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# Short Communication

## Basic Fibroblast Growth Factor: Its Role in the Control of Smooth Muscle Cell Migration

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***The formation of an intimal lesion in an injured artery is the consequence of the replication and migration of smooth muscle cells. Recent studies have implicated basic fibroblast growth factor (bFGF) as an important mediator of replication in the arterial media, and platelet-derived growth factor as an important mediator of migration. However, the degree of arterial trauma produced during injury has a significant influence on the time of onset of intimal thickening, suggesting that factors released from damaged smooth muscle cells may affect migration. We have investigated the role of one of these factors, bFGF, in smooth muscle cell migration*** **in vivo.** ***We found that 1) deendothelialization of the rat carotid artery results in significantly more migration when it is accompanied by traumatic injury to the underlying smooth muscle; 2) the rate of migration in arteries that have been gently deendothelialized is significantly stimulated by systemic injection of bFGF; and 3) inhibition of bFGF with a blocking antibody significantly reduces the amount of migration after traumatic deendothelializing injury with a balloon catheter. These findings suggest that bFGF plays an important role in the mediation of smooth muscle cell migration after arterial injury. (Am J Pathol 1993, 143:1024–1031)***

Recent reports have shown that the replication and migration of smooth muscle cells are both key events in the development of arterial lesions, and there is evidence to suggest that these events are controlled by different factors. For example, platelet-derived growth factor (PDGF), a potent mitogen for smooth muscle cells *in vitro*, has only a minimal effect on smooth muscle cell replication in injured rat arteries yet significantly increases the migration of smooth muscle cells from the media into the intima.[1,2] In contrast, basic fibroblast growth factor (bFGF), which is expressed and synthesized by rat arterial smooth muscle cells,[3] markedly increases smooth muscle cell replication in injured arteries[4] but little is known about its effect on smooth muscle cell migration. With respect to the role of bFGF in migration, in one study we found that a single administration of neutralizing antibody to this growth factor immediately before balloon catheter injury did not inhibit intimal lesion development and by inference was thought not to block smooth muscle cell migration.[5]

We have observed a close correlation in injured arteries between the onset of smooth muscle cell migration and the synthesis of tissue plasminogen activator (tPA).[6] Furthermore, the presence of a plasmin inhibitor, tranexamic acid, markedly inhibits the ability of smooth muscle cells to migrate into the intima. bFGF has been shown to influence the synthesis of plasminogen activators[7] and certain collagenases.[8,9] It has been suggested that the effect of PDGF, a known chemotactic agent, on smooth muscle cell migration is indirect and is mediated via synthesis of bFGF.[10] One possibility therefore is that bFGF, which is known to be released after vascular injury, not only stimulates smooth muscle cells to replicate but also induces their migration toward the intima.

In this study, we examined smooth muscle cell migration in arteries subjected either to balloon catheter injury or to gentle denudation, a procedure that pro-

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duces total endothelial denudation with little medial damage.[11] We found that gentle denudation caused significantly less smooth muscle cell migration than balloon catheter injury. The administration of bFGF at the time of gentle denudation caused a marked increase in smooth muscle cell migration 4 days after surgery. Finally, smooth muscle cell migration after balloon injury was inhibited by administration of a blocking antibody to bFGF. These results strongly suggest that bFGF plays a role in smooth muscle cell migration and potentiates the movement of smooth muscle cells into the intima.

## Materials and Methods

### Administration of bFGF

Immediately after arterial injury by the gentle denudation technique, human recombinant bFGF (a generous gift from Synergen, Inc., Boulder, CO) was administered in a bolus intravenous dose of 60 µg. Control animals received an equivalent volume of vehicle (26 mmol/L glycerol, 0.9 mmol/L sodium phosphate, pH 7.3).

### Administration of Neutralizing Antibody to bFGF

An antibody to bFGF was raised in rabbits, as previously described.[5] Immunoglobulin G (IgG) was purified by protein G-Sepharose (Pharmacia LKB, Uppsala, Sweden) chromatography and was dissolved in phosphate-buffered saline (PBS) at a concentration of 7.5 mg/ml. Nonimmune rabbit IgG was produced the same way. To facilitate the penetration of the IgG into the arterial wall, the endothelium was removed from the left common carotid artery by the gentle denudation technique, and 10 mg of IgG or 1.33 ml of PBS was injected intravenously. Five minutes later, the artery was injured with a balloon catheter. Every 24 hours thereafter, 5 mg of IgG or 0.67 ml of PBS was injected intravenously.

