Hypertension-enhanced monocyte adhesion in experimental atherosclerosis

Bradford I. Tropea, MD, Phil Huie, MS, John P. Cooke, MD, PhD, Philip S. Tsao, PhD, Richard K. Sibley, MD, and Christopher K. Zarins, MD, Stanford, Calif.

Purpose: Hypertension is a known clinical risk factor for atherosclerosis. In experimental atherosclerosis, monocyte adhesion to the endothelial surface is enhanced and is considered to be an important early stage in plaque formation. We tested the hypothesis that hypertension enhances monocyte adhesion in experimental atherosclerosis.

Methods: Twenty-two New Zealand White rabbits were fed an atherogenic diet for 3 weeks to induce plaque formation. Aortic coarctation was created in eight rabbits by wrapping a Dacron band around the midportion of the descending thoracic aorta (stenosis group), whereas six rabbits underwent banding without aortic constriction (no stenosis group). Eight rabbits served as nonoperated controls. Monocyte binding to the aortic endothelial surface was counted with epifluorescent microscopy on standard aortic segments proximal and distal to the band. Immunohistochemistry was performed for the following antibodies: VCAM-1, RAM11, CD11b, and factor VIII.

Results: Mean blood pressure was 89 ± 3 mm Hg in the aorta proximal to the stenosis, compared with 64 ± 4 mm Hg in the no stenosis group and 74 ± 3 mm Hg in the control group (p < 0.01). The mean aortic blood pressure gradient across the stenosis was 16 ± 2 mm Hg in the stenosis group, whereas the aortic blood pressure gradient was 0.2 ± 0.6 mm Hg in the no stenosis group and -0.3 ± 0.4 mm Hg in the control group (p < 0.001). Monocyte adhesion to the aortic endothelial surface proximal to the stenosis was increased twofold compared with adhesion to the aorta distal to the stenosis and compared with the proximal aorta in the control group (p < 0.02). The proximal-to-distal aortic ratio of monocyte binding was enhanced in the stenosis group (2.2) compared with the no stenosis (0.76) and control (0.83) groups (p < 0.01). The intima area of the aorta proximal to the stenosis was significantly increased compared with the proximal aortas in the no stenosis and control groups (p < 0.01). RAM11, CD11b, and endothelial VCAM-1 expression were enhanced in the hypertensive region proximal to the stenosis.

Conclusions: In the hypertensive region in the aorta proximal to the stenosis, monocyte adhesion and endothelial VCAM-1 expression were increased, with intimal thickening and accumulation of macrophages. These findings suggest that hypertension may promote atherosclerotic plaque formation by enhancing monocyte adhesion. (J VASC SURG 1996;23:596-605.)

Hypertension is a well-known clinical risk factor for atherosclerosis. The mechanism by which hypertension enhances atherosclerosis is unknown. Monocytosis are believed to be important in early atherogenesis. Circulating monocytes adhere to the endothelial surface, migrate through endothelial junctions, and then take up lipid. Early atherosclerotic lesions are described as containing lipid-laden macrophages and smooth muscle cells. Leading theories on the pathogenesis of atherosclerosis describe endothelial dysfunction that may lead to enhanced monocyte adhesion. Experimental studies have suggested that monocyte adhesion is enhanced in hypertensive-enhanced atherosclerosis. The mechanism by which hypertension enhances atherosclerosis is unknown.
A few studies have examined leukocyte accumulation in a region of hypertension, but no specific study has addressed the effect of hypertension on monocyte adhesion. We tested the hypothesis that hypertension enhances monocyte adhesion in experimental atherosclerosis. Monocyte adhesion, CAM-1 expression, and macrophage accumulation were measured in a region of hypertension that is known to develop intimal plaque proximal to surgically induced aortic stenosis.

METHODS

Twenty-two adult male New Zealand White rabbits weighing 2.5 to 3.5 kg were fed an atherogenic diet that consisted of 1% cholesterol and 4% corn oil in standard rabbit chow. Eight rabbits underwent tight aortic banding (stenosis group), six underwent aortic banding without constriction (no stenosis group), and eight served as nonoperated controls (control group). After 3 weeks on an atherogenic diet all animals were killed. Total serum cholesterol levels were obtained perioperatively and at the time of death.

