Strain-Dependent Vascular Remodeling Phenotypes in Inbred Mice

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We have recently established a mouse model of arterial remodeling in which flow in the left common carotid artery of FVB mice was interrupted by ligation of the vessel near the carotid bifurcation, resulting in a dramatic reduction of the lumen as a consequence of a reduction in vessel diameter and intimal lesion formation. In the present study we applied this model to various inbred strains of mice. Wide variations in the remodeling response with regard to reduction in vessel diameter, intimal lesion formation, lumen area, and medial hypertrophy were found. On carotid artery ligation SJL/J mice revealed the most extensive inward remodeling leading to an approximate 78% decrease in lumen area while lumen narrowing in FVB/NJ mice was largely due to extensive neointima formation as a result of smooth muscle cell (SMC) proliferation. Significant positive remodeling in the contralateral right carotid artery with a >20% increase in lumen area was observed in SM/J and A/J mice. An *in vitro* comparison of growth properties of SMC isolated from FVB/NJ mice and a strain that exhibited very little SMC proliferation (C3H/HeJ) demonstrated accelerated growth of SMC from FVB/NJ following serum stimulation. *In vivo*, SMC proliferation in the FVB/NJ strain was preceded by a 37% loss of medial SMC occurring within the 2 days after ligation, however, cell death was not detectable in C3H/HeJ mice. These findings suggest that the mechanisms leading to lumen narrowing in the vascular remodeling process are genetically controlled. (*Am J Pathol* 2000, 156:1741–1748)

Vascular remodeling is a response of blood vessels to both physiological and pathological stimuli, leading to either vessel enlargement (positive remodeling) or reduction in vessel diameter (negative remodeling). Examples of remodeling have been observed in fetal development¹ and after graft placement^{2–5} or angioplasty.^{6–8} In humans, vascular remodeling but not intimal lesion formation was shown to account for the majority of the restenosis in response to angioplasty procedures.^{9,10}

We have recently established and characterized a mouse model of arterial remodeling.¹¹ In this model, flow

in the common carotid artery is interrupted by ligation of the vessel near the carotid bifurcation. Using FVB/NJ mice, this resulted in a dramatic reduction in vessel diameter and formation of an intimal lesion. Neointima formation and the influx of inflammatory cells in this model are reduced in P-selectin-deficient mice, while the reduction in vessel diameter is not affected by the lack of P-selectin.¹² Additional specific factors that mediate the remodeling response are beginning to emerge. Several studies have implicated nitric oxide (NO) as an inhibitor of remodeling events.^{13–17} Our own studies demonstrated that alterations in blood flow also lead to changes in gene expression of platelet-derived growth factor A-chain and B-chain, factors known to modulate proliferation and migration of smooth muscle cells (SMC).¹⁸

Preliminary experiments in our laboratory indicated that there is wide qualitative and quantitative variation in the vascular remodeling response of different mouse strains. To provide the basis for a genetic analysis, we subjected 11 different strains of inbred mice to carotid artery ligation for analysis of the remodeling response. Large differences were found between strains with regards to negative as well as positive remodeling and intimal lesion formation. The magnitude of neointima formation correlated with increased loss of SMC occurring immediately after ligation of the carotid artery as well as enhanced growth properties of SMC *in vitro*.

Materials and Methods

Animals

All animal studies were approved by the Institutional Animal Care and Use Committee. All mice were housed in Thoren (Hazleton, PA) Maxi Miser Duplex cages with HEPA filtered air, fed Pro Lab 3000 chow, and acidified (pH 2.8–3.1) chlorinated water. Only female mice, 18 to 27 weeks old, were used in these experiments (Table 1). 129/SvJ, A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ, SJL/J, SM/J, and SWR/J mice were from Jackson Laboratories (Bar Harbor, ME). The animals

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Table 1. Summary of Data on Inbred Mouse Strains Examined in the Left Carotid Artery Ligation Model