### Arterial Injury

Male Sprague-Dawley rats were obtained from Tyler Laboratories (Bellevue, WA) and were surgically anesthetized by intraperitoneal injection under ether anesthesia of a mixture of ketamine hydrochloride (Ketaset, Aveco Company, Fort Dodge, IA; 71.7 mg/kg) and xylazine (AnaSed, Lloyd Laboratories, Shenandoah, IA; 4.6 mg/kg). The common carotid arteries were injured by three rotating passes with either a size 2 French arterial embolectomy catheter (American Edwards Laboratories, Añasco, Puerto Rico) inflated with saline, or in the case of gentle denudation, with a loop of 5-0 nylon monofilament suture.

### Quantification of Smooth Muscle Cell Migration

Animals were killed 4 days after arterial injury by intravenous overdose of sodium pentobarbital (Anthony Products Company, Arcadia, CA) and were exsanguinated by perfusion with Lactated Ringer's Injection U.S.P. (Baxter Healthcare Corporation, Deerfield, IL) at a pressure of 120 mmHg. The animals were fixed by perfusion with Chi's fixative (1% paraformaldehyde, 2% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.2). The common carotid arteries were removed, and in one study, a small piece was removed for assay of smooth muscle cell proliferation as described below. The remaining tissue was postfixed overnight in Chi's fixative at 4 C. The vessels were opened longitudinally and pinned out on teflon cards. They were dehydrated through an ethanol series before being dried in a critical point drier (Tousimis Research Corporation, Rockville, MD). The dried specimens were mounted on aluminum stubs with colloidal silver paste. After sputter coating with gold/palladium, the specimens were examined in a JEOL 35C scanning electron microscope at an accelerating voltage of 15 kV and at 86× magnification. An acetate sheet with a ruled grid was placed over the electron microscope screen. Each square of the grid was 81 $mm^2$ in area, corresponding to 4,133 $\mu^2$ on the specimen. The total area of the specimen and the area occupied by intimal smooth muscle cells were determined by counting squares. Intimal smooth muscle cells were clearly distinguishable from adherent platelets on the criteria of size, morphology, and orientation. Smooth muscle cells were generally arranged in a longitudinal orientation parallel to the direction of blood flow. The long axis was approximately 20 µ to 40 µ, and the short axis was approximately 5 µ to 15 µ, giving an average surface area of about 270 $\mu^2$. Platelets were spheres of approximately 3 µ diameter before spreading, after which they took on a stellate appearance with an approximate diameter of 6 µ. Leukocytes were occasionally seen adhering to the intimal surface. They were, in contrast to smooth muscle cells, highly electron-reflective. They were spherical in shape with a diameter of approximately 10 µ.

### *Quantification of Smooth Muscle Cell Proliferation*

In animals treated with neutralizing anti-bFGF antibody, osmotic minipumps (Alza Corporation, Palo Alto, CA) were implanted intraperitoneally at the time of arterial injury. The pumps delivered [methyl-$^3$H]thymidine (6.7 mCi/mmol/L; Du Pont Company, NEN Research Products, Boston, MA) at a constant rate of 10 µl/hour. Four days after balloon injury, the animals were killed with an intravenous overdose of sodium pentobarbital and then perfusion-fixed with 4% paraformaldehyde at a pressure of 120 mmHg for 5 minutes. A small piece of the left common carotid artery was removed and embedded in paraffin. Cross-sections were deparaffinized in xylene and then dipped in NTB-2 emulsion (Eastman Kodak Company, Rochester, NY). After exposure for 2 weeks at 4 C, the slides were developed, counterstained with hematoxylin, and mounted under coverslips. The numbers of labeled and unlabeled nuclei were counted, and the thymidine index was calculated as the percentage of total nuclei bearing autoradiographically detectable label.

