Thrombogenic Effects of Antiphospholipid Antibodies Are Mediated by Intercellular Cell Adhesion Molecule-1, Vascular Cell Adhesion Molecule-1, and P-Selectin

Silvia S. Pierangeli, Ricardo G. Espinola, Xiaowei Liu, E. Nigel Harris

Abstract—Recent studies have shown that antiphospholipid (aPL) enhances expression of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on endothelial cells (ECs) and that these effects are correlated with increased adhesion of leukocytes to endothelium in cremaster muscle in vivo and with thrombosis in a mouse model. Activation of ECs by aPL may create a hypercoagulable state that precedes and contributes to thrombosis in patients with aPL syndrome (APS). This study proposed to examine whether this in vivo activation of ECs and enhanced thrombosis by aPL are mediated by ICAM-1, P-selectin, or VCAM-1. The dynamics of thrombus formation and the number of adhering leukocytes were studied in ICAM-1–deficient (ICAM-1−/−) mice or ICAM-1−/−/P-selectin−/− (ICAM-1−/−/P-selectin−/−) mice treated with affinity-purified aPL antibodies (ap IgG-APS) or with control IgG and compared with wild-type mice treated in a similar fashion. In another set of experiments, the adhesion of leukocytes to cremaster muscle and the dynamics of thrombus formation were studied in CD1 mice treated with aPL or control IgG before and 30 minutes after intravenous infusion with 100 μg monoclonal antibody anti–VCAM-1. The results indicate that the enhanced adhesion of leukocytes to endothelium in wild-type mice was significantly reduced in ICAM-1−/− and completely abrogated in ICAM-1−/−/P-selectin−/− mice treated with ap IgG-APS compared with wild-type mice treated with ap IgG-APS (6.9±2.3, 0.4±0.4 versus 35±12, respectively). More importantly, this correlated with a significant reduction in thrombus size compared with wild-type mice treated with ap IgG-APS (895±259 μm², 859±243 μm² versus 3816±672 μm², respectively). Infusion of the mice with anti–VCAM-1 antibodies significantly reversed the enhanced adhesion of leukocytes (14.9±3 to 11.3±2.1) and thrombus size (3830±1008 μm² versus 876±548 μm²) in mice treated with ap IgG-APS. The data indicate that ICAM-1, P-selectin, and VCAM-1 expression are important in thrombotic complications by aPL antibodies and may provide novel targets for therapy in patients with APS. (Circ Res. 2001;88:245-250.)

Key Words: antiphospholipid antibodies • intercellular adhesion molecule-1 • vascular adhesion molecule-1 • P-selectin

Antiphospholipid (aPL) antibodies are associated with aPL syndrome (APS), which is a syndrome of thrombosis, pregnancy loss, and thrombocytopenia.1 aPL antibodies have been shown to enhance thrombus formation and induce fetal resorption in mice, but the mechanisms involved are not completely understood.2–5 Several studies have demonstrated that aPL antibodies activate endothelial cells (ECs) in vitro, as demonstrated by enhanced expression of adhesion molecules (intercellular cell adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], and E-selectin) on human umbilical vein endothelial cells (HUVECs) and enhanced monocyte adherence to ECs in vitro.6,7 Our group recently showed that upregulation of expression of ICAM-1, VCAM-1, and E-selectin on ECs8 by aPL correlated directly with an increased adhesion of leukocytes to endothelium of mouse cremaster muscle, an indication of EC activation in vivo, and with enhanced thrombosis in vivo. In another study, George et al9 showed that upregulation of adhesion molecules by some murine monoclonal anti-β2 glycoprotein I (anti-β2GPI) antibodies correlated with fetal resorption in mice producing aPL antibodies. As additional support for the hypothesis that aPL antibodies activate ECs and may create an hypercoagulable state in APS patients, 2 recent studies indicated that the levels of soluble ICAM-1 and VCAM-1 were significantly increased in the plasma of patients with APS and recurrent thrombosis.10,11