Strain	Age (weeks)	Body weight (g)	No. of ligated animals	No. of control animals
129/SvJ	19	21.6	6	4
A/J	19	20.6	6	4
AKR/J	18	31.4	5	5
BALB/cJ	24	24.4	6	4
C3H/HeJ	26	21.6	6	3
C57BL/6J	23	21.6	6	4
DBA/2J	20	22.3	6	4
FVB/NJ	20	22.3	6	4
SJL/J	18	21.8	5	5
SM/J	27	18.4	6	4
SWR/J	18	23.3	6	3

were anesthetized by intraperitoneal injection with a solution of xylazine (5 mg/kg, AnaSed, Lloyd Laboratories, Shenandoah, IA) and ketamine (80 mg/kg body weight, Ketaset, Aveco Co., Fort Dodge, IA). BALB/cJ mice were very sensitive to this anesthetic combination and received only one-third of this dose. The left common carotid artery was dissected and ligated near the carotid bifurcation as described.¹¹ After 4 weeks all animals were fixed for 5 minutes by perfusion at physiological pressure with 4% paraformaldehyde in 0.1 Mol/L sodium phosphate buffer, pH 7.3, as described.¹⁹ The perfusate was allowed to drain from a very small incision in the left carotid artery just proximal of the ligature. The left and right common carotid arteries were embedded in paraffin, and serial sections 5 μ m thick were cut and Orcein stained for analysis by morphometry. Between 7 and 10 sections spanning the entire length of the vessel (with the exception of the 1-mm segment adjacent to the ligature where clotting occurs) were analyzed by morphometry. For the right carotid artery, all sections were cut from the vessel distal to the subclavian artery branch point. For each animal the mean value for all parameters was calculated using all measurements obtained from the vessel sections. Age matched unmanipulated animals from each strain were also analyzed in the same manner (Table 1) and the data were used to express the parameters as ratios normalized to measurements from unmanipulated mice.

In addition, groups of C3H/HeJ and FVB/NJ mice were harvested 2 days after ligation to assess the loss of SMC in response to cessation of blood flow. The number of nuclear profiles in the media was counted on cross sections of ligated as well as normal control vessels. Ten sections per vessel were analyzed with 5 animals in each group. The average number of SMC in the media of ligated vessels was expressed as a percentage of the cell number present in normal control vessels.

Morphometry

Morphometric analysis was carried out on the ligated left common carotid artery and on the contralateral right common carotid artery 4 weeks after ligation. In the unmanipulated control animals morphometric analysis was

performed on the left and right common carotid arteries. Digitized images of these vessels were analyzed using image analysis software for Apple Macintosh computers (NIH Image 1.60). The circumference of the lumen, the lengths of the internal elastic lamina (IEL) and the external elastic lamina (EEL) were determined by tracing along the luminal surface, the perimeter of the neointima (IEL) and the perimeter of the tunica media (EEL). Very small folds were not included in the IEL and EEL data and therefore the IEL and EEL measurements more accurately reflect the perimeter of the neointima and media, respectively. Assuming a circular structure the circumference of the lumen was used to calculate the lumen area. The medial area was calculated by subtracting the area defined by the IEL from the area defined by the EEL and intimal area was determined as the area defined by the luminal surface and IEL. Taking the measurements from all sections per vessel into account, a mean value was calculated for each animal. From these values the means $\pm$ SE for all parameters was then determined for each strain. This was done both for the ligated mice and the corresponding unmanipulated mice. Within each strain the means of the control mice and ligated mice were compared and analyzed by Student's *t*-test. The results were considered significantly different if $P \leq 0.05$ and the *p* values are indicated for $P \leq 0.1$. The remodeling responses in the ligated mice were then also normalized to the control mice by determining the ratio of the value for each ligated mouse divided by the mean obtained for the corresponding control mice. This approach allowed for comparisons between strains.

SMC Culture

The aortae from C3H/HeJ and FVB/NJ mice were removed and minced into small pieces for explant culture. The cells were cultured in DMEM containing 10% fetal calf serum. All cells stained with an antibody against smooth muscle α -actin (clone 1A4; Sigma, St. Louis, MO). For growth curves, cells were seeded at 10^4 cells/cm² in 12-well plates and grown in 10% fetal calf serum. For each time point, cells from three wells were harvested and pooled for cell counting with a hemocytometer. Cell counts from two separate cell isolations are shown.

