Endothelial cell adhesion molecule expression in gene-targeted mice

MICHAEL J. EPPIHIMER,¹ JANICE RUSSELL,¹ DONALD C. ANDERSON,² BARRY A. WOLITZKY,³ AND D. NEIL GRANGER³

¹Department of Molecular and Cellular Physiology, Louisiana State University Medical Center, Shreveport, Louisiana 71130; ²Discovery Research, Pharmacia and Upjohn Inc., Kalamazoo, Michigan 49001; and ³Division of Inflammation/Autoimmune Diseases, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

This study was designed to determine whether gene-targeted mice that are deficient in CD11/CD18, intracellular adhesion molecule-1 (ICAM-1), or P-selectin exhibit an altered constitutive or induced expression of the endothelial cell adhesion molecules E- and P-selectin. The gene-targeted mice were all developed in the 129Sv mouse strain and backcrossed into C57Bl/6J mice. The number of backcrosses ranged between 8 (P-selectin) and 10 (CD18 and ICAM-1) generations. The dual-radio-labeled monoclonal antibody technique was used to quantify E- and P-selectin expression in different vascular beds. In the unstimulated state, E-selectin expression was significantly elevated (relative to wild-type mice) in the stomach, large intestine, and brain of mutants deficient in ICAM-1. In general, constitutive expression of P-selectin did not differ between wild-type, ICAM-1-deficient, and CD11/CD18-deficient mutants. In CD11/CD18-deficient mice, tumor necrosis factor-α (TNF-α) administration elicited a more profound upregulation of P-selectin in several vascular beds, compared with wild-type and ICAM-1-deficient mice. E-selectin expression in brain of TNF-α-stimulated, ICAM-1-deficient, and P-selectin-deficient mice was attenuated compared with wild-type mice. These findings indicate that chronic deficiency of some of the adhesion glycoproteins that mediate leukocyte recruitment alters basal and induced surface expression of other adhesion molecules on endothelial cells.

β₂-integrins; E-selectin; P-selectin; intracellular adhesion molecule-1

Egress of leukocytes from the vasculature involves a cascade of adhesive events that begins with leukocyte rolling movement along the vessel wall and the subsequent firm adhesion to the endothelial cells. It is generally recognized that selectins mediate leukocyte rolling in inflamed postcapillary venules. The selectins represent a family of three (L-, E-, and P-selectin) structurally similar carbohydrate-binding lectins, consisting of an NH₂-terminal lectin domain, an epidermal growth factor domain, and a series of consensus repeats similar to those in complement proteins (10). L-selectin is expressed on the surface of all leukocytes and is shed by proteolysis with leukocyte activation (12). Recent studies (6) have demonstrated that P-selectin, but not E-selectin, is constitutively expressed on the surface of endothelial cells in many tissues. After stimulation of endothelial cells and platelets with agents such as histamine or thrombin, P-selectin is rapidly (within minutes) translocated from the secretory storage granules to the cell surface (7, 21). In addition, P-selectin appears to be regulated by transcription-dependent mechanisms that function in parallel to, but independent of, the rapidly induced translocation of P-selectin from storage granules in endothelial cells (9). E-selectin expression, on the other hand, is controlled almost exclusively by activation pathways that require de novo synthesis of new adhesion glycoprotein (2).

Numerous studies have attempted to define the specific leukocyte and endothelial cell adhesion molecules that sustain the leukocyte trafficking which occurs in inflamed microvessels (8, 17, 29). Experimental strategies that have been used to delineate these molecular determinants of adhesion include immunoneutralization of adhesion glycoproteins with monoclonal antibodies (MAbs) and gene-targeted mice that are deficient in one or more cell adhesion molecules. Gene-targeting technology has resulted in the production of mice that are deficient in each of the three selectins (L-, E-, and P-selectin) (1, 4, 16, 20). Studies of P-selectin-deficient mice have revealed that leukocyte accumulation is significantly attenuated 2–4 h after Streptococcus pneumoniae injection, compared with that observed in wild-type mice (4). Intravital microscopic observations of postcapillary venules in P-selectin-deficient mice have demonstrated a significant reduction in the number of rolling leukocytes under control and inflammatory conditions, compared with venules of wild-type mice (15). Similar leukocyte trafficking studies have been performed using mice that are deficient in either intracellular adhesion molecule-1 (ICAM-1) or the leukocyte adhesion glycoprotein CD11/CD18 (Mac-1) (25, 28). For example, it has been shown that ICAM-1-deficient mice have elevated circulating neutrophil counts and are protected against lethal doses of lipopolysaccharide (LPS). Furthermore, S. pneumoniae-induced leukocyte emigration into the peritoneum of ICAM-1-deficient mice is significantly attenuated compared with that in wild-type mice (4). Similar reductions in leukocyte recruitment have been reported in mice that are genetically deficient in CD11/CD18 (25).