Before surgery the rabbits were sedated with 50 μg/kg ketamine and 5 to 7 mg/kg xylazine. Rabbits that underwent thoracotomy were intubated and ventilated with a volume ventilator under halothane anesthesia. Catheters were introduced into the central artery and the femoral artery to measure the blood pressure proximal and distal to the stenosis. The descending thoracic aorta was exposed with a left thoracotomy. A 5-mm band of Dacron was wrapped around the aorta and sutured to itself. The band was tightened; the proximal and distal catheters were placed on the rocking platform for 5 minutes. A volume ventilator under halothane intubation and 5 to 7 mg/kg xylazine. Rabbits were sedated with 50 μg/kg ketamine and 5 to 7 mg/kg xylazine. Rabbits that underwent thoracotomy were intubated and ventilated with a volume ventilator under halothane anesthesia. Catheters were introduced into the central artery and the femoral artery to measure the blood pressure proximal and distal to the stenosis. The descending thoracic aorta was exposed with a left thoracotomy. A 5-mm band of Dacron was wrapped around the aorta and sutured to itself. The band was tightened; the proximal and distal catheters were placed on the rocking platform for 5 minutes. The rabbits then were killed with intravenous sodium pentobarbital (150 mg/kg). The aortas were immediately excised, and the following standard anatomic segments of aorta were sampled (Fig. 1): (1) an aortic ring 2 cm proximal to the stenosis, (2) a segment of aorta extending from 0.5 cm to 2.0 cm proximal to the stenosis, (3) a segment of aorta extending from 0.5 cm to 2.0 cm distal to the stenosis, and (4) an aortic ring 2 cm distal to the stenosis. The aortas from the stenosis and control groups were harvested simultaneously; the aortas from the no stenosis group were harvested separately. All protocols were approved by the Administrative Panel on Laboratory Animal Care at Stanford University, and these studies were performed in accordance with the recommendations of the American Association for the Accreditation of Laboratory Animal Care.

Monocyte adhesion. Human mononuclear cells (THP-1) were grown in Dulbecco's modified Eagle’s medium supplemented with 10% (vol/vol) fetal calf serum and were kept in an atmosphere of 5% CO₂/95% air. Human mononuclear cells have been shown in our laboratory to adhere to rabbit aortas equally with rabbit mononuclear cells, and thus were used for binding studies. Before binding studies, mononuclear cells were fluorescently labeled and allowed to incubate for 15 minutes at room temperature in RPMI medium (Gibco/BRL, Tulsa, Okla.) that contained TRITC (3 μg/ml; Molecular Probes). The cell suspension was carefully underlaid with a layer of fetal calf serum and then centrifuged at 400g to separate labeled cells from the remaining dye. The cells were washed in a complete medium and resuspended in Hanks’ balanced salt solution (HBSS; Irvine Scientific; Santa Ana, Calif.), which contains 2 mmol/L Ca²⁺, 2 mmol/L Mg²⁺, and 20 mmol/L HEPES.

After removal of the adventitia, the aortic segments were placed in cold physiologic saline solution. The vessels were opened longitudinally and placed on 35-mm culture dishes that contained 2 ml HBSS. Each aortic segment was fixed to the culture dishes with 25-gauge needles to expose the endothelial surface to the medium; the segments were placed on a rocking platform for 5 minutes. The HBSS then was replaced with binding medium (HBSS supplemented with 2 mmol/L Ca²⁺, 2 mmol/L Mg²⁺, and 20 mmol/L HEPES) that contained THP-1 cells (2 ml, containing 10⁶ cells/ml). The aortic specimens then were incubated for 30 minutes on the rocker platform, and the cell-culture dishes were rotated 120 degrees every 10 minutes. The medium was aspirated and replaced by 2 ml fresh binding medium and placed on the rocking platform for 5 minutes. A
second wash with binding medium then was performed. The aortic segments were removed from the culture dishes and placed on glass slides with the endothelial side up. Mononuclear cells that adhered to the endothelial surface were counted from 24 high-power fields on each aorta with epifluorescent microscopy. The 24 fields were selected from a standard, predetermined grid pattern that was the same for each segment examined. The data is expressed as the number of adherent cells per mm² of aortic surface.

**Morphometry.** Light microscopic sections were prepared from the closed aortic rings 2 cm proximal and distal to the stenosis and were stained with hematoxylin. The intima and media areas were calculated with a commercial computer-controlled digitized tracer (Microcomp Image Analysis; Southern Micro Instruments; Atlanta, Ga.). Area calculations were determined by tracing contours of the lumen, inner media, and outer media.