Results

Remodeling

Ligation of the left carotid artery leads to cessation of net forward flow, whereas the contralateral right carotid artery is expected to experience a compensatory increase in flow. As was previously reported, this leads to dramatic changes in vessel wall morphology in FVB/NJ mice¹¹ within 4 weeks. To determine whether arterial remodeling is genetically controlled, we examined the response to carotid artery ligation in 11 different inbred strains of mice. Morphometric analyses was performed on the left carotid artery 4 weeks after ligation and on the contralateral right carotid artery as well as on the left and right

carotid artery of unmanipulated mice. Comparison of morphometric data between ligated mice and unmanipulated mice allowed us to detect changes in morphometric parameters both in the ligated vessels and in the contralateral artery.

The changes occurring in the ligated left carotid artery normalized to the left carotid artery of control mice are shown in Figure 1. A significant reduction in lumen area of the ligated carotid artery was observed in all strains. Although lumen area was reduced in FVB/NJ and SJL/J by nearly 80%, C3H/HeJ mice showed only a 54% reduction compared to the corresponding controls (Figure 1A). Because lumen area is determined both by the amount of neointimal lesion formation as well as negative or inward remodeling, we measured the perimeter of the media (EEL) and intima (IEL) as indicators of the remodeling response. Significant negative remodeling was observed in all strains with the exception of the FVB/NJ mice, despite the fact that this strain showed the largest reduction in lumen area (Figure 1, E and F). Determination of neointima formation (Figure 1C) and the ratio of intimal area over medial area from control vessels (Figure 1B) demonstrated that FVB/NJ mice are unique in that they achieve the reduction in lumen area largely by neointima formation and less so by inward remodeling. Much less intimal lesion formation was seen in SJL/J mice even though the reduction in lumen area in this strain was comparable to the FVB/NJ mice. This puts the SJL/J strain at the high end of the negative remodeling response (Figure 1, E and F). No intimal lesion formation was observed in the SM/J strain and very little in the C3H/HeJ mice. It should be mentioned that intimal lesion formation is not uniform along the ligated vessel; rather, there is a gradient with increased intimal lesion formation toward the ligation.¹¹ The data presented here reflect the average amount of lesion formation for the entire length of the vessel. Representative photomicrographs of the three prototype remodeling phenotypes are shown in Figure 2: FVB/NJ mice showing extensive neointima formation and little inward remodeling, SJL/J mice with pronounced negative remodeling and little intimal hyperplasia, and C3H/HeJ mice with the largest lumen area due to little inward remodeling and very little intimal lesion formation.

A significant increase in medial area, ie, medial hypertrophy, was seen in some strains. The strains that showed medial hypertrophy in the ligated vessel, FVB/NJ and BALB/cJ, were the same strains that also formed extensive intimal lesions.

We also examined the right carotid artery to determine whether a potential compensatory increase in blood flow led to positive remodeling in this vessel. These data were normalized to the right carotid artery from control animals and are shown in Figure 3. A significant increase in lumen area was seen in SM/J and A/J mice. Compared to control vessels, lumen area of the carotid artery contralateral to the ligated vessel showed increases of 20.3 and 32.7% for the SM/J and A/J strains, respectively (Figure 3A). Corresponding increases were also seen in the IEL and EEL for these strains (Figure 3, C and D).

Growth Properties of SMC in Vitro

The differences in intimal lesion formation between strains suggested that the proliferative response of SMC might be strain-dependent. We therefore examined whether SMC isolated from aortae by explant technique exhibited different growth properties in response to 10% fetal calf serum. We focused on the C3H/HeJ strain as an example with little intimal lesion formation and on the FVB/NJ strain with large neointimae. Growth curves from two independent cell isolations shown in Figure 4 demonstrate faster growth in the SMC derived from FVB/NJ mice.

