The Endothelium During Cuff-Induced Neointima Formation in the Rabbit Carotid Artery

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Intimal thickening in human arteries is considered as a site of predilection for atherosclerosis. The placement of a flexible, physically nonconstrictive, silicone cuff around the rabbit carotid artery induced a neointima composed of smooth muscle cells (SMCs) within 14 days. To investigate possible alterations of the endothelial cells (ECs) during neointima formation, their morphology was examined with scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal microscopy. In the early postoperative period (6 hours), both cuffed and sham-operated arteries demonstrated small foci (5 to 200 μm) of denudation, presumably as a consequence of the manipulation. Within 24 hours the luminal surface of the cuffed and sham-operated arteries was completely covered with endothelium, which remained continuous throughout the study. However, after 1 week the ECs of the cuffed arteries contained a pronounced rough endoplasmic reticulum. From 6 hours until 3 days, polymorphonuclear leukocytes infiltrated the cuffed but not the sham-operated arteries from the lumen. Subendothelial SMC accumulation in the cuffed arteries began after this time period. At day 14 a full-blown neointima composed of longitudinally oriented SMCs had formed in the cuffed arteries. The sham-operated arteries did not develop a neointima. During neointima formation immunoreactivity for von Willebrand factor (vWF) increased in the ECs, and vWF was deposited in the extracellular spaces of the neointima. At day 14 the area of vWF deposits correlated positively with the area of the neointima (r = .73, P < .001). In subsequent weeks, the intimal area did not increase, and vWF deposits vanished from the neointimal matrix. The endothelium of the sham-operated arteries showed no change in vWF immunoreactivity compared with untreated arteries throughout the study. The altered ultrastructural morphology of the ECs and the concurrent vWF deposition in cuffed but not in sham-operated arteries point to alterations in EC function during the development of the neointima. The vWF secretion could possibly lead to increased adhesiveness of the extracellular matrix for the ECs as well as modulate neointima formation.

KEYWORDS • intima • von Willebrand factor • experimental neointima formation • arteriosclerosis • endothelium • polymorphonuclear leukocytes • confocal microscopy • extracellular matrix

Considerable evidence has accumulated indicating that intimal thickening may predispose humans to atherosclerosis.1-3 Many authors experimentally induce intimal thickening in arteries by removing the endothelium with a balloon, although endothelial denudation does not seem to be a common initiating event in human athrogenesis.4 Placing a physically nonconstrictive, flexible cuff around the rabbit carotid artery induces a neointima composed of smooth muscle cells (SMCs) within 14 days.5-6 This perivascular approach avoids direct injury to the vessel wall, particularly to the endothelium. Previously, we distinguished three phases in neointima formation.7 A first phase begins within 2 hours, with a polymorphonuclear leukocyte (PMN) infiltration from the luminal surface toward the intima and the inner media. In the second phase, which starts within 12 hours, the replication rate of SMCs in the media increases about 20-fold compared with unmanipulated arteries. The third phase is characterized by the appearance from day 3 onward of subendothelial SMCs that are immunoreactive for α-SMC actin and vimentin.

The aim of the present study was to examine whether the endothelium remained intact after cuff placement by means of scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal microscopy. To assess metabolic changes in the endothelial cells (ECs), von Willebrand factor (vWF) was demonstrated and quantified immunohistochemically during the time course of the neointima formation.

Methods

Male New Zealand White rabbits (2.5 to 3 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV). Both carotid arteries were surgically exposed and dis-
ected from the surrounding tissues. A nonocclusive, flexible silicone cuff was placed around the left carotid artery and closed with silicone glue.4-7 The right carotid artery was sham operated; i.e., it was isolated from the surrounding connective tissue and the vagus nerve and was exposed to a similar stretch as the contralateral cuffed artery. Animals were killed by perfusion fixation at 6 hours (n=5), 1 day (n=5), 3 days (n=4), 7 days (n=5), and 14 days (n=4) after cuff placement. For this purpose, the chest and the abdominal cavity were opened, and a cannula was inserted into the left ventricle. The aorta and the vena cava were ligated below the renal arteries. Krebs-Ringer solution was infused via the cannula into the left ventricle for 1 minute followed by 1500 mL paraformaldehyde 3% in a cacodylate buffer (pH 7.35) for 10 minutes at a pressure of 100 mm Hg. The vena cava was opened below the diaphragm and functioned as the outflow tract.

