There were no complications associated with intraperitoneal injections, and the carrier PBS did not alter the course of colitis in the disease control group (Fig. 4). Treatment with 2PH1 significantly reduced DAI compared with DSS controls (0.8 ± 0.1 vs. 2.1 ± 0.2 points, \( P < 0.05 \)). Administration of PS/2 also led to a significant decrease of DAI (0.9 ± 0.1 points, \( P < 0.05 \)) that was not significantly different from 2PH1. However, the combination of both antibodies reduced DAI stronger (0.4 ± 0.1 points), which was significantly better than either antibody alone (\( P < 0.05 \)), but did not reach the levels of healthy controls. MPO activity was significantly increased in both the proximal and distal colon of mice with chronic DSS colitis compared with healthy controls [26.9 ± 6.3 vs. 7.8 ± 1.0 U/g, \( P < 0.05 \) (proximal), respectively, 28.9 ± 8.4 vs. 3.8 ± 0.8 U/g, \( P < 0.05 \) (distal)]. There was no statistical significance in MPO levels between the proximal and distal colon. Treatment with 2PH1 significantly reduced MPO levels in both the proximal (7.1 ± 1.7 U/g) and the distal (7.2 ± 2.3 U/g, \( P < 0.05 \); Fig. 5, A and B) colon. Treatment with PS/2 also significantly decreased MPO activity in both the proximal (9.1 ± 2.7) and distal (13.8 ± 4.5 U/g) colon compared with diseased controls, but not as strong as 2PH1. However, there was no significant difference between either treatment group. The combination of both antibodies also reduced MPO levels in the proximal (7.2 ± 2.3 U/g) and distal (7.8 ± 2.2 U/g) colon compared with diseased controls, but there was no significant difference compared with either 2PH1 or PS/2 alone. The severity of histological inflammation in chronic DSS colitis increased toward the distal colon. The
distal colon (21.4 ± 4.6 points) was significantly more inflamed than the proximal colon (12.2 ± 1.1 points, P < 0.05). Administration of 2PH1 led to a significant reduction of inflammation in all parts of the colon (Fig. 6). This was most pronounced in the distal colon. PS/2 also reduced inflammation in all parts of the colon, which was similar to the results obtained with 2PH1. Combined treatment of both 2PH1 and PS/2 was significantly more effective in reducing colitis than either antibody alone in the middle colon (7.7 ± 0.3) and in the distal colon (9.7 ± 0.9, P < 0.05; Figs. 6 and 7).

**DISCUSSION**

In this study, we examined whether PSGL-1 is important for the recruitment of inflammatory cells in the inflamed colon using a murine model of IBD. An mAb against the NH2 terminus of mouse PSGL-1 that blocks adhesion of myeloid cells to P-selectin under static and flow conditions was used. The effects of PSGL-1 blockade were compared with an mAb against VLA-4. We were able to show that anti-PSGL-1 was responsible for almost an 80% reduction of rolling leukocytes in chronic intestinal inflammation in vivo and that long-term blockade of PSGL-1 ameliorated chronic colitis. This implicates chronic inflammation can be improved merely by reduction of leukocyte rolling. Furthermore, we showed that combined treatment with anti-VLA-4 (PS/2) and anti-PSGL-1 (2PH1) was more effective in terms of reduction of intestinal inflammation than either antibody alone.

Chronic DSS colitis is mediated by both Th1 and Th2 lymphocytes. The proinflammatory cytokines IFN-γ, IL-4, and IL-6 are increased in the colonic tissue of DSS-induced colitis (8, 13). In chronic DSS colitis, both ICAM-1 and VCAM-1 are upregulated in colonic venules, and VLA-4 has been detected on the surface of neutrophils, lymphocytes, and monocytes (13). Anti-neutrophil serum attenuates DSS colitis in rats, indicating that neutrophils are involved in the inflammatory process as well (9). This is also reflected by increased colonic MPO levels, which correlate with the amount of neutrophils in the tissue (22). During the acute phase of DSS colitis, the inflammatory infiltrate in the colon consists mainly of neutrophils, whereas in later stages of DSS colitis there is a change toward a lymphocytic infiltrate, as shown by histological studies (27, 29). PSGL-1 is expressed on B and T lymphocytes, and peripheral neutrophils also express PSGL-1 on their surface (6, 11, 40). VLA-4 is expressed on various leukocytes, such as lymphocytes, neutrophils, monocytes, and natural killer cells (3, 15). Because both 2PH1 and PS/2 diminished inflammation in chronic DSS colitis, we conclude that both adhesion molecules play an important role in the progression and maintenance of inflammation in this model.