Loss of SMC in Response Carotid Artery Ligation

In a rat carotid artery model we have previously demonstrated that the onset of medial SMC proliferation 2 days after denudation correlates with the amount of trauma inflicted on the vessel wall.^{20,21} We have also shown that the trauma-induced proliferation of SMC in the rat balloon injury model is mediated by the release of fibroblast growth factor-2 from damaged cells.^{22,23} Furthermore, we have previously noted a loss of medial SMC in the carotid artery of FVB/NJ mice occurring within the first 2 days after ligation of the vessel.¹¹ Because this loss of SMC with concomitant release of growth factors could be involved in the induction of SMC proliferation, we determined the number of medial SMC in the C3H/HeJ and FVB/NJ strain 2 days after ligation of the carotid artery. Compared to the unmanipulated carotid artery, no loss of SMC was detectable in the C3H/HeJ mice; however, in the FVB/NJ mice an approximate 37% loss of medial SMC was apparent (Figure 5).

Discussion

Transgenic and gene-targeted mice can be bred on different genetic backgrounds, resulting in differences in phenotype.^{24,25} Similarly, we have observed differences among mouse strains with regard to the remodeling response in the carotid artery ligation model. This led us to examine remodeling in a number of different inbred strains including those commonly used for gene targeting. It is apparent that different strains adjust their lumen in ways that are quantitatively and qualitatively different. The reduction in lumen area in the ligated vessel ranges from 54% in C3H/HeJ mice to 79% in the FVB/NJ strain. Furthermore, although some strains achieve the adjustment in lumen size almost exclusively by inward remodeling (decreased IEL and EEL ratios) without acquiring intimal mass, other strains form extensive intimal lesions with concomitantly less inward remodeling. We believe that the inward remodeling component in the ligation model, like models of flow reduction, can be viewed as a physiological response. The proliferative response of SMC leading to neointima formation, however, is probably a pathological response caused by the near-stasis conditions, in that it is not seen in models with 90% flow

Figure 1. The response of the left common carotid artery from inbred mouse strains 4 weeks after ligation is shown. The data were normalized to the left carotid artery from unmanipulated control mice by expressing them as a ratio of ligated vessel over control vessel. Absolute values for neointima formation are also shown in C. The values reflect means $\pm$ SE. The P value is indicated for $P \leq 0.1$ for comparisons between ligated and control animals.

Figure 2. Representative photomicrographs of cross-sectioned mouse carotid arteries from FVB/NJ, C3H/HeJ, and SJL/J mice 4 weeks after ligation of the left carotid artery. The unmanipulated artery from control mice is shown on the left. Note the marked decrease in lumen area with extensive neointima formation but little inward remodeling in the FVB/NJ mice (**A** and **B**), relatively little inward remodeling with no intimal hyperplasia in C3H/HeJ mice (**C** and **D**), and extensive inward remodeling with little neointima formation in SJL/J mice (**E** and **F**). Hematoxylin-eosin stain; original magnification, $\times 200$.

reduction.¹⁸ Potential factors that could contribute to the proliferative events include hypoxia, accumulation of metabolites, platelet activation, and inflammation, among others. Thus, at least some of the stimuli leading to inward remodeling in the ligation model (eg, SJL/J mice) may be the same as those mediating the remodeling response in flow reduction models with residual forward flow. Additional factors unique to the ligation model, however, are likely to be involved in the proliferative response of SMC, particularly in the FVB/NJ mice. The exaggerated proliferative response of the FVB/NJ mice in the ligation model, on the other hand, may also be a consequence of the inability to undergo significant inward remodeling for adjustment of shear stress. As an alternative explanation, the massive intimal proliferation may be impeding medial inward remodeling in these mice. The notion that addi-

tional factors not regulated by flow contribute to the response seen in FVB/NJ mice is further supported by the finding of extensive SMC loss in the media preceding the onset of SMC proliferation.¹¹ It is thought that the reduction in diameter is mediated by the endothelium in models of flow reduction with residual net flow.²⁶ This raises the issue of whether the endothelium in the ligated vessel of FVB/NJ mice is dysfunctional due to the near-stasis conditions. It should be emphasized that large scale loss of endothelium is usually not seen in the ligated vessel.