It has generally been inferred that gene targeting of cell adhesion molecules in embryonic stem cells results in either the deletion or an attenuated expression of the
targeted glycoprotein in the resultant mutant mice. This assumption is supported by an absence of the gene encoding for the targeted adhesion molecule, as assessed by Southern blot analysis (1, 4, 16, 20, 25, 28). It remains unclear, however, whether gene-targeted mice exhibit an altered expression of only those adhesion molecules that have been genetically deleted or whether the chronic deletion of one major leukocyte homing receptor affects the basal or stimulated expression of other adhesion glycoproteins on endothelial cells. The recent development of a method that employs radio-labeled MAbs to quantify the expression of adhesion molecules on endothelial cells in different vascular beds provides a means to address these unresolved issues related to gene-targeted mice (6, 11, 23). Hence, in the present study we measured the expression of E- and P-selectin in different vascular beds of mice that are deficient in P-selectin, ICAM-1, or CD11/CD18 and in wild-type mice. E- and P-selectin expression were measured in these mice under unstimulated conditions and after challenge with either tumor necrosis factor-α (TNF-α) or bacterial endotoxin.

**METHODS**

Mabs. MAbs used for the in vivo assessment of P- and E-selectin were RB40.34, a rat immunoglobulin (Ig) G1 against mouse P-selectin (PharMingen, San Diego, CA) (3), and 10E6, a rat IgG2 against mouse E-selectin (22), respectively. The antibody, RB40.34, directed against P-selectin has been shown by immunohistochemical staining to be localized on endothelial cells and platelets in blood vessels of wild-type mice and to be absent in mice deficient of the P-selectin gene (4). P-23, a nonbinding murine IgG1 directed against human P-selectin in different vascular beds of mice that are deficient in P-selectin, ICAM-1, and CD18-deficient mice were prepared and provided by Pharmacia-Upjohn (Kalamazoo, MI) (4, 25, 28). All of the mice were obtained at 4 wk of age and maintained on standard mouse diet until before the experiment.

Mice were anesthetized intraperitoneally with a mixture of ketamine and xylazine at a dose of 150 and 7.5 mg/kg, respectively. The left jugular vein and descending abdominal aorta were cannulated with polyethylene tubing (PE-10). To assess endothelial cell adhesion molecule (ECAM) expression, a mixture of 10 µg of either 125I-P-selectin MAb (RB 40.34) or 125I-E-selectin MAb (10E6) and a dose (0.5–5.0 µg) of 123I-nonbinding MAb (P-23) were injected through the jugular vein catheter. A blood sample was obtained through the abdominal aorta catheter at 5 min after injection of the MAb mixture. The animals were then heparinized (40 U heparin sodium) and rapidly exsanguinated by perfusion with bicarbonate-buffered saline (BBS) through the jugular vein catheter with simultaneous blood withdrawal through the abdominal aorta catheter. This was followed by perfusion of 10 ml BBS through the abdominal aorta catheter after severing the inferior vena cava at the thoracic level. Entire organs were harvested and weighed.

Calculation of E- and P-selectin expression. The method for calculating the expression of E- and P-selectin has been described previously (6, 23). In brief, the 125I (binding MAb) and 131I (nonbinding MAb) activities in different tissues and in 50-µl samples of cell-free plasma were counted in a 14800 Wizard 3 gamma-counter (Wallac, Turku, Finland), with automatic correction for background activity and spillover. The total injected activity in each experiment was calculated by counting a 4-µl sample of the radiolabeled MAb mixture. The radioactivities remaining in the tube used to mix the MAbs and the syringe used to inject the mixture were subtracted from the total injected activity and were on average <1% of the total injected activity. The accumulated activity of each MAb in an organ was expressed as the percentage of the injected activity per gram of tissue (% D. g).

E- and P-selectin expression were calculated by subtracting the accumulated activity per gram of tissue of the nonbinding MAb (123I-MAb P-23) from the activity of the binding anti-E-selectin MAb (125I-MAb 10E6) or anti-P-selectin MAb (125I-MAb RB40.34), respectively. Previous studies have shown that MAbs retain their functional activity after radioiodination as evidenced by a similar effectiveness of labeled and unlabeled MAbs to block leukocyte adherence in rat mesenteric venules (23).