Although there is a wealth of data suggesting that aPL antibodies activate ECs in vitro and in vivo and they enhance thrombosis, it is unclear what the relative roles are of the EC
adhesion molecules ICAM-1, VCAM-1, and P-selectin in these processes. To examine these questions, the present study used ICAM-1−deficient (ICAM-1−/−) or ICAM-1−/P-selectin−deficient (ICAM-1−/−/P-selectin−/−) mice to determine whether aPL antibodies enhance thrombus formation or increase leukocyte adhesion to ECs of mouse cremaster muscle compared with the corresponding wild-type mice. In both series of experiments, the thrombogenic effects were also examined by using a previously described mouse model of induced thrombosis2−4 and correlated with EC activation in vivo.8 Because VCAM-1−/− mice are not available, another series of experiments were conducted using monoclonal anti-VCAM-1 antibodies to determine the involvement of VCAM-1 in EC activation and enhancement of thrombus formation by aPL. The activation of ECs and the enhancement of thrombus formation in vivo induced by aPL in mice were measured before and after the infusion of monoclonal antibody anti–VCAM-1. Our findings suggest that all 3 adhesion molecules play a role in aPL-induced EC activation and thrombus formation in vivo. This study provides new significant information in the molecular events mediating activation of ECs and thrombosis by aPL antibodies.

Materials and Methods

Mice

C57BL/6J–ICAM-1tm1Bay (ICAM-1−/−) male mice weighing ~30 g and ICAM-1−/−/P-selectin−/− and wild-type C57BL/6J mice of the same weight and genders were purchased from Jackson Laboratories (Bar Harbor, Mass). CD1 male mice weighing ~30 g were purchased from Charles River Laboratories (Wilmington, Del). Animals were handled by trained personnel according to institutional Animal Care and Use Committee guidelines under the supervision and care of veterinarians.

Affinity Purification of aPL Antibodies

aPL antibodies (ap IgG-APS) were obtained from the sera of one APS patient by affinity purification using precipitation with cardiolipin liposomes and elution with 1.5 mol/L NaCl followed by protein G Sepharose chromatography, as previously described.8 The patient was a 42-year-old man with 3 deep-vein thrombi, an anti-cardiolipin titer of 405 GPL units, which was lupus anticoagulant–positive (determined as described elsewhere), and anti-β2-GPI antibody titer of 93.5 SGU detected by ELISA (Quanta-Lite, INOVA Diagnostics, Inc). The ap IgG-APS preparation was also positive for IgG and anti-β2-GPI antibodies (98.5 GPL and 54.6 SGU units, respectively) and was lupus anticoagulant–positive.

Presurgical Treatments

The ability of aPL antibodies to activate ECs in vivo and enhance thrombus formation was studied by examination of leukocyte (white blood cell [WBC]) adhesion to endothelium in exposed cremaster muscle and study of the dynamics of thrombus formation in exposed femoral vein in the same mouse.2−4,8,12 ICAM-1−/−, ICAM-1−/−/P-selectin−/−, or wild-type C57BL/6J groups in 9 were treated by intraperitoneal injection with ap IgG-APS preparation at time 0 and 48 hours later (500 µg antibody per injection in 1 mL of sterile saline solution). Mice in control groups were treated in a similar fashion with control IgG (IgG-NHS). The 2 surgical procedures described in next paragraph were performed in the same mouse 72 hours after the first injection. Samples of blood to determine the titer of aCL antibodies by ELISA were drawn at that time (72 hours after the first injection with ap IgG-APS or IgG-NHS).

In some experiments, CD1 male mice in groups of 9 were treated with ap IgG-APS or IgG-NHS as described elsewhere.2−4 Seventy-two hours after the first injection, mice were infused intravenously in the jugular vein with 100 µg/0.1 mL of monoclonal antibody anti–VCAM-1 (Pharmingen Laboratories) or sterile saline solution.

Surgical Procedures

Analysis of EC Activation in the Microcirculation of the Exposed Cremaster Muscle in Mice

Activation of ECs in the pretreated mice was assessed by direct visualization and quantitation of leukocytes (WBCs) adhering to ECs in the microcirculation of the exposed cremaster muscle of mice, as described elsewhere.8,12 After a stabilization period of 30 minutes, the number of adhering WBCs that remained stationary for a period of ≥30 seconds (sticking) within 5 different venules (diameter, 25 to 35 µm) was determined. (Figure 1). The means were calculated and compared between treated and control groups.

Analysis of Thrombus Dynamics: Effects of aPL on Thrombus Formation

The analysis of thrombus dynamics in a mouse model has been described previously.2−4 In brief, mice were anesthetized 72 hours after the first injection with the ap IgG-APS or IgG-NHS, and the right femoral vein was exposed. The vein was pinched with a standardized pressure to introduce an injury and induce a clot. Clot formation and dissolution in the transilluminated vein were visualized with a microscope equipped with a closed-circuit video system (including a color monitor and a recorder). Thrombus size (in square micrometers) was measured when the thrombus reached the maximum size by digitizing the image and tracing the outer margin of the thrombus. Three to five thrombi were successfully induced in each animal, and mean values were analyzed. Mean thrombus area and mean times for formation, disappearance, and total times were then computed for each group of injected animals. The person performing the surgery and measurements (X.L.) was blinded to what treatment had been given to each animal.