Several studies have implicated NO as an inhibitor of remodeling events.^{13–16} Using endothelial nitric oxide synthase (eNOS)-deficient mice, Rudic et al¹⁷ recently reported that inward remodeling was reduced in these mice. It is unlikely, however, that eNOS is the only major factor responsible for regulating flow-mediated remodel-

Figure 3. The response of the contralateral right carotid artery 4 weeks after ligation of the left carotid artery is shown. The data were normalized to the right carotid artery from unmanipulated control mice by expressing them as a ratio of right carotid from the ligated animal over right carotid from control animals. The values reflect means $\pm$ SE. The *P* value is indicated for $P \leq 0.1$ for comparisons between ligated and control animals.

ing. Inward remodeling was most extensive in SJL/J and 129/SvJ mice (Figure 1, E and F), and it is interesting to note that the 129/SvJ strain is known to have a duplication of the renin gene.²⁷ Medial hypertrophy in the ligated vessel showed a high degree of variability between strains, and it should be pointed out that strains forming the largest neointimas, ie, FVB/NJ and BALB/cJ, also developed the most extensive medial hypertrophy/hyperplasia.

The large differences in intimal lesion formation between strains were surprising. In the present study we addressed this issue in two ways. First, we compared the proliferative response of SMC from FVB/NJ and C3H/HeJ *in vitro*. It became obvious very soon that these cells behaved very differently under standard tissue culture conditions. Compared to the FVB/NJ cells, the SMC from

Figure 4. Growth of aortic SMC isolated from FVB/NJ and C3H/HeJ mice in response to 10% fetal calf serum. Equal numbers of cells were plated at day 0 and cell numbers were counted at the indicated time points after plating. Growth curves from two separate cell isolations (1 and 2) are shown.

Figure 5. The number of medial SMC on cross-sectioned common carotid arteries from C3H/HeJ and FVB/NJ mice was determined 2 days after ligation of the vessel. The data are expressed as a percentage of medial SMC in the unmanipulated contralateral artery. There was a significant loss of medial SMC in FVB/NJ but not in C3H/HeJ mice. Values represent means $\pm$ SE, 5 animals per group.

C3H/HeJ mice grew so slowly that it was problematic to obtain enough cells to perform the growth curve assays. Second, we examined the initial loss of SMC from the tunica media after ligation of the vessel. This experiment was based on our experience with vascular injury models in which the magnitude of medial SMC proliferation correlates with the amount of damage (loss of DNA) inflicted on the vessel wall.^{20,21} As we have previously shown, fibroblast growth factor-2 released from lethally and sublethally damaged cells is a key player in the onset of medial SMC proliferation. Nearly 37% of medial SMC were lost from the media within 2 days after ligation (ie, before SMC division occurs) in FVB/NJ carotid arteries, whereas no loss was detectable in the C3H/HeJ mice. It should be noted that in a study using immature rabbits, Cho and coworkers²⁸ reported apoptosis of medial SMC after a reduction in flow. An emerging question that we cannot answer at present is why SMC from FVB/NJ mice are more susceptible to medial SMC loss in the ligation model. Another factor that is likely to play a role in SMC proliferation and intimal lesion formation is inflammation. From our studies carried out in P-selectin-deficient mice¹² we know that the accompanying inflammatory response may contribute to intimal lesion formation after carotid artery ligation, because fewer infiltrating leukocytes and smaller intimal lesions were observed in the P-selectin-deficient mice.

