Vein interposition cuffs decrease the intimal hyperplastic response of polytetrafluoroethylene bypass grafts

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Purpose: The modification of the distal anastomosis of polytetrafluoroethylene (PTFE) bypass grafts with vein interposition cuffs (VCs) has been reported to increase graft patency. However, the mechanisms that are responsible for this improved patency are unclear. Because intimal hyperplasia (IH) is a primary cause of prosthetic graft failure, we hypothesized that VCs affect the distal anastomosis by decreasing the IH response of the outflow artery.

Methods: Twenty-three female domestic Yorkshire pigs (mean weight, 35 kg) underwent 42 femoral PTFE bypass grafting procedures. The PTFE bypass grafts were separated into the following three groups according to distal anastomotic configuration: end-to-side anastomoses (ES), VCs, and cuffs constructed with PTFE (PCs). Four femoral arteries from two pigs served as healthy controls. At sacrifice, the grafts were perfusion fixed, and the distal anastomoses harvested at 1 and 4 weeks. The specimens were hemisected and serially sectioned to identify the heel, toe, and mid-anastomotic regions. The sections were cut into 5-μm segments and analyzed for intima and media thickness and area, intima/media area ratio, and the distribution of IH in the vein cuff. The roles of transforming growth factor–β1 and platelet-derived growth factor–BB in IH development were assessed with immunohistochemistry.

Results: IH development was significantly lower at all areas of the anastomosis, with VCs compared with ES and PCs at 4 weeks ($P \leq .001$). IH decreased in VCs from 1 to 4 weeks in all areas of the anastomosis ($P \leq .001$). PCs showed pronounced IH at the mid-anastomosis as compared with VCs and ES ($P \leq .001$). IH was most pronounced at the toe with ES and PCs ($P \leq .001$). Qualitatively, VCs altered the site of IH development, sparing the recipient artery with preferential thickening of the vein cuff and formation of a pseudointima at the vein-PTFE interface. Immunohistochemistry results showed positive staining for transforming growth factor–β1, platelet-derived growth factor–BB, and smooth muscle α-actin in the hyperplastic intima.

Conclusion: PTFE bypass grafts with VCs had less IH develop than did grafts with ES and PC anastomoses. IH regression in VCs at 4 weeks suggests compensatory vessel wall remodeling mediated by the presence of the VC. Furthermore, VCs caused a redistribution of hyperplasia to the vein-PTFE interface, delaying IH-induced outflow obstruction in the recipient artery. The marked increase in IH with PCs, despite a similar geometric configuration to VCs, suggests that the biologic properties of autogenous tissue dissipate IH development. Similarly, the flow patterns in PCs and VCs should be identical, which suggests a less important role of hemodynamic forces in VC-mediated protection. (J Vasc Surg 2000;31:69-83.)

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Competition of interest: nil.

This research was supported by grants from the National Institutes of Health (Pappas, KO8 HL03354-04; Hobson, KO7 HL03437-03; and DeFouw, HL47936-06) and an industry grant from Boston Scientific/Vascular (Pappas).

Presented at the Forty-seventh Scientific Meeting of the International Society for Cardiovascular Surgery, North American Chapter, Washington, DC, Jun 8–9, 1999.

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0741-5214/2000/$12.00 + 0 24/6/102731

The autologous saphenous vein remains the conduit of choice for patients who require infrainguinal revascularization.[1,2] The lack of a suitable or available autologous saphenous vein as the result of phlebitis, varicosities, previous limb revascularization, coronary artery bypass grafting, or poor caliber emphasizes the need for alternative bypass graft conduits.[3] Accessory saphenous veins, arm veins, lesser saphenous veins, composites (spliced vein vs a prosthetic plus a vein), and synthetic grafts alone have all shown acceptable short-term patency rates and limb salvage rates.[4-9] Similar results have been reported with adjunctive modifications at the distal anastomosis of prosthetic grafts and the concomitant use of warfarin therapy.[9-13] However, poor long-term patency rates with alternative conduits and prosthetic graft adjuncts and the lack of randomized, prospective, multicenter trials with these techniques have tempered their widespread use.[13]