### *Measurement of Plasminogen Activator Activity*

Rats were killed by intravenous injection of sodium pentobarbital and were exsanguinated by perfusion with Lactated Ringer's Injection U.S.P. at a pressure of 120 mmHg. The left common carotid artery was dissected and removed. Adventitial fat and connective tissue were carefully removed, and each artery was homogenized in 1 ml of ice-cold 50 mmol/L Tris-HCl buffer, pH 9.0. Insoluble matter was removed by centrifugation at 14,000*g*. Parallel aliquots of the supernatant were incubated with human plasminogen (essentially plasmin-free, Sigma Chemical Co., St. Louis, MO; 0.3 U/ml), des-AA-fibrinogen (Desafib, American Diagnostica Inc., Greenwich, CN; 100 µg/ml), amiloride dihydrate (Sigma: 1 mmol/L) and a chromogenic substrate for plasmin (Spectrozyme-PL, American Diagnostica Inc., Greenwich, CN; 0.5 µmol/L). Standard curves were prepared using human high-molecular weight urokinase-type plasminogen activator (uPA; Calbiochem Corporation, La Jolla, CA) and human single-chain tPA (American Diagnostica Inc., Greenwich, CN). The absorbances of the reaction mixtures were determined at the signal wavelength of 405 nm and at a reference wavelength of 630 nm using a microplate reader (Bio-Tek Instruments Inc., Winooski, VT). Absorbances were measured again after incubation of the reaction mixtures for 1 hour at 37 C. The change in the absorbance differences between the signal and reference wavelengths was calculated. The protein concentrations of the tissue samples were determined using the bicinchoninic acid method (Pierce, Rockford, IL) against a calibration curve constructed with bovine serum albumin (Sigma). The total plasmin-generating activity of each tissue sample was expressed in terms of the activity of human uPA and was normalized in terms of the protein content. The relative contributions of uPA and tPA to the total plasmin-generating activity were determined by comparing parallel aliquots incubated with and without amiloride, which specifically quenches uPA.

### *Statistical Analysis*

The significances of differences between group mean values were determined using Student's *t*-test. Two-tailed probability values less than 0.05 were considered to be significant.

## Results

### *Rate of Smooth Muscle Cell Migration after Injury*

The number of smooth muscle cells that migrated into the rat carotid arterial intima was measured 4 days after denuding injury. Arteries were injured either with the balloon catheter or by gentle denudation.[11] As shown in Figure 1, there were fewer smooth muscle cells in the intima following gentle injury (4.0 ± 1.4% of the intimal surface area) compared with balloon injury (10.5 ± 2.0% of the intimal surface area: $P < 0.05$). The plasmin activity was also determined in these two sets of arteries, and significant decreases in tPA activity (−46%: $P < 0.01$) and plasmin activity (−47%: $P < 0.01$) were found after gentle injury, when compared with balloon injury (Table 1). No significant change was observed in the uPA activity of these vessels.

We have suggested that the low rate of smooth muscle cell replication after gentle injury is due to the lack of smooth muscle cell injury[11] and a consequent reduction in the release of bFGF.[3,5] We tested this hypothesis by injecting bFGF into animals whose carotid arteries were subjected to gentle injury. The number of smooth muscle cells that migrated into the intima was quantitated after 4 days,

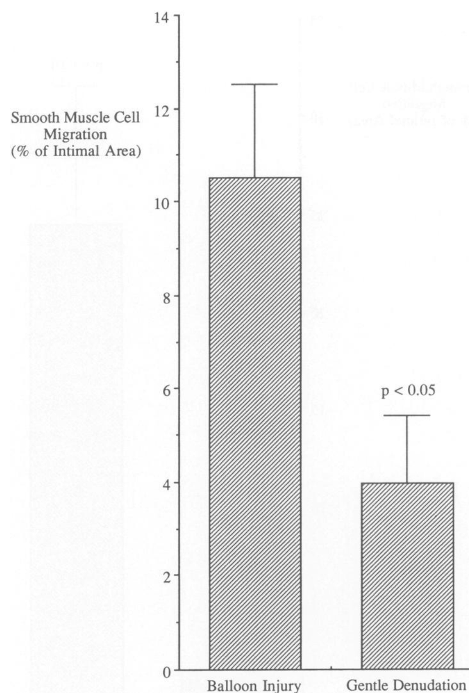


**Figure 1.** *Rat carotid arteries were injured either with a balloon catheter or by gentle injury with a rotating loop of fine nylon monofilament. The animals were killed 4 days after injury. Smooth muscle cell migration was determined by surveying with a scanning electron microscope the proportion of the intimal surface area that was occupied by smooth muscle cells.*

and, as is shown in Figure 2, there was a 15-fold increase in migration in animals treated with bFGF ($P < 0.01$). The addition of bFGF significantly increased total plasmin activity in these arteries (+164%: $P < 0.05$): both uPA activity (+256%: $P < 0.05$) and tPA activity (+141%: $P < 0.05$) were significantly increased (Table 2).