Segments of the cuffed arterial region and the sham-operated artery were cut in small arterial rings for TEM, SEM, and confocal microscopy. The rings were opened, and a cannula was inserted into the left ventricle. The aorta and the vena cava were ligated below the renal arteries. Krebs-Ringer solution was infused via the cannula into the left ventricle for 1 minute followed by 1500 mL paraformaldehyde 3% in a cacodylate buffer (pH 7.35) for 10 minutes at a pressure of 100 mm Hg. The vena cava was opened below the diaphragm and functioned as the outflow tract.

Segments of the cuffed arterial region and the sham-operated artery were cut in small arterial rings for TEM, SEM, and confocal microscopy. The rings were rinsed in a cacodylate buffer and were postfixed in glutaraldehyde 0.5% in a cacodylate buffer for 24 hours. For en face staining of F actin, the arterial rings were left in buffered paraformaldehyde for 1 hour.

To demonstrate the expression of vWF, rabbits were killed after 6 hours (n=5), 24 hours (n=5), 7 days (n=7), 14 days (n=19), 6 weeks (n=5), and 12 weeks (n=4) of cuff placement. The cuffed and sham-operated carotid arteries of these animals were fixed in methanol-carn fixative (methanol 60%, 1,1,1-trichloroethane 30%, and glacial acetic acid 10%). After embedding in paraffin, serial sections were cut in order to use adjacent sections for staining with antibodies against vWF and α-SMC actin.

**TEM**

After fixation, the specimens were washed with sucrose phosphate buffer, postfixed in 1% OsO4, dehydrated in ethanol and propylene oxide, and embedded in Epon. Selection of the zones most characteristic for the lesions was done on semithin sections stained with toluidine blue. Thin sections were cut with an LKB ultratome, stained with 2% uranyl acetate, and examined in a Balzers sputter coater. They were photographed in a Jeol 1200 EX electron microscope at 80 kV.

**SEM**

After fixation, the sections were dehydrated in an ethanol gradient up to 70% and were further dehydrated in acetone. They were dried in a critical-point dryer (Balzers) with CO2 as the transition fluid. The specimens were mounted on an aluminum stub with colloidal silver paint and coated with a 20-nm gold layer in a Balzers sputter coater. They were photographed in a JSM 220 SEM at 20 kV.

**Confocal Microscopy for F Actin**

For demonstrating F actin in en face preparations, the surrounding tissues were removed from the rings, which were then opened. These segments were permeabilized with 0.1% Triton X-100 for 20 seconds and stabilized with 0.1 mol/L glycine for 10 minutes. After washing with phosphate buffer (pH 7.4), the segments were placed in a bodipy phallacidin solution (0.165 µmol/L) (Molecular Probes, Eugene, Ore). Bodipy phallacidin is a fluorescent phallotoxin conjugate that binds to large and small F-actin filaments.8 The segments were halved, and one half was additionally stained with propidium iodide for the identification of nuclei. The segments were mounted en face in a glycerine-gelatin solution and examined with a confocal microscope (Biorad MRC-600).

**Immunohistochemistry**

The reactions were carried out by an indirect peroxidase antibody conjugate method using a monoclonal anti-α-SMC actin antibody, dilution 1:3000 (Sigma, St Louis, Mo) and a polyclonal antibody raised in sheep against human factor VIII–related antigen (vWF), dilution 1:250 (Binding Site, Birmingham, UK). To enhance antigenicity the sections were pretreated with 50 mg trypsin/50 mL tris(hydroxymethyl)aminomethane buffer, pH 7.8, at 37°C for 10 minutes (No. 93613, Fluka Switzerland). The specificity of the primary antibody against vWF was tested by absorbing the antibody with purified vWF (gift of the Belgian Red Cross). Liquid-phase absorption of the antibody for 12 hours at 4°C with different concentrations of human vWF gradually abolished staining of the endothelium, the subendothelium, and neointimal space. Preabsorbing the antibody with fibrinogen or fibronectin did not influence staining. The specificity of the antibody was further confirmed by double diffusion against a vWF preparation and Dako antisera against human vWF. Final confirmation of specificity was achieved by isoelectric focusing and counter-current isoelectric focusing.

For the detection of the monoclonal antibody against α-SMC actin, the sections were incubated with rabbit anti-mouse peroxidase for 45 minutes or rabbit antimouse Texas Red (Jackson) for double immunofluorescence. The polyclonal sheep anti-vWF antibody was visualized by a pig anti-sheep peroxidase as a secondary antibody (Binding Site) by using aminothiolcarbazol as a chromogen or donkey anti-sheep fluorescein (Jackson) for double immunofluorescence. For double immunofluorescence both primary antibodies were used in parallel. For negative controls the primary antibody was omitted.