Prosthetic expanded polytetrafluoroethylene (ePTFE) grafts are commonly used alternative conduits because of their biocompatability, good handling properties, ability to be manufactured in large quantities, lack of aneurysmal dilatation, and absence of intrinsic intraluminal defects.[1] In an effort to improve long-term graft patency, vein patches or interpositon vein cuffs have been added as adjunctive modifications to the distal anastomoses of prosthetic bypass grafts.[10-12,14-16] A recent review of our institution's clinical experience with vein interposition cuffs (VCs) and of one single-center randomized clinical trial has documented improved patency rates as compared with the rates of non-cuffed ePTFE bypass grafts.[16-19]

Although the clinical data suggest improved efficacy with VCs, few investigations have identified the mechanisms responsible for this improved response.[20,21] Because prosthetic grafts accelerate the development of intimal hyperplasia (IH) at the heel, the toe, and the base of the recipient outflow artery, we hypothesized that VCs improve patency by modifying the degree and distribution of distal anastomotic IH.[22-24] Therefore, the purpose of this investigation was to quantitatively and qualitatively assess the IH response of recipient outflow arteries in a porcine model after prosthetic bypass grafting anastomoses, with and without adjunctive modifications.

METHODS

Study design and rationale. Forty-two 6-mm ePTFE bypass grafts (Exxcel, Boston Scientific/Vascular, Oakland, NJ) were placed in 23 female domestic Yorkshire pigs (30 to 36 kg). A porcine model was chosen for the following reasons: (1) numerous investigators have demonstrated reproducible IH lesions after balloon angioplasty of the porcine coronary, femoral, and carotid arteries; (2) porcine arteries are histologically and biochemically similar to those of humans; (3) an intima consisting of elastic tissue, collagen, and scattered smooth muscle cells and endothelia is present; (4) porcine arteries are large enough to receive prosthetic bypass graft conduits; and (5) pigs have a coagulation system that is similar to that of humans, and therefore they avoid the hypercoaguable states that are observed with canine models.[25,26]

The pigs were separated into the following three groups according to distal anastomotic configuration, which consisted of the following at 1 and 4 weeks, respectively: (1) traditional end-to-side anastomoses (ES; n = 5; n = 6); (2) VCs fashioned in the manner described by Raptis and Miller[16] (n = 5; n = 9); and (3) PTFE interposition cuffs (PCs), which were constructed in the laboratory and autoclaved before insertion (n = 6; n = 6). The controls consisted of healthy femoral arteries harvested from pigs that had not undergone bypass grafting (n = 4). The PC group was included to address issues of anastomotic angle and compliance and control for flow pattern influences. The VC and PC groups had identical configurations, with essentially 90° interfaces between the prosthetic graft and the recipient artery. With the graft configuration and angle kept constant, the flow pattern forces were eliminated as independent variables in the development of distal anastomotic IH between these two groups. The biologic properties of the interposition cuff material become the determinate variable and an indirect assessment of compliance. The ES group similarly addresses compliance issues and angulation. All the ES anastomoses were less than 90° and greater than 30°. The differences in IH between the ES and PC groups should therefore represent the effect of anastomotic angulation because both grafts were in direct contact with the target outflow artery, which resulted in compliance mismatch.

Surgical procedures. After intramuscular sedation with ketamine hydrochloride (33 mg/kg), acepromazine maleate (0.1 mg/kg), and atropine (0.02 mg/kg), a 20-gauge intravenous needle was placed in an ear vein, and animal hair at the surgical site was shaved. Tetracycline (22 mg/kg) was administered intravenously during surgery and after surgery for two doses, and maintenance intravenous fluids were administered. After intubation, the animals were

anesthetized with isoflurane and oxygen at a maximum alveolar concentration between 1 and 3. Pulse oximetry, bovie cautery, grounding pads, temperature mats, sheepskin, and rectal temperature probes were placed before the prepping and draping.