**Immunohistochemistry.** Five-millimeter aortic rings approximately 2 cm proximal and distal to the stenosis were immersed in ornithine carbamoyltransferase (OCT) and then snap-frozen in liquid nitrogen and stored at -75° C. Six-micrometer thick aortic cross-sections were cut with a cryostat and mounted onto gelatin-coated glass microscope slides. The sections were air dried and fixed overnight in absolute acetone at -20° C. Before staining, the slides were removed from the acetone, air dried, and rehydrated.

---

**Fig. 1.** Diagram of thoracic aorta with creation of stenosis by aortic banding. 1.5-cm segments of aorta both proximal and distal to stenosis were used for monocyte adhesion assay. Histologic sections used for quantitative morphometry and immunohistochemistry are listed.
Table I. Hemodynamic and metabolic parameters

<table>
<thead>
<tr>
<th></th>
<th>Proximal BP (mm Hg)</th>
<th>Distal BP (mm Hg)</th>
<th>Gradient BP (mm Hg)</th>
<th>Cholesterol at baseline (mg/dl)</th>
<th>Cholesterol at 3 weeks (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenosis</td>
<td>89 ± 3*</td>
<td>73 ± 2</td>
<td>15.6 ± 2.2*</td>
<td>43 ± 5</td>
<td>847 ± 157</td>
</tr>
<tr>
<td>No stenosis</td>
<td>64 ± 4†</td>
<td>64 ± 3</td>
<td>0.2 ± 0.6</td>
<td>41 ± 6</td>
<td>1285 ± 84</td>
</tr>
<tr>
<td>Control</td>
<td>74 ± 3</td>
<td>74 ± 3</td>
<td>-0.3 ± 0.4</td>
<td>53 ± 6</td>
<td>938 ± 176</td>
</tr>
</tbody>
</table>

Expressed as mean arterial blood pressure or total cholesterol level ± SEM. Gradient BP is the difference between proximal and distal arterial pressure. The cholesterol level at time of death (3 wks) was statistically elevated compared with the baseline cholesterol level for all three groups.

BP, Blood pressure.

*p < 0.01; Stenosis vs both no stenosis and control.
†p < 0.05; No stenosis vs control.

Table II. Morphologic changes in proximal and distal aorta

<table>
<thead>
<tr>
<th></th>
<th>Intima area mm²</th>
<th>Media area mm²</th>
<th>Ratio × 100 intima to media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>0.712 ± 0.2*</td>
<td>3.6 ± 0.6</td>
<td>19.1 ± 4.4*</td>
</tr>
<tr>
<td>Distal</td>
<td>0.000 ± 0.0</td>
<td>3.3 ± 0.5</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>No stenosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>0.045 ± 0.03</td>
<td>4.4 ± 0.4‡</td>
<td>1.2 ± 1.0‡</td>
</tr>
<tr>
<td>Distal</td>
<td>0.003 ± 0.0†</td>
<td>2.8 ± 0.4</td>
<td>0.1 ± 0.1†</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>0.001 ± 0.0</td>
<td>3.5 ± 0.6</td>
<td>0.00 ± 0.0</td>
</tr>
<tr>
<td>Distal</td>
<td>0.000 ± 0.0</td>
<td>2.3 ± 0.3</td>
<td>0.00 ± 0.0</td>
</tr>
</tbody>
</table>

Ratio is defined as intima area divided by media area.

*p < 0.01; Proximal stenosis vs distal stenosis, proximal no stenosis, and proximal control.
†p < 0.05; Distal no stenosis vs distal stenosis and distal control aorta.
‡p < 0.05; Proximal vs distal.

Table III. Antibody expression

<table>
<thead>
<tr>
<th>Antibody location</th>
<th>Stenosis (%)</th>
<th>No stenosis (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>74 ± 13*†</td>
<td>18 ± 10</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Distal</td>
<td>11 ± 11</td>
<td>23 ± 15</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>RAM11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>100 ± 0*†</td>
<td>51 ± 9*‡</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Distal</td>
<td>3 ± 2</td>
<td>15 ± 6§</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>CD11b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>87 ± 5*†</td>
<td>13 ± 12</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Distal</td>
<td>0 ± 0</td>
<td>2 ± 1</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Factor VIII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>97 ± 2</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Distal</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

Expressed as percentage antibody staining the circumference of the endothelial surface ± SEM.