Experimental protocols. As demonstrated previously, the dose of anti-E- and anti-P selectin MAbs that saturated all adhesion receptors was found to be 10 µg for each MAb (6). The following protocols were employed to assess potential differences in constitutive TNF-α-induced expression of E- and P-selectin in different vascular beds between wild-type and gene-targeted mice. Constitutive and induced expression of E- and P-selectin were assessed by injecting a mixture of radiolabeled binding and nonbinding MAbs into the mouse circulation. The mixture consisted of either 10 µg of labeled anti-E-selectin (10E6) or anti-P-selectin MAb (RB40.34) and a dose of labeled nonbinding MAb (P-23) ranging from 0.5 to 5 µg. A variable dose of nonbinding MAb was used to compensate for the decay in activity of the 123I isotope, which has a half-life of ~8 days. The amount of MAb P-23 injected into a mouse was determined so as to have a total injected radioactivity of ~500,000 cpm. On the basis of previously determined kinetics of E- and P-selectin expression, we chose to measure E- and P-selectin expression in different tissues of wild-type and gene-targeted mice at 3 and 4 h after the mice received an intraperitoneal injection of recombinant murine TNF-α (Sigma) at a dose of 25 µg/kg. The 125I-MAb 10E6 and 123I-MAb RB40.34 bind specifically to its ligand, as evidenced
by an absence of $^{125}$I-MAb 10E6 and $^{125}$I-MAb RB40.34 accumulation in tissues of mice deficient of E- and P-selectin, respectively, compared with wild type (6).

Statistics. Average E- and P-selectin expression in a tissue (%I.D./g) was compared between genotypes of mice under unstimulated or TNF-$\alpha$-stimulated conditions using one-way analysis of variance, followed by the Bonferroni test for multiple comparisons. Comparison of the levels of selectin expression between an unstimulated and a TNF-$\alpha$-stimulated tissue of a given murine genotype was performed using a two-tailed $t$-test. Statistical significance for all tests was set at a value of $P < 0.05$.

RESULTS

Constitutive and TNF-$\alpha$-induced P-selectin expression in genetargeted mice. Significant differences were found in the accumulation of $^{125}$I-MAb RB40.34 (P-selectin-specific MAb) in various vascular beds of wild-type and gene-targeted mice under TNF-$\alpha$-stimulated conditions (Table 1). However, no significant differences in the constitutive expression of P-selectin was observed between wild-type and gene-targeted mice (P > 0.05). In the heart, stomach, large intestine, muscle, and brain of wild-type mice, an insignificant accumulation of $^{125}$I-MAb RB40.34 was observed under unstimulated conditions (P > 0.05). In all tissues of mutant mice, except in the lung and muscle of CD18- and ICAM-1-deficient mice, respectively, the accumulation of $^{125}$I-MAb RB40.34 in unstimulated tissues was significantly greater than zero (P < 0.05). In both mutant strains, there was an insignificant accumulation of $^{125}$I-MAb RB40.34 in unstimulated brain tissue. After TNF-$\alpha$ stimulation, a significant accumulation of $^{125}$I-MAb RB40.34 was observed in all tissues, compared with unstimulated conditions (P < 0.05). In TNF-$\alpha$-stimulated CD18-deficient mice, a significant increase in P-selectin expression was observed in the small intestine compared with TNF-$\alpha$-stimulated ICAM-1-deficient mice (P < 0.05) and in the heart compared with wild-type mice (P < 0.05). The expression of P-selectin in TNF-$\alpha$-stimulated ICAM-1-deficient mice was also significantly attenuated in the pancreas, stomach, and large intestine compared with that observed in TNF-$\alpha$-stimulated CD18-deficient mice. In addition, a significant elevation in P-selectin expression in the stomach and large intestine was observed in CD18-deficient mice, compared with wild-type mice.