A study on the genetic control of platelet activation in inbred mouse strains by Ault and coworkers²⁹ revealed large differences among strains with regard to spontaneous platelet activation. Interestingly, SJL/J mice showed the highest levels of spontaneous platelet activation, followed by AKR/J mice, whose levels were still more than twofold higher than any of the other strains tested. In the present study, SJL/J and AKR/J mice also showed extensive inward remodeling. This interesting correlation raises

the question whether platelets or platelet factors are mediating the decrease in arterial diameter. Potential vasoconstrictors present in platelets include serotonin, thromboxane, and platelet-derived growth factor.^{30–34} Platelet adhesion and subsequent release of platelet products are likely to occur in the ligation model, since we have frequently seen discontinuities in the endothelial layer¹¹ that would lead to the exposure of the thrombogenic subendothelial matrix. Immunostaining with an anti-platelet antibody has also revealed the presence of platelet immunoreactivity in the ligated vessels (data not shown). The possibility that platelet products are mediating vasoconstriction in this model would also agree with the notion that the initial vessel constriction after flow reduction is thought of as a vasoactive response.¹

The data presented here suggest that the remodeling response is genetically controlled, making linkage analysis a valuable approach for future studies. With the increasing use of this model by other investigators, the present study provides important data on the influence of genetic background on physiological remodeling as well as SMC proliferation in the carotid artery. These results have widespread implications for the interpretation of vascular injury studies performed in mice with different genetic backgrounds.

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References

1. Brownlee RD, Langille BL: Arterial adaptations to altered blood flow. *Can J Physiol Pharmacol* 1991; 69:978–983
2. Geary RL, Kohler TR, Vergel S, Kirkman TR, Clowes AW: Time course of flow-induced smooth muscle cell proliferation and intimal thickening in endothelialized baboon vascular grafts. *Circ Res* 1994; 74: 14–23
3. Kim YH, Chandran CB, Bower TJ, Corson JD: Flow dynamics across end-to-end vascular bypass graft anastomoses. *Ann Biomed Eng* 1993; 21:311–320
4. Ojha M: Wall shear stress temporal gradient and anastomotic intimal hyperplasia. *Circ Res* 1994; 74:1227–1231
5. Losordo DW, Rosenfield K, Kaufman J, Pieczek A, Isner JM: Focal compensatory enlargement of human arteries in response to progressive atherosclerosis: in vivo documentation using intravascular ultrasound. *Circulation* 1994; 89:2570–2577
6. Kakuta T, Currier JW, Haudenschild CC, Ryan TJ, Faxon DP: Differences in compensatory vessel enlargement, not intimal formation, account for restenosis after angioplasty in the hypercholesterolemic rabbit model. *Circulation* 1994; 89:2809–2815
7. Lafont AM, Chisolm GM, Whitlow PL, Goormastic M, Cornhill JF: Post-angioplasty restenosis in the atherosclerotic rabbit: proliferative response or chronic constriction? *Circulation* 1993; 88:1–521
8. Nunes GL, Sgoutas DS, Redden RA, Sigman SR, Gravanis MB: Combination of vitamins C and E alters the response to coronary balloon injury in the pig. *Arterioscler Thromb Vasc Biol* 1995; 15:156–165
9. Mintz GS, Popma JJ, Hong MK, Pichard AD, Kent KM, Satler LF, Leon MB: Intravascular ultrasound to discern device-specific effects and mechanisms of restenosis. *Am J Cardiol* 1996; 78:18–22
10. Mintz GS, Popma JJ, Pichard AD, Kent KM, Satler LF, Wong C, Hong MK, Kovach JA, Leon MB: Arterial remodeling after coronary