**Quantification of the Area of vWF Expression**

The images were analyzed by using a Kontron image-processing system (IBAS software; Kontron Bildanalyse GmbH, Eching, FRG). In both the cuffed and sham-operated carotid arteries the endothelial-subendothelial (neointimal) area and the vWF immunoreactive areas were quantified. The arterial wall was divided into six separate, equally sized sectors. In the central region of each sector a rectangle with a long side of 100 µm along the luminal margin was selected. The parallel segment of the internal elastic lamina formed the opposite outer border of this rectangle (Fig 1A). The demarcation of these intimal rectangles was done interactively. The area and percentage of the vWF-immunoreactive area within each intimal rectangle were measured. The segmentation of the vWF-immunoreactive area was done by interactive selection of the gray-level zone corresponding with the brown color of the immunoreactive regions. The gray level of the cytoplasm of the ECs was used as the intensity cutoff value for the segmentation of the vWF-immunoreactive area. The procedure is explained in Fig 1A.
**Fig 1.** A, Low-power (×150) photomicrograph of a cuffed carotid artery at 14 days stained for von Willebrand factor (vWF). A neointima (NI) has formed, and a dense immunoreactivity is present in the NI. The area between the four arrowheads shows an intimal rectangle used for quantification (see "Methods"). The dotted line demonstrates the segment of the internal elastic lamina (IEL) that forms the outer border of the rectangle. M indicates media; ADV, adventitia. Bar=100 μm. B, A higher magnification of an intimal rectangle of the neointima in a cuffed carotid artery at 14 days stained for vWF. C, Same image as 1B but viewed with a green filter (580 nm); afterward the picture was digitized. This gray-level image is used for segmentation. The gray-level range of the immunohistochemical reaction product within the cytoplasm of the endothelial cells (arrowheads) was used as a threshold for the whole image. D, Binary image of the gray levels that exceeded this threshold. E, Composite image of C and D. The green-colored area represents the threshold gray-level area.

1B through 1E. By comparing the interactively measured lengths of the internal elastic lamina contained in the 100-μm rectangle, a correction could be made for the contractile state of the arteries.

**Statistical Analysis**

All data are expressed as mean±SEM; n refers to the number of rabbits. The six measurements of the area of the intimal rectangle, of the vWF-immunoreactive area, of the percentage of vWF-immunoreactive area within the intimal rectangle, and of the length of the internal elastic lamina of the cuffed arteries were averaged and then compared with the average values of the contralateral sham-operated arteries by using a paired t-test. The parameters of the different time points studied were compared by an ANOVA followed by a Newman-Keuls test. A regression analysis was done comparing the means (six measurements per artery) of the areas of the intimal rectangles with the vWF-immunoreactive areas within these rectangles at week 2. The SPSS FOR WINDOWS package (SPSS, Chicago, Ill) was used for these purposes. A 5% level of significance was selected.
Results

Morphology of the Endothelium

Sham-operated arteries. SEM of the sham-operated arteries demonstrated a luminal surface that was lined by a continuous flat sheet of elongated ECs with their long axes parallel to the blood flow. Six hours after the operation, small foci devoid of ECs were present. These areas measured 5 to 200 μm in diameter, and some platelets and a few white blood cells were stuck to the subendothelium. These lesions, which were variable within different parts of an artery and between different arteries, were not observed in animals that were not surgically treated. At 24 hours and at 3 days after surgery, SEM demonstrated a continuous EC layer without gaps. A few scattered white blood cells stuck to the endothelial surface.

TEM at all time points confirmed the presence of a continuous layer of ECs. White blood cells beneath the endothelium were never detected at any time points. The subendothelial space contained small proteoglycan granules and variable amounts of cross-banded collagen fibers. The luminal side of the internal elastic membrane demonstrated small elastic buds. En face staining for F actin showed a continuous EC layer at all time points. The ECs were outlined by peripheral actin bands and contained variable amounts of stress fibers. Beneath the EC layer discrete groups of longitudinally oriented resident SMCs were present, but PMNs were never detected.