*p < 0.05 for proximal vs distal in the same column.
†p < 0.05 for proximal stenosis vs both no stenosis and control proximal aorta.
‡p < 0.05 for proximal no stenosis vs proximal control.
§p < 0.05 for distal no stenosis vs both distal stenosis and distal control.

in phosphate-buffered saline solution for 5 minutes. Individual sections were incubated with 50 μl of the following mouse monoclonal primary antibodies in diluent: anti-CD11b (Biosource; Camarillo, Calif.), RAM11 (Dr. A. M. Gown, Univ. of Washington, Seattle), and anti-VCAM-1 (Dr. M. I. Cybulsky, Brigham and Women’s Hospital, Boston). The sections were rinsed briefly and washed in phosphate-buffered saline solution for 20 minutes. This wash was followed by incubation with rabbit anti-mouse immunoglobulins conjugated with peroxidase (Dako; Carpinteria, Calif.). The tissue was washed for an additional 20 minutes and developed with 3,3'-diaminobenzidine, H₂O₂ (Sigma Chemical; St. Louis). The sections were washed in water and counterstained with Gill's hematoxylin no. 3 (Sigma).

Polyclonal goat anti-human factor VIII (Atlantic Antibodies; Silverwater, Mich.), which cross-reacts with rabbit endothelial cells, was used to evaluate the integrity of the endothelium. Incubation and washing procedures similar to those described previously were used. RAM11 and CD11b are both mouse monoclonal antibodies to rabbit macrophages. VCAM-1, the vascular cell-adhesion molecule, is a member of the immunoglobulin gene superfamily and functions as a receptor for monocytes, lymphocytes, and other molecules.

Quantitation for VCAM-1, RAM11, CD11b, and factor VIII expression was performed by quantitative morphometry. Total length of antibody staining at the endothelial surface and perimeter of lumen were both calculated. The expression of antibody staining is expressed as percentage staining of the endothelial surface (total length of antibody staining + perimeter × 100).

Statistics. Results are expressed as the mean ± SEM except for ratio data, which is expressed as the geometric mean. Statistical analysis was performed either with one-way analysis of variance, Student’s t test, or Mann-Whitney rank sum test. For the ratio
data, one-way analysis of variance was performed on the logarithmic transformed data. Significance was assumed when \( p < 0.05 \).

**RESULTS**

**Diet.** Animals in each of the three groups maintained stable and comparable body weights throughout the experiment. Before the atherogenic diet, the average serum cholesterol level in the 22 rabbits was 46 ± 4 mg/dl. Institution of the special diet resulted in a rise in serum cholesterol to 1000 ± 93 mg/dl at 3 weeks (\( p < 0.001 \)). The cholesterol level was similar in all three groups.

**Hemodynamic data.** Immediately before each rabbit was killed, blood pressure was measured proximal and distal to the stenosis and was recorded as mean arterial pressure (Table I). The proximal aorta of the stenosis group was exposed to hypertension (89 ± 3 mm Hg) compared with the proximal aorta in the no stenosis group (64 ± 4 mm Hg) and the control group (74 ± 3 mm Hg) (\( p < 0.01 \)). Mean arterial pressure was greater in the proximal aorta of the control rabbits than in the proximal aorta of the no stenosis rabbits because 40% more xylazine was administered to the no stenosis rabbits before blood pressure measurements. The mean arterial blood pressure in the distal aortas of the three groups did not differ. The stenosis group had a mean gradient of 15.6 ± 2.2 mm Hg (mean car pressure – mean femoral artery pressure), which was significantly elevated compared with 0.2 ± 0.6 mm Hg in the no stenosis group and – 0.3 ± 0.4 mm Hg in the control group (\( p < 0.001 \)).

**Intima and media area.** The 0.71 ± 0.2 mm\(^2\) intimal area in the proximal thoracic aorta of the stenosis group was significantly increased compared with 0.05 ± 0.03 mm\(^2\) in the no stenosis group and 0.001 ± 0.0 mm\(^2\) in the control group (Table II). The proximal aortic intimal area in the stenosis group was significantly greater than the distal aortic intimal area. Distal aortic intimal area was greater in the no stenosis group than in either the stenosis or control groups. Medial area in the proximal aorta was similar among the three groups, as was the medial area in the distal aorta. As expected, the proximal aorta tended to have a larger medial area than did the distal aorta because the proximal thoracic aorta is larger than the distal thoracic aorta. The ratio of intima to medial area was markedly increased in the stenosis group (>15-fold) compared with the no stenosis and control groups in the proximal aorta.