There is evidence that P-selectin may be the most important selectin responsible for leukocyte rolling in the model of DSS colitis (43). This implicates a comparable role for its ligand PSGL-1. In chronically inflamed animals, a single intravenous injection of 2PH1 led almost immediately to an 80% decrease in rolling leukocytes in submucosal venules in the distal colon. This shows that leukocyte rolling is mainly mediated by PSGL-1 in this model, although it remains unclear whether it interacted exclusively with P-selectin. Because 2PH1 did not completely block leukocyte rolling although given in saturating amounts, it becomes clear that further selectin ligands or integrins ought to be involved in leukocyte rolling. To a lesser degree, α4-integrin is involved in leukocyte rolling. Interestingly, the reduction of leukocyte rolling after intravenous injection of PS/2 developed slowly and became more obvious after 20 min. This indicates that the role of α4-integrin is an indirect one, for example, resulting from a reduction of leukocyte activation and release of cytokines. However, in murine carotid arteries, it has been shown that the numbers and velocity of rolling mononuclear cells could be reduced by an antibody directed to α4-integrin (32). Based on these findings,
we expected a stronger reduction of leukocyte rolling when both antibodies 2PH1 and PS/2 were injected simultaneously. However, no additional effects on leukocyte rolling were observed in our series, although the results of each single mAb for rolling and sticking were repeated and, in summation, leukocyte adhesion was clearly diminished. 2PH1 did not influence leukocyte firm adhesion in this model. The function of PSGL-1 seems to be very specific and limited to leukocyte rolling. This does not necessarily implicate that PSGL-1 exclusively binds to P-selectin. It has been shown that PSGL-1 also interacts with E-selectin and possibly also to other selectins (16, 44). There is also evidence that neutrophils expressing

Fig. 5. Myeloperoxidase (MPO) activity in the proximal colon (A) and distal colon (B) expressed as U/g tissue. Treatment with 2PH1 significantly reduced MPO activity compared with diseased controls ($P < 0.05$). PS/2 was also able to decrease MPO activity, but to a lesser extent. The combination of both antibodies did not reach beneficial effects in terms of MPO activity.
the α4-integrin can directly roll and adhere to endothelial cells expressing VCAM-1 under certain inflammatory conditions (17). Therefore, it was surprising that the combination of 2PH1 and PS/2 could not further decrease leukocyte rolling in our model. However, this could also indicate that PSGL-1 and VLA-4 share functions in leukocyte recruitment in chronic DSS colitis. This is supported by observations in other models of inflammation, for example, experimental autoimmune encephalitis, where their ligands P-selectin and α4-integrin have overlapping roles (19). In contrast, PS/2 clearly diminished leukocyte firm adhesion. There was a gradual reduction of adherent leukocytes in colonic submucosal venules, which reached a maximum ~45 min after intravenous injection of PS/2. α4-Integrin binds to VCAM-1 expressed on endothelial cells, and VCAM-1 is upregulated under inflammatory conditions. There is also evidence that α4-integrin mediates leukocyte adherence independent from VCAM-1. The decrease in leukocyte firm adherence could be explained by a loosening of α4-VCAM-1 connections and a detachment of already adherent leukocytes from the endothelial cells. Alternatively, the development of de novo α4-VCAM-1 interactions was inhibited. In our experimental setting, a differentiation of these two mechanisms was not possible. However, it is most likely that both mechanisms are involved. Also, in terms of leukocyte firm adherence, the combination of both antibodies did not cause a further decrease, although both antibodies were given in saturating amounts. The effects of each single mAb were repeated exactly when given together. This raises the question of how long a leukocyte rolls along the endothelium until firm adhesion by activation of CD18 β2-integrins occurs. Obviously, other cell adhesion molecules are able to take over functions in leukocyte adherence when PSGL-1-P-selectin and VLA-4-VCAM-1 interactions are blocked. Candidates are E- or L-selectin and ICAM-1, respectively, which could develop increasing importance in the absence of PSGL-1 or VLA-4.