Cuffed arteries. Six hours after cuffing (n=5), SEM demonstrated a luminal surface lined by ECs with their long axes parallel to the blood flow. Small foci of EC denudation measuring 5 to 200 μm were present (Fig 2). As in the sham-operated arteries, these lesions were variable within different parts of an artery and between different arteries. Some platelets and a few white blood cells adhered to the small denuded foci. The ECs at the border of these foci showed wrinkled margins. Adhesion of PMNs to the intact EC layer was demonstrated. TEM demonstrated numerous foci of subendothelial polymorphonuclear cells. In these regions the ECs were lifted from the internal elastic membrane. Cell junctions were intact. The extracellular subendothelial space contained a dense granulofibrillar material between the proteoglycan granules.

En face staining for F actin showed a continuous EC layer with a few foci of EC loss. The ECs contained a dense peripheral band of F actin and decreased amounts of stress fibers. Immediately beneath the EC layer numerous foci of PMNs, as identified by their nuclear shape, were present, confirming the TEM data (Fig 3A and 3B).

On day 1 after cuff placement (n=5), SEM demonstrated a luminal surface completely covered with ECs in all cuffed arteries. Individual ECs bulged into the lumen (Fig 4). Foci of EC denudation were not detectable. TEM confirmed the integrity of the EC layer with numerous subendothelial PMNs that corresponded to the bulging luminal surfaces. En face staining for F actin showed an intact EC layer. The ECs contained a dense peripheral actin band and variable amounts of longitudinally oriented stress fibers. Beneath the EC layer numerous PMNs were present. Confocal microscopy demonstrated that the ECs covering foci of PMNs lay in a more luminal optical section.

Three days after cuff placement (n=4), SEM revealed a luminal surface completely covered by ECs. Individual ECs protruded above the luminal surface; some were loosened, leaving the cell attached by one or two ends. Few subendothelially located PMNs were present. The margins of adjacent ECs were often prominent, overlapping, and curled.

TEM confirmed the presence of protruding ECs. These cells were often lifted up by neighboring ECs and showed numerous ruffles extending into the vascular lumen. SMCs could be detected between the endothelium and the internal elastic membrane. The cytoplasm of some medial SMCs penetrated through gaps of the internal elastic membrane into the subendothelial space. En face staining for F actin demonstrated ECs with dense peripheral actin bands and stress fibers. Foci of SMCs with a strong F actin fluorescence that lay with
FIG 3. Confocal microscopy of en face stain of F actin combined with a nuclear stain of a carotid artery 6 hours after cuff placement. A, Optical section parallel to the endothelium. B, Parallel optical section 3 μm beneath (abluminal) section shown in A. N indicates endothelial cell nuclei; PMN, polymorphonuclear leukocyte; arrows, dense peripheral bands of F actin (original magnification ×1600).

their long axes in the direction of the blood flow were found beneath the endothelium.

Seven days after placement (n=5), SEM demonstrated a continuous EC layer. TEM showed that the ECs contained a pronounced rough endoplasmic reticulum, Weibel-Palade bodies, and stress fibers (Fig 5). Beneath the endothelium a neointima was present, consisting of SMCs with a well-developed rough endoplasmic reticulum. The intercellular space of the neointima contained proteoglycans and basal lamina-like material. En face staining for F actin showed neointimal SMCs that lay in the same direction as the ECs, ie, perpendicularly to the circularly oriented SMCs of the media.

Fourteen days after cuff placement (n=4), SEM demonstrated a continuous flat sheet of intact ECs (Fig 6). TEM showed that the neointima consisted exclusively of SMCs. The intercellular space between the SMCs of the neointima was less wide than on day 7 and contained fragments of elastic fibers and sparse bundles of cross-banded collagen fibers. The SMCs of the neointima were surrounded by basal lamina-like material. En face preparations for F actin showed the longitudinally oriented SMCs of the neointima and a continuous EC layer.

vWF Expression

Sham-operated arteries. The arteries were lined by ECs demonstrating a granular and punctate staining reaction throughout the period of investigation. A representative example is shown in Fig 7A. Subendothelial deposition was not found. The media and adventitia were negative. Vasa vasorum were not detectable in the media or adventitia. In the periadventitia numerous small blood vessels were present, demonstrating a granular staining of the ECs.

Cuffed arteries. Six hours after cuff placement (n=5), the cuffed arteries were lined by a continuous EC layer that demonstrated a granular and punctate immunoreactivity for vWF. In about one third of the arterial sections examined, subendothelial foci of vWF-immunoreactive material were present. These foci were located in gaps of the internal elastic membrane, often extending into the inner part of the media, and were frequently associated with accumulations of subendothelial polymorphonuclear cells (Fig 7B).