**Monocyte adhesion.** Monocyte adhesion to the

---

Fig. 2. Microscopy of fluorescent mononuclear cells adhered to aortic endothelial surface (magnification, 10x). **A**, Aorta proximal to stenosis. **B**, Aorta distal to stenosis.
Monocyte adhesion to aortic endothelial surface was significantly increased in the proximal aorta of the stenosis group compared with the distal aorta of the stenosis group; however, this difference was not present in the no stenosis and control groups. Monocyte adhesion also was significantly greater in the hypertensive proximal aorta of the stenosis group than in the proximal aorta of the control group (Figs. 2 to 4). Monocyte adhesion between the distal aortas of the stenosis and control groups did not differ. Direct comparison of monocyte

![Graph of monocyte adhesion](image)

**Fig. 3.** Monocyte adhesion to aortic endothelial surface in stenosis and control groups, expressed as number of monocytes per mm² ± SEM. Monocyte adhesion is enhanced in hypertensive aortic segment proximal to stenosis. *p < 0.02 compared with both groups distal to the stenosis and proximal control aorta.

![Graph of proximal to distal ratio](image)

**Fig. 4.** Geometric means for ratio of monocyte adhesion in proximal aorta to distal aorta for the three groups are listed as horizontal bars. Proximal-to-distal aortic ratio of monocyte adhesion is listed for each rabbit (circles). *p < 0.01 compared with no stenosis and control groups.
adhesion in the stenosis and control groups was possible because both aortas were harvested simultaneously (Fig. 3), which controlled for the variability inherent in the functional binding assay for monocytes. The monocyte adhesion study for the no stenosis group was done separately. The variability of monocyte adhesion was controlled for in this group by calculating the ratio of monocyte adhesion in the proximal aorta to the distal aorta and by comparing this ratio with a similar ratio in the stenosis and control groups. The ratio was 0.76 in the no stenosis group and 0.83 in the control group (Fig. 4). The proximal-to-distal ratio of monocyte adhesion in the stenosis group was 2.2; this enhancement was statistically significant when compared with the other two groups.

**Immunohistochemistry.** Integrity of the endothelial layer was verified in each animal by staining the proximal and distal aortas with factor VIII. Factor VIII staining in all the aortic segments of the three groups was 100% except for the proximal aorta in the stenosis group, which had 97% staining. This was not statistically different from the other groups (Table III).

Endothelial VCAM-1 expression was increased in the hypertensive proximal aorta compared with the distal aorta of the stenosis group (Fig. 5). VCAM-1 expression was also higher in the proximal aorta of the stenosis rabbits than in the proximal aortas in the other two groups. VCAM-1 staining in the distal aortas for the three groups of rabbits did not differ.

RAM11 antibody staining in the thoracic aorta proximal to a stenosis was significantly elevated compared with its distal aortic counterpart and with the proximal thoracic aorta in the control and no stenosis groups. There was very little RAM11 macrophage expression in the distal aortas of all three groups; however, there was less RAM11 staining in the aorta distal to the stenosis compared with the aorta distal to no stenosis. In the hypertensive proximal aorta of the stenosis group, significant staining of RAM11 in the entire intima was consistent with infiltration of a large number of macrophages.

CD11b is another marker for macrophages, and the findings were similar to those of RAM11, except that antibody staining in the distal aortic segments of the groups did not differ.

**DISCUSSION**

We found that monocyte adhesion is increased in a region of hypertension proximal to the stenosis, that RAM11, CD11b, and endothelial VCAM-1 expression are enhanced proximal to a stenosis, and that intimal area is significantly increased in the hypertensive region of the proximal thoracic aorta of the stenosis group of hypercholesterolemic rabbits.

The clinical manifestations of hypertension and its association with coronary artery disease \(^9\) and peripheral occlusive disease \(^10-12\) are well known. Experimental studies have also indicated that hypertension enhances atherosclerosis. Previous studies that used aortic banding to produce proximal aortic hypertension in hypercholesterolemic primates revealed increased plaque in the thoracic aorta proximal to the stenosis and increased coronary and carotid atherosclerosis. \(^13,14\) Similar studies that used hypercholesterolemic rabbits also showed increased aortic atherosclerosis proximal to an aortic coarctation. \(^15,16\) Plaque was well established after 3 to 6 months of an atherogenic diet in these studies. Our findings of increased intimal thickening is in agreement with these previous investigations. The mechanism by which hypertension potentiates atherosclerosis is unknown. Some investigators have suggested that hypertension induces alterations in sodium and calcium influx, wall composition, vasoactive hormones, and leukocyte adherence. \(^15,17,18\)