Statistical Analysis

The number of animals needed per group was determined by power analysis. It was established that 9 to 10 animals would provide a statistical power (1−β) for detection of test-group differences in both thrombus size and activation of ECs in vivo. The unpaired Student’s t test was used to compare the means of thrombus sizes and adhering WBC numbers between treated and control groups. Statistical significance was considered to be achieved when P<0.05.
TABLE 1. Effects of aPL Antibodies on Adhesion of Leukocytes to Endothelium in ICAM-1\(^{-/-}\) and ICAM-1\(^{-/-}\)/P-Selectin\(^{-/-}\) Mice

<table>
<thead>
<tr>
<th>Mice (n=9 per group)</th>
<th>Treatment</th>
<th>Adhering Leukocytes (No.) (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J (wild-type)</td>
<td>IgG-NHS</td>
<td>14.0±0.5</td>
</tr>
<tr>
<td>C57BL/6J (wild-type)</td>
<td>ap IgG-APS</td>
<td>35.0±2.1</td>
</tr>
<tr>
<td>ICAM-1(^{-/-})</td>
<td>IgG-NHS</td>
<td>5.9±2.1</td>
</tr>
<tr>
<td>ICAM-1(^{-/-})/P-selectin(^{-/-})</td>
<td>ap IgG-APS</td>
<td>6.9±2.3†</td>
</tr>
<tr>
<td>ICAM-1(^{-/-})/P-selectin(^{-/-})</td>
<td>IgG-NHS</td>
<td>0.3±0.2</td>
</tr>
</tbody>
</table>

For mice treated with ap IgG-APS, aCL titer at the time of surgery (72 hours after the first injection with ap IgG-APS) was 89.5±35.6 for C57BL/6J (wild-type) mice, 35.1±21.8 GPL units for ICAM-1\(^{-/-}\) and 73.5±18.1 GPL units for ICAM-1\(^{-/-}\)/P-selectin\(^{-/-}\). There was no statistically significant difference between aCL levels in the 3 groups of mice. All 3 groups of mice infused with IgG-NHS were negative for aCL antibodies at the time of surgery (72 hours after the first injection with IgG-NHS).

*Statistically significantly different from wild-type mice treated with IgG-NHS.
†Statistically significantly different from wild-type mice treated with ap IgG-APS.
‡Statistically significantly different from wild-type mice treated with ap IgG-APS.

Results

aPL-Induced EC Activation Is Impaired in ICAM-1\(^{-/-}\) and ICAM-1\(^{-/-}\)/P-Selectin\(^{-/-}\) Mice

Adhesion of leukocytes to endothelium was significantly enhanced in wild-type (C57BL/6J) mice treated with ap IgG-APS compared with wild-type mice treated with IgG-NHS (35±12 versus 14±5; \(P=0.002\)) (Table 1). The mean aCL titer of the mice treated with ap IgG-APS was 89.5 GPL units (high positive), and all of the control mice tested negative for aCL antibodies. In comparison with wild-type mice, adhesion of leukocytes to endothelium was significantly less in ICAM-1\(^{-/-}\) mice, whether treated with IgG-NHS or ap IgG-APS. For animals treated with IgG-NHS, adhesion was 5.9±2.1 for ICAM-1\(^{-/-}\) compared with 14±5 for wild-type, as noted above (Table 1). For animals injected with ap IgG-APS, adhesion was 6.9±2.3 for ICAM-1\(^{-/-}\) compared with 35±12 for wild-type, as noted above (\(P=0.0006\)). There was no statistical difference in leukocyte adhesion in ICAM-1\(^{-/-}\) mice infused with ap IgG-APS (6.9±2.3) compared with those infused with IgG-NHS (5.9±2.1) (\(P=NS\)). The titers of aCL antibodies in ICAM-1\(^{-/-}\) mice treated with aPL was medium-high positive (63.1±21.8).