Constitutive and TNF-$\alpha$-induced E-selectin expression in wild-type and gene-targeted mice. Significant differences were also noted in the tissue accumulation of $^{125}$I-MAb 10E6 (E-selectin-specific MAb) between wild-type and gene-targeted mice, under unstimulated and TNF-$\alpha$-stimulated conditions (Table 2). In all tissues except the heart, mesentery and small intestine, an insignificant level of E-selectin was observed in unstimulated tissues of wild-type mice (P > 0.05). In ICAM-1-deficient mice, all tissues except the lung and

Table 1. P-selectin expression under unstimulated and TNF-$\alpha$ conditions in wild-type and CD18- and ICAM-1-deficient mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Wild Type Constitutive</th>
<th>Wild Type TNF-$\alpha$</th>
<th>CD-18 Deficient Constitutive</th>
<th>CD-18 Deficient TNF-$\alpha$</th>
<th>ICAM-1 Deficient Constitutive</th>
<th>ICAM-1 Deficient TNF-$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>0.159 ± 0.052</td>
<td>0.954 ± 0.342</td>
<td>1.199 ± 0.148</td>
<td>0.212 ± 0.074</td>
<td>1.009 ± 0.300</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0</td>
<td>0.168 ± 0.033</td>
<td>0.022 ± 0.005</td>
<td>0.355 ± 0.048*</td>
<td>0.027 ± 0.006</td>
<td>0.207 ± 0.030</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.073 ± 0.009</td>
<td>0.669 ± 0.082</td>
<td>0.080 ± 0.004</td>
<td>0.967 ± 0.129</td>
<td>0.087 ± 0.008</td>
<td>0.549 ± 0.026†</td>
</tr>
<tr>
<td>Mesentery</td>
<td>0.155 ± 0.050</td>
<td>0.979 ± 0.231</td>
<td>0.073 ± 0.009</td>
<td>0.889 ± 0.190</td>
<td>0.063 ± 0.002</td>
<td>0.335 ± 0.032</td>
</tr>
<tr>
<td>Stomach</td>
<td>0</td>
<td>0.531 ± 0.033</td>
<td>0.089 ± 0.020</td>
<td>0.989 ± 0.150*</td>
<td>0.096 ± 0.032</td>
<td>0.446 ± 0.024†</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.088 ± 0.009</td>
<td>1.010 ± 0.160</td>
<td>0.100 ± 0.008</td>
<td>1.582 ± 0.303</td>
<td>0.119 ± 0.015</td>
<td>0.603 ± 0.099†</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0 ± 0.470 ± 0.067</td>
<td>0.455 ± 0.003</td>
<td>0.766 ± 0.094*</td>
<td>0.043 ± 0.010</td>
<td>0.311 ± 0.024‡</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.093 ± 0.012</td>
<td>0.008 ± 0.003</td>
<td>0.151 ± 0.021</td>
<td>0.002 ± 0.014</td>
<td>0.096 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0</td>
<td>0.045 ± 0.014</td>
<td>0.062 ± 0.012</td>
<td>0.025 ± 0.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. E-selectin expression under unstimulated and TNF-$\alpha$ Conditions in wild-type and CD18- and ICAM-1-deficient mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Wild Type Constitutive</th>
<th>Wild Type TNF-$\alpha$</th>
<th>CD-18 Deficient Constitutive</th>
<th>CD-18 Deficient TNF-$\alpha$</th>
<th>ICAM-1 Deficient Constitutive</th>
<th>ICAM-1 Deficient TNF-$\alpha$</th>
<th>P-Selectin Deficient Constitutive</th>
<th>P-Selectin Deficient TNF-$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>0</td>
<td>1.066 ± 0.185</td>
<td>0.125 ± 0.036</td>
<td>0.992 ± 0.160</td>
<td>0.841 ± 0.164</td>
<td>0.454 ± 0.133</td>
<td>0.454 ± 0.133</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.066 ± 0.006</td>
<td>0.403 ± 0.049</td>
<td>0.047 ± 0.017</td>
<td>0.308 ± 0.051</td>
<td>0.057 ± 0.011</td>
<td>0.451 ± 0.125</td>
<td>0.051 ± 0.014</td>
<td>0.237 ± 0.035</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.013 ± 0.004</td>
<td>0.316 ± 0.068</td>
<td>0.221 ± 0.036</td>
<td>0.113 ± 0.034</td>
<td>0.285 ± 0.026</td>
<td>0.708 ± 0.032</td>
<td>0.078 ± 0.029</td>
<td>0.261 ± 0.038</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.019 ± 0.008</td>
<td>0.289 ± 0.071</td>
<td>0.189 ± 0.033</td>
<td>0.040 ± 0.010</td>
<td>0.190 ± 0.035</td>
<td>0.044 ± 0.015</td>
<td>0.167 ± 0.033</td>
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</tr>
<tr>
<td>Small intestine</td>
<td>0.031 ± 0.003</td>
<td>0.326 ± 0.048</td>
<td>0.200 ± 0.031</td>
<td>0.316 ± 0.100*</td>
<td>0.276 ± 0.055</td>
<td>0.204 ± 0.059</td>
<td>0.389 ± 0.113</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.100 ± 0.050</td>
<td>0.032 ± 0.048</td>
<td>0.140 ± 0.050</td>
<td>0.102 ± 0.026*</td>
<td>0.139 ± 0.023</td>
<td>0.076 ± 0.028</td>
<td>0.263 ± 0.043</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0</td>
<td>0.083 ± 0.015</td>
<td>0.043 ± 0.014</td>
<td>0.059 ± 0.019</td>
<td>0.029 ± 0.010</td>
<td>0.066 ± 0.017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0</td>
<td>0.055 ± 0.008</td>
<td>0.029 ± 0.007</td>
<td>0.017 ± 0.004*</td>
<td>0.027 ± 0.003*</td>
<td>0.018 ± 0.006*</td>
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</table>