Monocytes are believed to play an important role in early and late atherogenesis. For this reason, much attention has been focused on monocyte adhesion and expression of monocyte adhesion molecules. Monocyte adhesion to the endothelial surface followed by diapedesis into the subendothelial space and migration can occur within 1 week of feeding a hypercholesterolemic diet in primates. \(^19\) This process is thought to lead to fatty streak formation. Monocytes isolated from human patients with hypercholesterolemia and hypertriglyceridemia adhere to cultured human umbilical-vein cells to a greater extent than monocytes harvested from normocholesterolemic patients. \(^5\) Monocyte adhesion to the thoracic aorta in rabbits increased threefold after 2 weeks of an atherogenic diet when compared with rabbits fed a normal diet. \(^4\)

In our study, monocyte adherence to the rabbit aortic endothelial surface in the hypertensive region proximal to the stenosis increased twofold compared with the nonhypertensive regions in the other groups. This is the first demonstration of enhanced monocyte adhesion in a region of hypertension. It is also noteworthy that monocyte adhesion did not differ between the no stenosis and control groups, in which blood pressure between the proximal and distal aortic segments did not differ. Although these findings suggest that hypertension may be an important modulator of monocyte adhesion, other factors such
as pulse pressure, wall motion, and wall stress also may play a role in this experimental model. Given that this study involved hypercholesterolemia, it would be difficult to rule out the possibility that the hypertension enhanced lipid ingress, which resulted in increased monocyte adhesion. Low shear has been correlated with enhanced monocyte adhesion; however, it is unlikely that shear was significantly affected proximal to the stenosis. Glass models of stenoses demonstrate little change in shear proximal to a stenosis. Distal to the stenosis, glass models demonstrate secondary flows at the wall with oscillatory shear, yet atherosclerosis was minimal distal to the stenosis. These findings suggest that wall shear stress is not the primary mechanism for the increased monocyte adhesion and atherosclerosis that is present proximal to the stenosis.

Only a few studies have examined the relationship between hypertension and leukocyte adhesion. Kowala et al. studied hypertension in the rat aorta by clipping one of the renal aortas or performing aortic ligation, and found that after 2 weeks atherosclerosis and leukocyte emigration were increased. The authors speculated that leukocyte adhesion may have been enhanced but did not specifically measure increased adhesion. McCarron et al., who used normotensive and spontaneously hypertensive rat brain endothelial cells under cytokine stimulation, demon-
strated increased intracellular adhesion molecule expression in the hypertensive endothelial cells. The constitutive expression of intracellular adhesion molecule, however, was not increased in the hypertensive rat. In contrast, Arndt et al. found no difference in leukocyte adherence to mesenteric venules in both normotensive and hypertensive rats, and demonstrated a reduction in leukocyte adherence in response to inflammatory exogenous stimuli. Monocytes are considered significantly more important than neutrophils and lymphocytes in the early stages of atherogenesis, which may explain the contradictory results in the literature that have not specifically examined monocyte adhesion in an area of hypertension.

The adhesive properties of vascular endothelium have received much attention recently, and it is believed that induction of endothelial-leukocyte adhesion molecules may contribute to monocyte recruitment in arteries. VCAM-1 is enhanced in animals who are fed an atherogenic diet. VCAM-1 is a cytokine inducible adhesion marker that is believed to bind to the β1 integrin receptor VLA-4 on the monocyte. Endothelial VCAM-1 expression was enhanced in the hypertensive segment of aorta. Our findings of increased RAM11 and CD11b macrophage expression further support the hypothesis that plaque development includes monocyte diapedesis and subendothelial accumulation of lipid-laden macrophages.

As indicated in Table III, RAM11 expression is higher (with a concomitant trend in VCAM-1 expression) in the distal aorta of the no stenosis group than in the control and stenosis groups. Surgical induction of cytokines and other inflammatory mediators may explain the difference seen in the no stenosis and control rabbits. The reduction in RAM11 distal to the stenosis may be related in part to the known effect of reduced atherogenesis distal to an aortic coarctation. As indicated in Table II, intima area was statistically reduced distal to the stenosis compared with that of the no stenosis group, even though both areas were very small.

CONCLUSION

We demonstrated that exposure of the aorta to increased blood pressure results in increased monocyte adhesion and endothelial VCAM-1 expression with intimal thickening and macrophage accumulation in hypercholesterolemic rabbits. These findings suggest that hypertension may promote atherosclerotic plaque formation by enhancing monocyte adhesion.

REFERENCES

18. Wolinsky H. Long-term effects of hypertension on the rat

Submitted March 13, 1995; accepted August 29, 1995.