In the ICAM-1\(^{-/-}\)/P-selectin\(^{-/-}\) mice, leukocyte adhesion was very low, namely 0.3±0.2 for IgG-NHS compared with 14±5 for wild-type and 5.9±2.1 for ICAM-1\(^{-/-}\) (Table 1). For mice infused with ap IgG-APS, the aCL titer was 73.5±18.9 GPL units and the leukocyte adhesion was 0.04±0.4, compared with 6.9±2.3 for ICAM-1\(^{-/-}\) and 35±12 for wild-type mice.

VCAM-1 Mediates the Activation of ECs by aPL

The adhesion of leukocytes to endothelium was measured in CD1 mice treated with ap IgG-APS or IgG-NHS. As expected, the adhesion of leukocytes to ECs in cremaster muscle was significantly increased in mice treated with ap IgG-APS compared with IgG-NHS-treated mice (mean 14.9±3 versus 8.6±1.9, \(P=0.001\)) (Table 2). After infusion with anti–VCAM-1, the adhesion of leukocytes to endothelium was decreased from 14.9±3 to 11.3±2.1 in mice treated with ap IgG-APS. This decrease, although partial, was significant (\(P=0.045\)). In mice infused with IgG-NHS, the intravenous infusion of anti–VCAM-1 antibodies also produced a significant decrease of the adhesion of leukocytes to endothelium to 5.8±1.1, compared with 8.6±1.9 in mice treated with only IgG-NHS (\(P=0.002\)) (Table 2). Thus, anti–VCAM-1 antibody infusion decreased leukocytes in IgG-NHS–treated mice to 5.8±1.1, whereas in the presence of ap IgG-APS, leukocyte adhesion was significantly higher at 11.3±1.1, suggesting that expression of EC adhesion molecules contributes to EC activation in vivo.

aPL-Enhanced Thrombosis in Mice is Abrogated in ICAM-1\(^{-/-}\) and ICAM-1\(^{-/-}\)/P-Selectin\(^{-/-}\) Mice

C57BL/6J (wild-type) mice treated with ap IgG-APS, as indicated in Materials and Methods, produced significantly larger thrombi (3816±672 \(\mu m^3\)) compared with mice treated

TABLE 2. Effects of Anti–VCAM-1 Antibody on aPL-Induced Adhesion of Leukocytes to Endothelial Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adhering WBCs Before Infusion With Anti–VCAM-1 Antibodies (No.) (Mean±SD)</th>
<th>Adhering WBCs After Infusion With Anti–VCAM-1 Antibodies (No.) (Mean±SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG-NHS</td>
<td>8.6±1.9</td>
<td>5.8±1.1†</td>
</tr>
<tr>
<td>ap IgG-APS</td>
<td>14.9±3‡</td>
<td>11.3±2.1†‡</td>
</tr>
</tbody>
</table>

Number of mice per group=9.

*Mean aCL titer of mice treated with ap IgG-APS antibodies immediately before the surgical procedure and after the infusion with anti–VCAM-1 monoclonal antibodies was 90.8±35.6 GPL units (high positive), and mice treated with IgG-NHS were negative for aCL antibodies.
†Statistically significant difference in adhesion of WBCs to endothelium after infusion with anti–VCAM-1 antibodies in mice treated either with IgG-NHS or ap IgG-APS.
‡Statistically significant difference between mice treated with ap IgG-APS and IgG-NHS (before infusion with anti–VCAM-1 antibody).
with IgG-NHS (mean thrombus size, 654±152 μm², P=0.0001). The ability of ap IgG-APS to enhance thrombus formation was significantly reduced in ICAM-1−/− mice and ICAM-1−/−/P-selectin−/− mice (mean thrombus size: 895±259 μm² and 859±243 μm², respectively [probability value: 0.0012 and 0.0011, respectively]) (Figure 2). These values were not statistically significantly different from the corresponding control groups treated with IgG-NHS, where thrombus size for ICAM-1−/− was 705±135 μm² and for ICAM-1−/−/P-selectin−/− was 1108±293 μm² (Figure 2).

aPL-Enhanced Thrombosis in Mice Is Abrogated After Infusion With Anti–VCAM-1 Monoclonal Antibodies

The size of induced thrombi was measured in CD1 mice treated with ap IgG-APS or IgG-NHS. As expected, the thrombus size in mice injected with ap IgG-APS was significantly larger (3830±1008 μm²) compared with mice treated with IgG-NHS (875±548 μm², P=0.002) (Figure 3). After infusion with anti–VCAM-1 monoclonal antibodies, the mean thrombus size in the mice injected with ap IgG-APS was significantly smaller (876±548 μm²) than the mean thrombus size before the infusion with the monoclonal antibodies (3830±1008 μm²) (P=0.0015) in the same group of animals. In mice infused with IgG-NHS, the intravenous infusion of anti–VCAM-1 antibodies did not produce a significant change in thrombus size after the infusion with anti–VCAM-1 antibodies (743±264 μm²) compared with thrombus size before the infusion of the antibodies (875±268 μm²) (P=0.2).