One day after cuff placement (n=5), the cuffed arteries were lined by a continuous EC layer immunoreactive for vWF. A dense immunoreactivity for vWF was present in regions of the media that were infiltrated by polymorphonuclear cells.

Seven days after cuff placement (n=7), the arteries were lined by ECs demonstrating a dense cytoplasmic immunoreactivity for vWF. Between the endothelium and the internal elastic lamina a neointima had formed composed of α-SMC actin–positive SMCs. About 20% of the extracellular space of the neointima was immunoreactive for vWF (Fig 8). Moreover, a variable diffuse immunoreactivity was found in the inner media. The dense foci of vWF-immunoreactive material present in the media after 1 day had disappeared.
Fourteen days after cuff placement ($n=19$), a neointima composed of $\alpha$-actin–positive SMCs was present (Fig 7C). The thickness of the neointima in different sectors of each section varied. The ECs showed a dense flocculent immunoreactivity for vWF. Beneath the endothelium extracellular immunoreactive material was found (Fig 7D). Double immunofluorescence for $\alpha$-SMC actin and vWF demonstrated that the subendothelial vWF deposits lay in the extracellular space between the neointimal SMCs (Fig 7E). The depth of the subendothelial accumulation varied. In some regions some focal immunoreactivity was found in the inner media.

The quantitative results are shown in Fig 8. The area of the intimal rectangle, the vWF area in this rectangle, and the relative area of vWF-positive material in the rectangle of the cuffed carotid arteries differed from the sham-operated arteries. However, the interactively measured length of the internal elastic lamina contained in the 100-μm rectangles in the cuffed arteries was not different from that of the sham-operated arteries (Fig 8D). This indicates that the contractile state of the cuffed arteries was not different from the sham-operated arteries, and therefore a correction for the contractile state was not necessary. In the cuffed carotid arteries there was a positive correlation between the areas of the intimal rectangles and the vWF-immunoreactive area within these rectangles (Fig 9).

Six weeks after cuff placement ($n=5$), the neointima was fully lined by ECs with a dense, flocculent immunoreactivity for vWF. The subendothelial vWF deposition was markedly reduced and decreased almost to the value for sham-operated arteries (Fig 8). The area of the neointima was statistically not different from the neointimal area at 14 days. The relative amount of vWF in the intimal rectangle was identical to the sham-operated vessels.

Twelve weeks after cuff placement ($n=4$), the neointima was completely lined by ECs with a granular cytoplasmic appearance and an immunoreactivity for vWF. The appearance of the ECs was comparable to that of the sham-operated arteries (Fig 7F). The subendothelial vWF deposition had disappeared. The area of the neointima was statistically not different from the neointima at 2 and 6 weeks. The relative amount of vWF-positive material in the intimal rectangle was again not different from the sham-operated arteries.

Discussion

The present study focused on the integrity of the ECs during cuff-induced neointima formation. It demonstrated that a transient focal loss of ECs was not sufficient to induce a local neointima in rabbit carotid arteries. Indeed, small patches of ECs disappeared in cuffed as well as in sham-operated arteries in the first hours after surgery. Because the cuffed as well as the sham-operated arteries, which underwent the same degree of stretching, showed these small endothelial defects, it is likely that they were the immediate result of manipulation. However, since the sham-operated arteries did not develop a neointima, this relatively small degree of denudation was apparently not sufficient by itself to induce neointima formation. These foci were of the same size as those seen after a distension injury. Similar small denudation foci have been induced exper-
Within 24 hours the luminal surface of the cuffed and sham-operated arteries was covered completely by a continuous sheet of ECs, as indicated by SEM and confocal microscopy. This observation confirmed the morphological findings of Booth et al. and was in accord with our functional data. This rapid repair also reflects the findings of authors who have used a nylon catheter or acute hydrostatic distension to remove a small zone of endothelium that is completely healed by new endothelium within 2 to 3 days.