The concept of blocking cell adhesion molecules to influence inflammation is based on the observation that there is a permanent influx of inflammatory cells at sites of inflammation, whereas only a few migrated leukocytes proliferate within the tissue. In the present study, repeated treatment with 2PH1 ameliorated established murine colitis, although this is a result of a bare decrease of leukocyte rolling by inhibition of selectin function. However, in animal models of IBD, results of therapeutic selectin blockade remain controversial. Although IVM studies showed that leukocyte rolling could be reduced by blocking selectins with P-selectin as the most effective selectin in acute colitis (36, 43), long-term treatment did not significantly alter or even deteriorate colitis (25, 36). So why is blockade of PSGL-1 more effective than blockade of P-selectin in this model? Probably because PSGL-1 may bind to additional selectins or other partner molecules that are different from P-selectin and thereby inhibit nearby all selectin-mediated rolling. These additional interactions seem to be relevant for in vivo neutrophil recruitment in chronic DSS colitis. In this context, there is evidence that limiting the initial steps of leukocyte rolling to a minimum also reduces further adhesion and migration into the tissue and thus reduces intestinal inflammation. Nevertheless, a baseline leukocyte adhesion capacity is required for health maintenance. This becomes obvious since double knockout mice for P-selectin and ICAM-1 showed an increased morbidity in 2,4,6-trinitrobenzenesulfonic acid colitis (25) and P-selectin-deficient mice have increased susceptibility to DSS as well (13). Mice lacking both P- and E-selectin have severe neutrophilia and spontaneous skin infections that limit their life span (18). However, in our model, combined treatment with anti-PSGL-1 and anti-VLA-4 was more effective in reducing colitis than either antibody alone. Therefore, it is important to determine which specific cell adhesion molecules are blocked to obtain sustained suppression of inflammation and to avoid deleterious effects. 2PH1 could not completely abrogate colitis compared with healthy animals. The absence of selectins specifically impairs neutrophil recruitment but leaves mononuclear cell recruitment relatively intact (18). Especially in chronic DSS colitis, CD+...
T cells with increased resistance toward apoptosis (8) play an important role, and these may have been responsible for maintaining inflammation. Interestingly, the amelioration of DSS colitis after treatment with 2PH1 was as strong as after treatment with PS/2. Blockade of VLA-4 is well known to inhibit inflammation in various models of colitis, since mAbs toward the α4-integrin attenuate colitis in the cotton top tamarin (31). In this context, downregulation of inflammation by PS/2 may be mediated by inhibiting both VCAM-1 and MAdCAM-1 interactions. However, treatment with PS/2 did not completely reduce inflammatory parameters. Maybe this would occur when a longer treatment schedule would have been chosen, but this remains speculative. Therefore, we also can not predict if inflammation relapses when cell adhesion blockade is discontinued. However, the successful suppression of inflammation by blockade of PSGL-1 and VLA-4 in this model of colitis raises the question if this might have impact on human IBD as well. For example, blockade of other adhesion molecules such as ICAM-1 by means of antisense oligonucleotides in patients with steroid-dependent Crohn’s disease resulted in long-term steroid-free remission (45). In fact, there are promising results from Natalizumab, a neutralizing humanized antibody vs. the α4-integrin (12). PSGL-1 has been detected in patients with IBD as well (35). Therefore, we postulate that a humanized antibody vs. PSGL-1 may have anti-inflammatory effects on human IBD as well. Targeting PSGL-1 with mAbs seems to be a promising novel concept for IBD treatment, which might be the subject of clinical studies in the future.

**GRANTS**

This work was supported by a grant of the Crohn and Colitis Foundation of Germany.
REFERENCES