The above data suggest that bFGF plays a role in stimulating smooth muscle cell movement. This issue was addressed by administering an antibody that blocks the mitogenic action of bFGF both *in vitro* and *in vivo*.[5] A bolus of immune IgG was given immediately after balloon catheter injury, and then every 24 hours until the end of the experiment. Control animals received nonimmune rabbit IgG. As Figure 3 shows, in animals receiving the antibody the number of smooth muscle cells that migrated to the intima was 80% lower than in controls ($P < 0.001$). Interestingly, no significant decrease in arterial wall tPA, uPA, or plasmin activity was detected after antibody treatment (Table 3). To investigate the possibility that the decrease in smooth muscle cell migration in these animals was an indirect consequence of inhibition of smooth muscle cell proliferation, we measured the [$^3$H]thymidine labeling index in the same vessels. As is shown in Figure 4, there was no significant effect of the neutralizing antibody on smooth muscle cell replication as assessed continuously over the 4-day period.

## Discussion

### Arterial Injury and Smooth Muscle Cell Migration

The migration of smooth muscle cells to the intima is an important and essential step for the development of intimal lesions in rat arteries. In common with many small mammals, rats do not have smooth muscle cells normally resident in the arterial intima.[12] Therefore, the development of intimal lesions in rat arteries requires the migration of smooth muscle cells from the media into the intima. The importance of this phase of lesion progression has been made clear in recent reports that show that inhibition of PDGF with a blocking antibody markedly reduces the size of intimal lesions though having little effect on smooth muscle cell replication.[2] This finding is supported by the observation that the administration of exogenous PDGF causes an approximate doubling of the size of intimal lesions but has no effect on smooth muscle cell replication.[1] bFGF is a growth factor known to be capable of stimulating the replication of smooth muscle cells in injured arteries,[4] and there is some evidence from work in this laboratory that it may also be active in influencing smooth muscle cell migration. This was inferred from a study in which intimal lesion growth was compared in rats whose carotid arteries had either been injured with a balloon catheter or by the gentle injury procedure.[11] Not only was there a lower rate of smooth muscle cell replication following gentle injury, but also there was a marked delay in the onset of smooth muscle cell migration into the intima. Because we have proposed that a major difference between these injuries is the degree of cellular injury and release of bFGF,[13] it seemed appropriate to determine the influence of bFGF on smooth muscle cell migration.

In the present study, smooth muscle cell migration was studied in arteries that were subjected to total endothelial denudation. Two types of injury were used, both of which cause similar endothelial cell loss and similar platelet interaction with the exposed subendothelial surface.[11] These injuries have also been found to be similar in terms of stimulation of expression of certain genes, and of particular note is the finding that they do not differ in terms of the expression of PDGF-A chain and

PDGF-B chain.[11] If PDGF alone were responsible for controlling smooth muscle cell migration, we might expect to see similar rates of migration into the intima in these injured arteries. However, the rates of smooth muscle cell migration were markedly different. This suggests that PDGF cannot be totally responsible for smooth muscle cell migration and also suggests that the rate of migration is dependent upon the severity of the denuding injury. We have previously shown that a major difference between these two injury protocols is the extent of medial damage and the amount of bFGF released. This fact is important in explaining the differences in smooth muscle cell replication, and it could possibly explain the difference in the observed rates of smooth muscle cell migration. After balloon injury, where there is cell trauma and release of bFGF,[5] smooth muscle cell migration occurs at a significantly higher rate than in arteries subjected to gentle injury, where little trauma and little release of bFGF is thought to occur. Thus the rate of smooth muscle cell migration in injured arteries is influenced by the severity of the initial denuding injury, which suggests that factors other than PDGF also play a role in stimulating smooth muscle cell migration.