The external jugular vein or the hind limb greater saphenous vein was used to construct the VC. Once harvested, the vein was transected longitudinally and inspected for the presence of valves or phlebitis. The acceptable segments were sutured onto the distal anastomosis of a 6-mm ePTFE graft (Exxcel). The grafts with PTFE cuffs were constructed in the laboratory and autoclaved before surgery. The venous donor sites were closed with a running 2-0 polyglactin 910 subcutaneously and a running 2-0 silk for the skin closure. Incisions were subsequently made in each hind limb groin, and the common femoral, profunda femoris, and superficial femoral (SFA) arteries were identified and dissected out. The branches of the SFA were individually ligated with 4-0 silk. Porcine heparin (100 mg / kg) was administered intravenously, and 6-mm grafts were sutured into 4-mm arteries in the usual manner with 6-0 Prolene (Ethicon, Somerville, NJ). The proximal anastomosis was sutured at the common femoral artery–SFA junction. The distal anastomosis was sutured to the distal SFA. The length of the graft was between 7 and 8 cm. At the completion of the anastomoses, a 2-0 tie was placed in the mid-portion of the bypassed femoral artery to ensure preferential flow through the bypass graft. The immediate graft patency was confirmed with continuous wave Doppler scan results. Identical graft configurations within each animal were avoided. The wounds were subsequently closed as described previously. The incisions were covered with an occlusive dressing and maintained for 24 hours.

Postoperative pain was managed with intravenous buprenorphine hydrochloride (0.3 mg / mL / ampule) every hour as needed. Graft patency was determined with manual palpation, weekly duplex ultrasound scanning, and a duplex scan on the day of harvest. For surveillance ultrasound scanning, the pigs were sedated as stated previously and mask ventilated.

On the day of harvest, the pigs were sedated and an ear vein was cannulated. After the dissection of the proximal portions of the graft, the animals were killed with an intravenous injection of 60 mL of saturated potassium chloride (Fischer Scientific, Springfield, Ill). Clamps were placed on the proximal portions of the graft, and the distal portions were perfusion fixed with a 10% formalin solution (Sigma, St Louis, Mo) at a pressure of 100 mm Hg for 15 to 20 minutes. After perfusion fixation, the distal anastomosis and adjacent veins were harvested en bloc and placed in a 10% formalin solution. All the animal studies were approved by the University of Medicine and Dentistry of New Jersey–New Jersey Medical School's Institutional Animal Care Committee and performed within the guidelines of the National Institutes of Health.

Specimen processing. The specimen blocks were removed from the formalin fixatives within 24 hours, after which they were trimmed, hemisected, and transferred to a 70% alcohol solution. The specimens were subsequently moved to a tissue processor (rotary open Auto-Technicon tissue processor model 2A, The Technicon Company, Tarrytown, NY) for overnight processing in 70%, 80%, 95%, and 100% graded ethyl alcohol solutions, two exchanges with a xylene (Sigma, St Louis, Mo) clearing agent, and molten paraffin. After the tissues were oriented, the specimens were embedded in paraffin blocks, allowed to solidify, chilled, and cut into 5-μm sections on a rotary microtome (Olympus 4060) (Olympus America Inc, Melville, NY). After sectioning, the specimens were mounted on Superfrost Plus slides (EMS, Fort Washington, Pa) for morphometric analyses and immunohistochemistry.

Morphometric analysis. Serial 5-μm sections from paraffin-embedded blocks were analyzed until areas corresponding to the middle, heel, and toe of the anastomoses were identified (Fig 1). The representative sections were deparaffinized in xylene and rehydrated with 100%, 95%, 90%, 70%, and 50% graded ethyl alcohol solutions, followed by distilled water. After hydration, the slides were stained with Verhoeff's elastic stain and van Gieson's counterstain. Measurements were obtained with videomicroscopy (Nikon Optiphot-2 microscope, Garden City, NY) and image analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, Md). The morphometric variables that were assessed were intima and media thickness, intima and media area, and intima / media area ratios (intima area / intima area + media area). The intimal and medial boundaries were determined by means of digital planimetry. Four sections from each heel, toe, and mid-anastomosis per specimen were inspected. For each section, 16 intima and media thickness measurements and four non-overlapping intima and media area measurements were performed for a total of 64 intima and media thickness and 16 intima and media areas per specimen. The area measurements were standardized with a fixed micrometer for a length of 100 μm.