- angioplasty: a serial intravascular ultrasound study. *Circulation* 1996, 94:35–43
11. Kumar A, Lindner V: Remodeling with neointima formation in the mouse carotid artery after cessation of blood flow. *Arterioscler Thromb Vasc Biol* 1997, 17:2238–2244
12. Kumar A, Hoover JL, Simmons CA, Lindner V, Shebuski RJ: Remodeling and neointimal formation in the carotid artery of normal and P-selectin-deficient mice. *Circulation* 1997, 96:4333–4342
13. Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, Tedgui A: Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arterioscler Thromb Vasc Biol* 1996, 16:1256–1262
14. Xue C, Johns RA: Upregulation of nitric oxide synthase correlates temporally with onset of pulmonary vascular remodeling in the hypoxic rat. *Hypertension* 1996, 28:743–753
15. Mitani Y, Maruyama K, Sakurai M: Prolonged administration of L-arginine ameliorates chronic pulmonary hypertension and pulmonary vascular remodeling in rats (see comments). *Circulation* 1997, 96:689–697
16. Takemoto M, Egashira K, Tomita H, Usui M, Okamoto H, Kitabatake A, Shimokawa H, Sueishi K, Takeshita A: Chronic angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade: effects on cardiovascular remodeling in rats induced by the long-term blockade of nitric oxide synthesis. *Hypertension* 1997, 30:1621–1627
17. Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, Sessa WC: Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *J Clin Invest* 1998, 101:731–736
18. Mondy JS, Lindner V, Miyashiro JK, Berk BC, Dean RH, Geary RL: Platelet-derived growth factor ligand and receptor expression in response to altered blood flow in vivo. *Circ Res* 1997, 81:320–327
19. Lindner V, Fingerle J, Reidy MA: Mouse model of arterial injury. *Circ Res* 1993, 73:792–796
20. Lindner V, Reidy MA, Fingerle J: Regrowth of arterial endothelium. Denudation with minimal trauma leads to complete endothelial cell regrowth. *Lab Invest* 1989, 61:556–563
21. Fingerle J, Au YP, Clowes AW, Reidy MA: Intimal lesion formation in rat carotid arteries after endothelial denudation in absence of medial injury. *Arteriosclerosis* 1990, 10:1082–1087
22. Lindner V, Reidy MA: Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. *Proc Natl Acad Sci USA* 1991, 88:3739–3743
23. Lindner V, Olson NE, Clowes AW, Reidy MA: Inhibition of smooth muscle cell proliferation in injured rat arteries. Interaction of heparin with basic fibroblast growth factor. *J Clin Invest* 1992, 90:2044–2049
24. Sibilia M, Wagner EF: Strain-dependent epithelial defects in mice lacking the EGF receptor [published erratum appears in *Science* 1995, 269:909]. *Science* 1995, 269:234–238
25. Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, LaMantia C, Mourton T, Herrup K, Harris RC: Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 1995, 269:230–234
26. Langille BL: Remodeling of developing and mature arteries: endothelium, smooth muscle, and matrix. *J Cardiovasc Pharmacol* 1993, 21(suppl 1):S11–S17
27. Sharp MG, Fettes D, Brooker G, Clark AF, Peters J, Fleming S, Mullins JJ: Targeted inactivation of the Ren-2 gene in mice. *Hypertension* 1996, 28:1126–1131
28. Cho A, Mitchell L, Koopmans D, Langille BL: Effects of changes in blood flow rate on cell death and cell proliferation in carotid arteries of immature rabbits. *Circ Res* 1997, 81:328–337
29. Ault KA, Knowles C, Mitchell J, Brown CL, Schultz KL, Beamer WG: Genetic control of platelet activation in inbred mouse strains. *Platelets* 1997, 8:235–241
30. Vanhoutte PM, Houston DS: Platelets, endothelium, and vasospasm. *Circulation* 1985, 72:728–734
31. Schumacher MA, Alksne JF: Mechanisms of whole blood-induced cerebral arterial contraction. *Neurosurgery* 1981, 9:275–282
32. Yamada K, Kubo K, Shuto K, Nakamizo N: Inhibition of thromboxane A₂-induced vasoconstriction by KF4939, a new anti-platelet agent, in rabbit mesenteric and dog coronary arteries. *Jpn J Pharmacol* 1984, 36:283–290
33. Vanhoutte PM, Cohen RA, Van Nueten JM: Serotonin and arterial vessels. *J Cardiovasc Pharmacol* 1984, 6(suppl 2):S421–S428
34. Berk BC, Alexander RW, Brock TA, Gimbrone MAJ, Webb RC: Vasoconstriction: a new activity for platelet-derived growth factor. *Science* 1986, 232:87–90