Discussion

In these studies, ap IgG-APS enhanced leukocyte adhesion to endothelium and enhanced thrombus formation in wild-type (C57BL/6J) mice. In ICAM-1−/− mice, ap IgG-APS did not increase leukocyte adhesion to ECs nor did these antibodies enhance thrombus formation. Similarly, in ICAM-1−/−/P-selectin−/− mice, ap IgG-APS had no effect on leukocyte adhesion nor did it enhance thrombus formation. These data lead to a few interesting conclusions. The first is that aPL activation of ECs in vivo and aPL-induced enhancement of thrombus formation seem to be dependent on expression of ICAM-1 and P-selectin. Adhesion of leukocytes to endothelium was not only impaired in ICAM-1−/− and ICAM-1−/−/P-selectin−/− mice with ap IgG-APS but also in mice treated with IgG-NHS. However, the findings of this study clearly indicate that the enhanced aPL-mediated adhesion of leukocytes to ECs in vivo was abrogated in the knockout strains of mice, indicating that this effect is mediated by the adhesion molecules missing in the 2 strains of knockout mice. Second, leukocyte adhesion was lower and thrombus size was significantly smaller in ICAM-1−/−/P-selectin−/− mice compared with ICAM-1−/− mice. The latter finding was observed both in mice infused with ap IgG-APS and mice infused with IgG-NHS. In line with these findings, other studies have indicated that the rolling and acute emigration of neutrophils into the peritoneum is completely absent in ICAM-1−/−/P-selectin−/− mice. Investigators have also shown that P-selectin and ICAM-1 deficiencies together substantially protect against atherosclerosis in apolipoprotein E–deficient mice. Hence, the complete abrogation of leukocyte adhesion in our experiments, even when mice were treated with IgG-NHS, is not surprising.

Although from these studies it is clear that P-selectin and ICAM-1 play a role in aPL-induced adhesion of leukocytes to endothelium and thrombus formation, the relative or individual effect of each adhesion molecule separately could not be clearly established. It is known that the rolling and adhesion of leukocytes to the endothelium involves several sequential steps. Initially, the interaction of selectins (P-selectin and
E-selectin) allows leukocytes to adhere reversibly to the vessel wall, so that circulating leukocytes can be seen to roll along the endothelium. This first adhesive interaction permits the stronger interaction mediated by ICAM-1 and VCAM-1. In this study, leukocytes that remain stationary for 30 seconds in the ECs of the cremaster muscle are counted as adhering leukocytes. Thus, early disruption of the interaction of leukocytes to ECs in ICAM-1−/−/P-selectin−/− mice may lead to a complete abrogation of the adhesion of leukocytes to the vessel wall, as observed in our studies. As expected, in ICAM-1−/− mice (in which only one step in the adhesion process is impaired), the adhesion of leukocytes to endothelium was decreased significantly but not completely abrogated, as was the case with double-knockout mice.

What is relevant in this study is that the significant increase of adhesion of leukocytes to endothelium induced by aPL was abrogated in the knockout mice. Most importantly, these studies show that the enhancement of the thrombus size mediated by aPL was abrogated in the 2 strains of knockout studies show that the enhancement of the thrombus size mediated by aPL was abrogated in the 2 strains of knockout mice. As expected, there was also a decrease in the adhesion of leukocytes in control mice treated with IgG-NHS. Hence, this study shows for the first time that VCAM-1 is a mediator of aPL-induced thrombosis.

This study also investigated the role of VCAM-1 on ap IgG-APS effects on leukocyte adhesion to ECs and thrombus size. Because of the unavailability of VCAM-1−/− mice, anti–VCAM-1 antibodies were used. The design of the experiments allowed us to measure adhesion of leukocyte to endothelium and effects of thrombus formation in ap IgG-APS–treated mice before and after 30 minutes of an intravenous infusion with anti–VCAM-1 antibodies in the same animal. In this study, the infusion of monoclonal antibodies anti–VCAM-1 decreased significantly the adhesion of leukocytes to ECs in vivo induced by aPL and reversed the aPL-mediated enhancement of thrombus formation, indicating that VCAM-1 is also a mediator in these processes. As expected, there was also a decrease in the adhesion of leukocytes in control mice treated with IgG-NHS (indicating that the anti–VCAM-1 antibody used was functional). Hence, this study shows for the first time that VCAM-1 is a mediator in aPL-induced EC activation and thrombosis.