Values are means ± SE expressed as %I.D./g. Values not significantly different from 0 (P > 0.05) are reported as 0. *Significantly different from wild-type mice, P < 0.05.

Values are means ± SE expressed as percentage of the injected activity per g tissue (%I.D./g). TNF-$\alpha$, tumor necrosis factor-$\alpha$; ICAM-1, intracellular adhesion molecule-1. Values not significantly different from 0 (P > 0.05) are reported as 0. *Significantly different from CD-18 deficient mice, P < 0.05.
small intestine were noted to have a constitutive level of E-selectin that was significantly greater than zero (P < 0.05), whereas P-selectin-deficient mice exhibited significant elevations in constitutive E-selectin expression in all tissues except the lung, small intestine, and brain. In the stomach, large intestine and brain, the constitutive expression of E-selectin was noted to be significantly greater in ICAM-1-deficient mice, compared with wild-type mice (P < 0.05). After TNF-α injection, significant upregulation of E-selectin was noted in all tissues of ECAM-1-deficient mice except stomach, large intestine and muscle (P < 0.05). In these tissues, significant elevations in constitutive E-selectin expression were observed. However, after TNF-α stimulation, significant differences in E-selectin expression between mouse strains were observed only in the brain. In this tissue, E-selectin was noted to be significantly attenuated in P-selectin and ICAM-1-deficient mice compared with wild-type mice (P < 0.05).

DISCUSSION

Gene-targeted mice that are deficient in cell adhesion molecules are rapidly gaining acceptance as a tool for assessing the relative contribution of specific adhesion glycoproteins to leukocyte trafficking during inflammatory conditions (1, 4, 16, 20, 25, 28). An assumption that is inherent in the interpretation of data derived from these gene-targeted mice is that chronic deletion of one major leukocyte homing receptor does not affect the basal or stimulated expression of other adhesion glycoproteins on endothelial cells. This study represents the first effort to test this assumption using a dual radiolabeled MAb technique that provides quantitative measures of E- and P-selectin expression in different vascular beds of the mouse. Our findings indicate that, compared with wild-type mice, CD18-, ICAM-1-, and P-selectin-deficient mice exhibit an altered expression of E- and P-selectin under basal and/or TNF-α-stimulated conditions and that these alterations are often vascular bed specific.

MAbs and gene-targeted mice have been successfully used in studies demonstrating that ICAM-1 is a essential for the adhesion and subsequent emigration of leukocytes through the venular wall during inflammatory conditions (8). In general, ICAM-1-deficient mice exhibit a strong leukophilic response, with a two- to threefold increase in the circulating neutrophil population (25). Intravital microscopic observations of leukocyte rolling in the cremaster muscle of ICAM-1 mutants indicate that the number of rolling leukocytes is not significantly different from control (15). This observation agrees favorably with our observation that E- and P-selectin expression in muscle of TNF-α-stimulated, ICAM-1-deficient mice is similar to that observed in TNF-α-stimulated, wild-type mice. Inasmuch as P-selectin appears to be responsible for the leukocyte rolling associated with tissue exteriorization, it is not entirely surprising that wild-type and ICAM-1-deficient mice exhibit similar leukocyte rolling characteristics in muscle tissue (13, 15, 17). Indeed, intravital microscopic analyses of leukocyte rolling in mouse cremaster suggest that both E- and P-selectin must be inhibited to block this adhesion process (15). In addition to the skeletal muscle vasculature, other regional circulations exhibit a similar expression of E- and P-selectin in ICAM-1-deficient mice. However, in the stomach and large intestine, an exacerbated level of constitutive E-selectin expression was observed, suggesting that an increased leukocyte rolling flux may be expected in these postcapillary venules of ICAM-1-deficient mice, compared with wild-type mice. Unfortunately, quantitative measurements of leukocyte rolling in mouse venules of these tissues are extremely difficult to obtain with intravital microscopy.