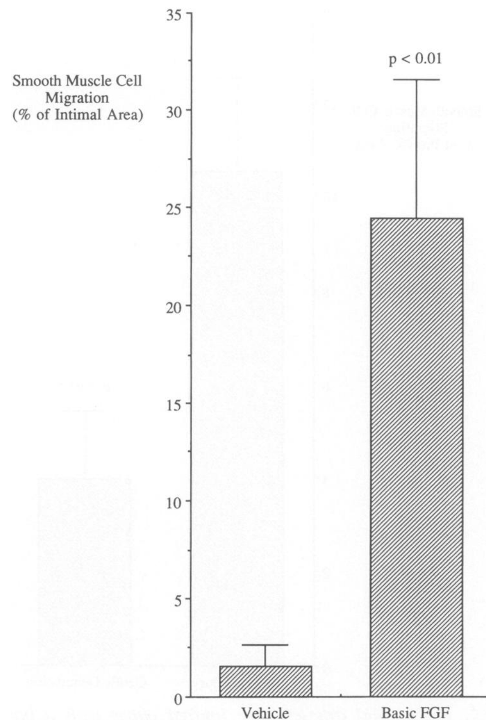


**Figure 2.** *Rat carotid arteries were injured with a rotating loop of fine nylon monofilament. Immediately after injury, an intravenous injection of 60 μg of bFGF or vehicle was given. The animals were killed 4 days after injury. Smooth muscle cell migration was determined by surveying with a scanning electron microscope the proportion of the intimal surface area that was occupied by smooth muscle cells.*

## *bFGF and Smooth Muscle Cell Migration*

The difference in smooth muscle cell migration caused by these two types of arterial injury suggests that bFGF is involved in this process. This hypothesis is supported by the data showing that administration of bFGF to rats significantly increases the number of migrating smooth muscle cells. If release of bFGF is important in stimulating the migration of medial smooth muscle cells into the intima, then migration should be inhibited by blocking endogenously released bFGF. We have previously shown that a single injection of a polyclonal antibody made against bFGF does not result in a smaller intimal lesion.[5] In the present study, we show that the use of this antibody reduces by 80% the number of smooth muscle cells migrating to the intima. On first inspection this result would seem to disagree with our earlier data. In the previous study, however, the antibody was given in a single bolus immediately before injury, whereas in this study it was also given every day during the experiment. We now know that migration starts after 3 days,[6] and a single injection of antibody given at the time of surgery would be cleared by this time. The need for multiple injections of the bFGF antibody to inhibit migration suggests that bFGF is active in the injured artery for several days following balloon catheterization. One explanation for the prolonged activity of bFGF activity may be that the released protein binds to the extracellular matrix and then is released from this site for several days after injury. In

**Table 1.** *Effects of Arterial Injury on Plasminogen Activator Activity*

| Group | *n* | tPA activity (IU uPA/mg protein) | | uPA activity (IU uPA/mg protein) | | Total plasmin activity (IU uPA/mg protein) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SEM | Mean | SEM | Mean | SEM |
| Balloon injury | 27 | 3.41 | 0.33 | 1.13 | 0.22 | 4.54 | 0.47 |
| Gentle denudation | 11 | 1.85* | 0.32 | 0.56 | 0.16 | 2.42* | 0.43 |

Rat carotid arteries were injured either with a balloon catheter or by gentle injury with a rotating loop of fine nylon monofilament. The animals were killed 4 days after injury. The activities of tPA and uPA were determined using an amidolytic assay on arterial homogenates, and were expressed in terms of the activity of a standard preparation of human uPA. SEM: standard error of the mean. * $P < 0.01$.

**Table 2.** *Effect of Exogenous bFGF on Arterial Plasminogen Activator Activity*

| | | tPA activity (IU uPA/mg protein) | | uPA activity (IU uPA/mg protein) | | Total plasmin activity (IU uPA/mg protein) | |
|---|---|---|---|---|---|---|---|
| Group | *n* | Mean | SEM | Mean | SEM | Mean | SEM |
| Vehicle | 5 | 2.20 | 0.59 | 0.55 | 0.24 | 2.75 | 0.80 |
| Basic FGF | 4 | 5.30* | 0.91 | 1.96* | 0.56 | 7.25* | 1.46 |

Rat carotid arteries were injured with a rotating loop of fine nylon monofilament. Immediately after injury, an intravenous injection of 60 μg of bFGF or of vehicle was given. The animals were killed 4 days after injury. The activities of tPA and uPA were determined using an amidolytic assay on arterial homogenates and were expressed in terms of the activity of a standard preparation of human uPA. SEM: standard error of the mean. * $P < 0.05$.