Immunohistochemistry. Immunohistochemistry analyses were performed with affinity purified poly-

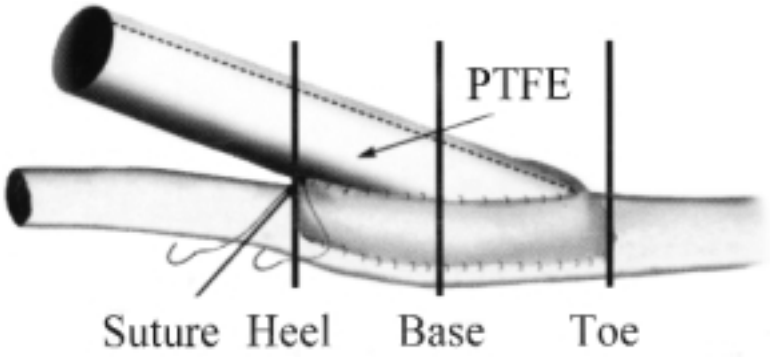

Fig 1. Areas of anastomosis corresponding to heel, base, and toe, where serial 5-μm sections for morphometric measurements and qualitative histologic and immunohistochemistry analyses were performed.

clonal antibodies to human transforming growth factor–β_1 (TGF-β_1), platelet-derived growth factor–BB (PDGF-BB), and smooth muscle α-actin. The following were the primary antibodies: (1) polyclonal, affinity purified, chicken anti-human TGF-β_1 immunoglobulin G (IgG) antibody (1:20 dilution; R&D Systems, Minneapolis, Minn); (2) polyclonal, affinity purified, goat anti-human PDGF-BB IgG antibody (1:20 dilution; R&D Systems); and (3) monoclonal, mouse anti–α actin IgG2a (smooth muscle) antibody (1:500 dilution; Boehringer-Manheim, Indianapolis, Ind). The biotinylated secondary antibody for TGF-β_1 was a goat anti-chicken IgG antibody (1:750 dilution; Vector Laboratories, Burlingame, Calif). Biotinylated secondary antibodies and blocking agents for PDGF-BB and smooth muscle α-actin staining were supplied as part of The Vectastain Elite ABC detection kits (Vector Laboratories) and the Histostain-Plus Broad Spectrum kit (Zymed, San Fancisco, Calif), respectively. The blocking reagent used for TGF-β_1 was a goat IgG antibody from goat serum (Vector Laboratories). For each antibody, the dilution that yielded the optimal specific staining was determined in pilot experiments.

As stated, the blocks were sectioned, mounted onto poly-L-lysine–coated slides, heated to 60°C, dehydrated with serial xylene, and rehydrated in serial ethyl alcohol dilutions. To facilitate antigen exposure, the sections were rinsed in 1× phosphate-buffered saline solution (PBS; Sigma), heated in a 1× saline sodium citrate solution (Sigma) in a microwave for 20 seconds, and left in the hot solution for 30 minutes. Once deparaffinized, the sections were digested with 0.2% hyaluronidase solution for 30 minutes at 37°C, followed by incubation in a 3% hydrogen peroxide solution for 10 minutes to exhaust endogenous peroxidase activity. The blocking reagents were applied for 20 minutes at room temperature and rinsed with PBS. The primary antibodies were applied for 3 hours at room temperature, followed by a washing with PBS. Biotinylated secondary antibodies for TGF-β_1 (2 μg/mL or 1:750 dilution) were applied for 30 minutes at room temperature. For PDGF-BB and smooth muscle α-actin staining, the supplied secondary antibodies were used as directed for 30 minutes and 10 minutes, respectively, at room temperature. After being rinsed with PBS, the slides were developed with a streptavidin-horseradish peroxidase complex for 30 minutes at room temperature, followed by diaminobenzidine substrate (Vectastain Elite ABC Kit). The positive reactions appeared to be dark brown with light microscopy. The smooth muscle α-actin–stained specimens were counterstained with hematoxylin and eosin for 1 minute. All the sections then were rehydrated with serially increasing ethyl alcohol dilutions before the placement of a cover slip. The sections of pig spleen underwent the same preparation and analysis and served as positive controls. The negative controls consisted of the previously mentioned investigations without the primary antibody.