Several studies have suggested that there may be differences in the expression of more than one adhesion molecule between ICAM-1-deficient mice and wild-type mice (5, 14, 24). This is evidenced by an invariance in Pseudomonas aeruginosa-induced neutrophil emigration in ICAM-1-deficient mice (compared with wild-type mice), whereas an ICAM-1-specific MAb reduces neutrophil emigration by 65% in P. aeruginosa-challenged wild-type mice (24). Similarly, neutrophil emigration into the peritoneum of LPS-stimulated, ICAM-1-deficient mice is similar to that observed in LPS-stimulated wild-type mice; however, treatment with either an anti-ICAM-1 MAb or an ICAM-1 antisense oligonucleotide effectively attenuates the LPS-induced neutrophil emigration (14). Furthermore, cobra venom factor-induced lung injury can be prevented by anti-ICAM-1 antibodies, but no such protection was demonstrated in mice deficient in ICAM-1, P-selectin, or both (5). When antibodies directed against ICAM-1 were administered to either of these gene-targeted mice, cobra venom factor-induced lung injury was not attenuated. It is possible that the antibodies and antisense oligonucleotides are inducing changes in endothelial function in addition to blocking the targeted adhesion molecule; however, this remains unclear and warrants further investigation. Regardless, these observations appear to suggest that ICAM-1-independent pathways of leukocyte recruitment may be altered in mice that are genetically deficient in ICAM-1. This possibility raises a concern about interpretation of data derived from some mutant mice. For example, it was recently shown that the size of cerebral infarctions after ischemia-reperfusion is significantly reduced in ICAM-1-deficient mice compared with wild-type mice (26). Although the authors appropriately attributed the blunted cerebral infarctions to an absence of ICAM-1, the results of our study suggest that the significant attenuation in E-selectin observed in the brain of ICAM-1-deficient mice may explain at least part of the attenuated leukocyte infiltration and microvascular injury observed after cerebral ischemia.

MAbs directed against the β2-integrin CD11/CD18 are known to be very effective in attenuating the leukocyte recruitment and tissue injury associated with several models of acute inflammation (8). Comparable findings have been reported using mice that are genetically deficient in CD11/CD18 (28). An interesting and potentially important observation in the present

...
study was the tendency for an enhanced expression of P-selectin in CD11/CD18-deficient mice compared with wild-type and ICAM-1-deficient mice. The enhanced levels of P-selectin in CD18-deficient mice may allow leukocytes to achieve a reduced rolling velocity that is sufficient for firm adhesion to endothelial cells via a CD11/CD18-independent pathway. The overexpression of P-selectin in splanchic tissues of CD18-deficient mice may account for the enhanced emigration of leukocytes into the peritoneum during inflammatory conditions (28).

The physiological basis for the altered expression of E- and P-selectin in gene-targeted mice is not readily apparent from the results of our study; however, there are several possible contributing factors that warrant some consideration. An outcome of our study that may shed light on underlying mechanisms is the general pattern of an enhanced expression (relative to wild-type and other genetically deficient mice) of selectins in mice that are genetically deficient in one of the endothelial cell adhesion molecules. A potential explanation for our findings is that endothelial cell adhesion molecule-deficient mice may produce greater levels of cytokines under basal conditions or after TNF-α to enhance selectin expression and retard leukocyte movement along the vascular endothelium. Yet another possibility for a disparity in the expression of endothelial selectins between ICAM-1 and wild-type mice may be explained (at least in part) by differences in the regulation of selectins in 129Sv vs. C57Bl/6 mice, since the adhesion molecule-deficient mice were originally bred on a 129Sv background and backcrossed to a C57Bl/6. However, this explanation appears unlikely for the data describing an attenuated expression of P-selectin in tissues of ICAM-1-deficient mice compared with CD18-deficient mice, since the backgrounds of these mice are similar.

Regardless of the molecular and cellular mechanisms that account for the observed alterations in E- and P-selectin expression in gene-targeted mice, the results of this study draw attention to the fact that chronic deletion of one major leukocyte homing receptor does indeed affect the basal or stimulated expression of other adhesion glycoproteins on endothelial cells. These responses of alternate endothelial cell adhesion molecules in gene-targeted mice should be considered when interpreting functional data derived from the same animal models.

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