At 6 hours and 1 and 3 days after cuff placement, polymorphonuclear cells were present between the ECs.
layer and the internal elastic lamina. At 1 and 3 days after cuff placement, SEM and TEM showed bulging, protruding, shrunken, and damaged single and small groups of ECs without exposure of the subendothelium. Indeed, the presence of neighboring cells under the margins of these altered ECs could suggest that they might have been replaced without exposing the subendothelium. Since these alterations were not noticed in the sham-operated arteries, they were due either to the transit of polymorphonuclear cells or to the presence of the cuff itself. The morphological changes of the EC layer pointed to lesions reminiscent of the nondenuding EC injury described by Reidy.13

Although the EC layer after 7 and 14 days of cuff placement was morphologically intact, the ECs were metabolically altered, as demonstrated by the pro-
Fig 8. Bar graphs showing quantification of the areas in which von Willebrand factor (vWF) was expressed and areas of the intimal rectangles at different time intervals after cuff placement. A, Area of the vWF-immunoreactive material within the intimal rectangle. This area was increased after 1 and 2 weeks in the cuffed arteries. At 6 weeks this area decreased in the cuffed arteries to the value of the sham-operated arteries. B, Areas of the intimal rectangle (as defined in "Methods") at different time intervals after cuff placement. At 2 weeks the intimal area was maximal and did not significantly change until the end of the experiment at week 12. C, Percentage of the area of the intimal rectangle occupied by vWF-immunoreactive material at different time intervals after cuff placement. After 1 week about 22% of the intimal area was occupied by vWF-immunoreactive material. At 2 weeks this percentage was 12%, and at 6 and 12 weeks the vWF deposits vanished from the matrix of the neointima. D, Length of the internal elastic lamina (IEL) measured interactively, that was contained in the 100-μm rectangle. The length was equal in cuffed and sham-operated arteries, which implies that no correction had to be made for the contractile state of the arteries. Data are mean±SEM (six measurements per artery). n indicates the number of rabbits. *P<.05, **P<.01, and ***P<.001 cuffed different from sham-operated arteries by paired t test; +P<.05 different from week 1 cuffed arteries by Newman-Keuls test; and #P<.05 different from week 2 cuffed arteries by Newman-Keuls test.

pronounced rough endoplasmic reticulum, by the intense presence of vWF-immunoreactive material throughout the cytoplasm compared with the granular aspect of normal ECs, and by the vWF accumulation within the subendothelial space. This vWF deposition in the extracellular matrix of the cuff-induced neointima was most likely a consequence of an increased vWF synthesis and release by the ECs. It is unlikely that platelets are a major source of vWF, since the endothelial layer was always continuous, and adhering platelets were not seen at 24 hours or at any later time point after surgery. Moreover, the cytoplasm of the intimal SMCs was negative for vWF. In this context it is interesting that after endotoxin treatment, which is known to produce a nondenuding injury to the endothelium, ECs demonstrate an increase in their vWF content.13 The deposition of vWF in the matrix of the neointima was a temporary phenomenon, since vWF disappeared from the matrix of the neointima 6 and 12 weeks after cuff placement. At these time points the neointima was lined by a continuous endothelium demonstrating the normal granular immunoreactivity for vWF.

At present a straightforward explanation cannot be given for the subendothelial and neointimal matrix deposition of vWF. From other models it is known that endothelial permeability increases after perivascular manipulation, and this could explain the deposition of molecules originating from the bloodstream.16 However, the deposition of vWF in the intima as a consequence of an increased permeability seems unlikely, because vWF...
sive protein is present in the basal lamina. Some earliest extracellular vWF deposition appears to be basolaterally under conditions of stress. In this respect both in the plasma and toward the subendothelial spatially related to migrating PMNs. The neointimal artery by using a Doppler technique and that the formation, but some indications point to a stimulation. It is interesting that hemodynamic and flow changes association between the time course of neointima formation and vWF deposition. It is not known which effect, if any, the increased subendothelial vWF deposition had on the neointima formation, but some indications point to a stimulation. First, the area of vWF deposition after 14 days of cuff placement correlated positively with the size of the neointimal areas. Second, it has been suggested that replicating ECs can modulate intimal hyperplasia and that ECs secrete vWF maximally when they are in their most active growth phase. A third argument comes from studies in pigs that lack vWF. These animals show a resistance to the initiation and progression of spontaneous and diet-induced atherosclerosis. This was attributed to the impaired platelet attachment to the vascular wall, resulting in a diminished release of platelet-derived growth factors in the subendothelial space. In the present study the EC layer was continuous, and platelet adhesion was absent when the deposition of vWF was most pronounced. Therefore, the cuff model suggested that the increased subendothelial deposition of vWF might by itself contribute to SMC multiplication and migration and neointima formation.

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Fig. 9. Scatterplot showing correlation between the area of the intimal rectangles and the von Willebrand factor (vWF)-immunoreactive areas within these rectangles at week 2. Each dot represents the mean value of six measurements.


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