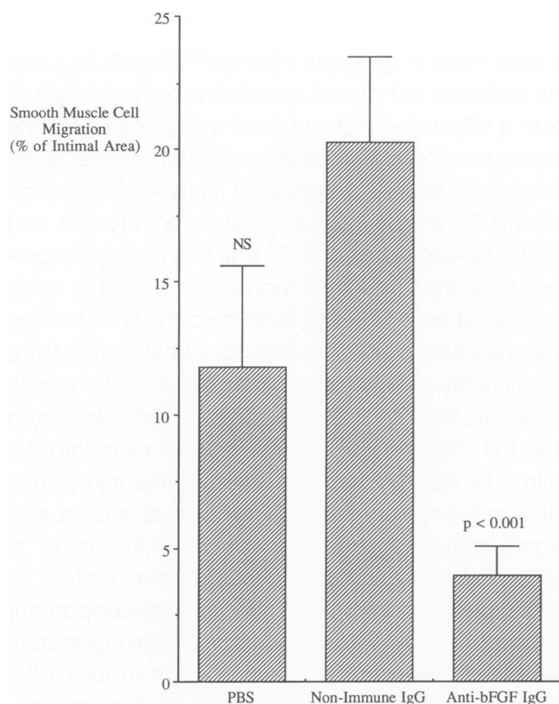


**Figure 3.** *Rat carotid arteries were injured with a rotating loop of fine nylon monofilament, and then an intravenous injection of PBS, nonimmune rabbit IgG, or a neutralizing anti-bFGF antibody was given. Five minutes later the vessels were reinjured with a balloon catheter. Further injections of antibody were given every 24 hours, and the animals were killed 4 days after injury. Smooth muscle cell migration was determined by surveying with a scanning electron microscope the proportion of the intimal surface area that was occupied by smooth muscle cells.*

this way, bFGF might be available to stimulate smooth muscle cell migration and replication at later times after injury. We have no direct evidence of this occurrence, but we do know that balloon catheter injury displaces most of the bFGF in the arterial wall into an extracellular pool.[14] This finding could explain why bFGF must be inhibited for several days to block smooth muscle cell migration.

In a previous study, we showed that neutralization of bFGF inhibited the first wave of smooth muscle cell proliferation after balloon injury.[5] A concern in the present study, therefore, was that the observed inhibition of smooth muscle cell migration might simply have been the consequence of an antiproliferative effect of antibody treatment. In fact, this was not the case, because migration was inhibited by 80%, but there was no effect on proliferation in the same vessels. However, the lack of effect on proliferation is surprising when considered in the light of our previous study.[5] One explanation for this difference relates to the protocols used for administration of [$^3$H]thymidine. In the earlier study, three injections of [$^3$H]thymidine were given between 23 and 39 hours after balloon injury, and animals were killed at 40 hours. In the present study, [$^3$H]thymidine was administered by continuous infusion from the time of injury until the time of killing at 96 hours. It is possible that the proliferation of medial smooth muscle cells was delayed by antibody treatment, so that the rate of proliferation measured at 40 hours was decreased but the total proliferation over the course of 4 days was not affected. Further studies will be required to resolve this issue. However, the data do suggest that treatment with the anti-bFGF antibody directly influenced smooth muscle cell migration in the absence of any overall change in cellular replication.

The data presented above reinforce our suggestion that bFGF plays an important role in smooth muscle cell migration. Interestingly, recent data has shown that PDGF also plays an important role in smooth muscle cell migration.[2] It would seem therefore that migration may be controlled by multiple factors that perhaps may act in concert. Cells normally migrate along a concentration gradient of a chemotactic agent and in a balloon-injured artery where bFGF seems to be ubiquitous,[3] it is difficult to envisage how a gradient of this factor could be established. Also, bFGF does not seem to be chemotactic for smooth muscle cells *in vitro*.[2,15] Platelet PDGF, on the other hand, is released on to the luminal surface of injured vessels and therefore should be present in high concentration at this site. Because bFGF is known to stimulate migration of cells by activating proteolytic enzymes,[9,10,16–19] it is possible that these two factors may regulate migration.