Our data showed that adhesion of leukocytes to endothelium was significantly increased in C57BL/6J mice and CD1 mice treated with ap IgG-APS compared with the corresponding controls treated with IgG-NHS. However, the mean values in the C57BL/6J mice were significantly larger than in the CD1 mice. It is likely that genetic or strain-related specific features may account for these differences.

This study did not enable conclusions to be drawn about the relative importance of any one adhesion molecule compared with the other two. Abrogation of any one molecule negates the aPL effect, suggesting that either all three adhesion molecules are required or that aPL stimulation of the expression of any of two molecules does not compensate for the abrogation of a third molecule. The data indicate for the first time that all three adhesion molecules seem to be involved in the aPL-induced activation of ECs and thrombosis in vivo, and this is in agreement with the hypothesis that aPL antibodies create a hypercoagulable state by activating ECs. Under normal conditions, vascular endothelium maintains an anticoagulant surface of blood vessels. The influence of ECs on the thrombosis pathway is complex and involves multiple cell-surface and secreted compounds. Key elements in this process are the expression of tissue factor, tissue factor–pathway inhibitor, and thrombomodulin and cell adhesion molecules on the surface of ECs. The upregulation of adhesion molecules on ECs induces increased monocyte adherence to endothelium with increased production of tissue factor and the generation of a hypercoagulable state in ECs as a consequence. Hence, our mouse model of microcirculation, the adhesion of leukocytes to endothelium, can be used as an indication of EC activation.

We do not exclude that mechanisms other than EC activation may be involved in the pathogenesis of the thromboembolic phenomena associated with aPL. Studies clearly indicate that abnormalities in platelet function or dysfunction or aPL interfering with phospholipid-protein complexes that play a critical role in regulation of the coagulation, such as protein C, thrombomodulin, or prothrombin, are undoubtedly important.

Studies by Del Papa and colleagues showed that aPL antibodies bind to ECs through interaction with β2 GPI and induce activation of ECs evaluated as increased adhesion molecule expression and upregulation of cytokine secretion and arachidonic acid metabolism. Simantov et al showed that the upregulation of adhesion molecules (ICAM-1, VCAM-1, and E-selectin) on HUVECs when cells were treated with aPL antibodies in the presence of β2 GPI in vitro and these effects were mediated through the F(ab′)2 fragment of the antibody. In another study, George et al showed that the upregulation of ICAM-1, VCAM-1, and E-selectin on HUVECs in vitro by some monoclonal aPL preparations correlated with increased fetal resorption in mice in vivo. We showed that affinity purified aPL antibodies from patients with APS and murine monoclonal aPL antibodies and upregulated expression of ICAM-1, VCAM-1, and E-selectin in vitro, and these effects correlated with enhanced thrombosis and leukocyte adhesion in vivo.

In line with these findings, one study indicated that increased soluble levels of VCAM-1 have been observed in patients with APS, and this correlated with recurrences in thrombosis. In a recent report by Meroni et al, the antihypercholesterolemic drug fluvastatin was shown to inhibit the endothelial proadhesive phenotype induced by aPL by reducing in vitro the enhanced expression of adhesion molecules on ECs, suggesting a possibility for a new therapeutical approach.

Increasing numbers of studies indicate a direct relationship of activation of ECs and vascular diseases. It has recently been shown that an increase of P-selectin on ECs occurs in human atherosclerotic plaques in classic atherosclerosis. In vivo, it has been shown that P-selectin plays a central role in neutrophil accumulation within thrombi, which is important for fibrin deposition. Previous studies have shown rapid expression of P-selectin in the venules of pulmonary vascular endothelium of rats subjected to infusion of cobra venom factor and in the myocardial venules of cats subjected to ischemia and reperfusion. In another study, expression of P-selectin on activated platelets was shown to be important in the recruitment of leukocytes to thrombi and in the induction
of fibrin production during hemostasis.\(^\text{35}\) This effect was inhibited by antibodies against P-selectin. In summary, this study provides strong evidence that activation of ECs is inhibited by antibodies against P-selectin. In summary, this study provides strong evidence that activation of ECs is inhibited by antibodies against P-selectin.

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**References**


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