**Table 3.** *Effect of Neutralizing Anti-bFGF Antibody on Arterial Plasminogen Activity*

| Group | n | tPA activity (IU uPA/mg protein) | | uPA activity (IU uPA/mg protein) | | Total plasmin activity (IU uPA/mg protein) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SEM | Mean | SEM | Mean | SEM |
| PBS | 6 | 3.69 | 0.63 | 1.24 | 0.35 | 4.93 | 0.97 |
| Nonimmune IgG | 5 | 3.59 | 0.61 | 1.43 | 0.55 | 5.02 | 1.16 |
| Anti-bFGF IgG | 7 | 2.47 | 0.24 | 0.78 | 0.16 | 3.24 | 0.31 |

Rat carotid arteries were injured with a rotating loop of fine nylon monofilament, and then an intravenous injection of PBS, nonimmune rabbit IgG, or a neutralizing anti-bFGF antibody was given. Five minutes later the vessels were reinjured with a balloon catheter. Further injections were given every 24 hours, and the animals were killed 4 days after injury. The activities of tPA and uPA were determined using an amidolytic assay on arterial homogenates, and were expressed in terms of the activity of a standard preparation of human uPA. SEM: standard error of the mean. There were no significant differences.

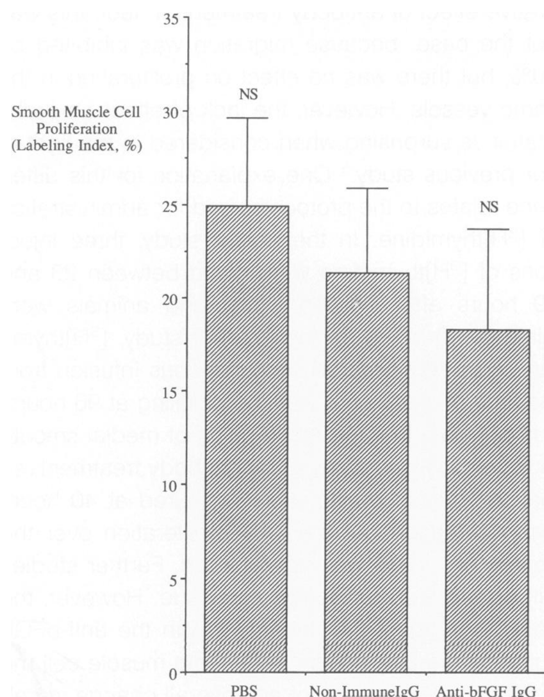


**Figure 4.** *Rat carotid arteries were injured with a rotating loop of fine nylon monofilament, and then an intravenous injection of PBS, nonimmune rabbit IgG, or a neutralizing anti-bFGF antibody was given. An osmotic minipump loaded with [methyl-$^3$H]thymidine was implanted into the peritoneal cavity. Five minutes later, the carotid arteries were reinjured with a balloon catheter. Further injections of antibody were given every 24 hours, and the animals were killed 4 days after injury. Smooth muscle cell proliferation in arterial sections was determined by autoradiography.*

bFGF may be required to switch on the machinery required for movement, such as plasminogen activators or other proteases, with PDGF providing the signal for directional migration. In the absence of either factor, migration of the cells could be reduced. The data from this and other studies would support this idea.

## *Smooth Muscle Cell Migration and Proteases*

In a recent study, we observed a relationship between smooth muscle cell migration and the ability of arterial cells to generate plasmin.[6] Plasmin is a serine protease with broad specificity that is thought to play a role in the metastasis of malignant cells. The expression of plasminogen activator genes is increased in arteries 3 days after injury and is accompanied by an increase in plasmin activity. We and others have proposed[6,20,21] that this change in proteolytic activity may be important for smooth muscle cell migration, a concept supported by data showing that tranexamic acid, an inhibitor of plasmin activity, significantly reduces the migration of smooth muscle cells into the intima. In the current study, we noted that the migration of smooth muscle cells into the intima is less pronounced after gentle injury than after balloon injury and that plasmin activity is also significantly reduced. Moreover, administration of bFGF markedly increases the plasmin activity in gently denuded arteries. These findings support our concept that bFGF can regulate plasminogen activators and plasmin generation, which in turn influence migration. We therefore anticipated that the addition of the bFGF antibody would significantly inhibit migration, but were surprised to find that plasmin activity was not reduced in this experiment. This finding could suggest that other proteolytic enzymes, also controlled by bFGF, are involved in smooth muscle cell migration. It is known that smooth muscle cells can synthesize a variety of metalloproteinases, and bFGF has been found to stimulate collagenases in other vascular cells.[8,9,16,19] Thus the bFGF antibody might interfere with smooth muscle cell migration by acting on other, as yet unidentified, proteolytic pathways. Regardless of the pathway involved in smooth muscle cell migration, this result reinforces the suggestion that bFGF plays a critical role in the migration of smooth muscle cells into the intima.

In summary, these studies show that bFGF plays an important role in stimulating the migration of smooth muscle cell into the intima. The administration of bFGF increased plasminogen activator and plasmin activity in denuded arteries and also stimulated smooth muscle cell migration. An antibody

that neutralizes bFGF significantly inhibited smooth muscle cell migration, but did not inhibit plasmin synthesis. Other bFGF-stimulated proteolytic pathways may therefore be involved in smooth muscle cell migration.

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## References

1. Jawien AD, Bowen-Pope DF, Lindner V, Schwartz SM, Clowes AW: Platelet-derived growth factor promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. J Clin Invest 1992, 89:507–511
2. Ferns GAA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R: Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 1991, 253:1129–1132
3. Olson NE, Chao S, Lindner V, Reidy MA: Intimal smooth muscle cell proliferation after balloon catheter injury: the role of basic fibroblast growth factor. Am J Pathol 1992, 140:1017–1023
4. Lindner V, Lappi DA, Baird A, Majack RA, Reidy MA: Role of basic fibroblast growth factor in vascular lesion formation. Circ Res 1991, 68:106–113
5. 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
6. Jackson C, Reidy M: Basic FGF is necessary for arterial smooth muscle cell migration after balloon catheter injury. J Cell Biochem 1992, (suppl) 16A:30
7. Presta M, Moscatelli D, Silverstein JJ, Rifkin DB: Purification from a human hepatoma cell line of a basic fibroblast growth factor-like molecule that stimulates capillary endothelial cell plasminogen activator production, DNA synthesis, and migration. Mol Cell Biol 1986, 6:4060–4066
8. Gross JL, Moscatelli D, Jaffe EA, Rifkin DB: Plasminogen activator and collagenase production by cultured capillary endothelial cells. J Cell Biol 1982, 95:974–981
9. Moscatelli D, Presta M, Rifkin DB: Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration. Proc Natl Acad Sci USA 1986, 83:2091–2095
10. Sato Y, Hamanaka R, Ono J, Kuwano M, Rifkin DB, Takaki R: The stimulatory effect of PDGF on vascular smooth muscle cell migration is mediated by the induction of endogenous basic FGF. Biochem Biophys Res Comm 1991, 174:1260–1266
11. Fingerle J, Au YTP, Clowes AW, Reidy MA: Intimal lesion formation in rat carotid arteries after endothelial denudation in absence of medial injury. Arteriosclerosis 1990, 10:1082–1087
12. Reidy MA, Schwartz SM: Endothelial regeneration. III. Time course of intimal changes after small defined injury to rat aortic endothelium. Lab Invest 1981, 44:301–308
13. 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
14. Lindner V, Olson NE, Reidy MA: Heparin removes endogenous bFGF released into the vessel wall after arterial injury. J Cell Biochem 1992, Suppl 16A:11
15. Grotendorst GR, Chang T, Seppä HEJ, Kleinman HK, Martin GR: Platelet-derived growth factor is a chemoattractant for vascular smooth muscle cells. J Cell Physiol 1982, 113:261–266
16. Tsuboi R, Sato Y, Rifkin DB: Correlation of cell migration, cell invasion, receptor number, proteinase production, and basic fibroblast growth factor levels in endothelial cells. J Cell Biol 1990, 110:511–517
17. Sato Y, Rifkin DB: Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J Cell Biol 1988, 107:1199–1205
18. Pepper MS, Belin D, Montesano R, Orci L, Vassalli J-D: Transforming growth factor-beta 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells in vitro. J Cell Biol 1990, 111:743–755
19. Mignatti P, Tsuboi R, Robbins E, Rifkin DB: In vitro angiogenesis on the human amniotic membrane: requirement for basic fibroblast growth factor-induced proteinases. J Cell Biol 1989, 108:671–682
20. Clowes AW, Clowes MM, Au YPT, Reidy MA, Belin D: Smooth muscle cells express urokinase during mitogenesis and tissue-type plasminogen activator during migration in injured rat carotid artery. Circ Res 1990, 67:61–67
21. Clowes AW, Clowes MM, Kirkman TR, Jackson CL, Au YPT, Kenagy R: Heparin inhibits the expression of tissue-type plasminogen activator by smooth muscle cells in injured rat carotid artery. Circ Res 1992, 70:1128–1136