Statistical analysis. The differences between the mean ± the standard error of the mean between groups were analyzed with a one-way analysis of variance and the Newman-Keuls post hoc tests. The differences between timepoints within groups were analyzed with a two-tailed Student *t* test. Significance was accepted at a *P* value of less than .05. The data were analyzed with the statistical software package GraphPad Instat (GraphPad Software, San Diego, Calif).

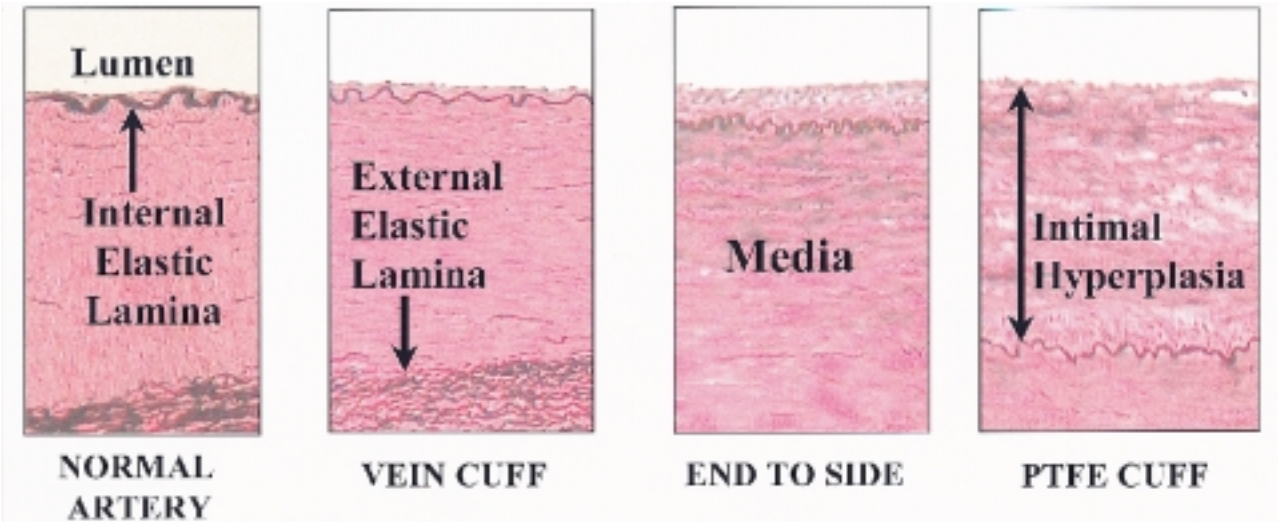

Fig 2. Verhoeff's elastic stain and van Gieson's counterstain results of mid-anastomotic sections at 4 weeks from each study group showing progressively increasing intimal thickening with vein cuff, end-to-side, and polytetrafluoroethylene *(PTFE)* grafts compared with control (magnification, 440×).

Table I. Morphologic data at the anastomotic heel at 1 week

	Control (n = 4)	*VC (n = 6)*	*ES (n = 5)*	*PC (n = 5)*	*P value*
Intima thickness (μm)	13 ± 0.6	42 ± 1.44	40 ± 1.54	84 ± 6.3	*,†,‡
Media thickness (μm)	268 ± 8	514 ± 5.1	355 ± 4.4	381 ± 6.2	*,†,‡
Intima area (μm^2)	1248 ± 123	4188 ± 237	4104 ± 274	8659 ± 1280	*,§
Media area (μm^2)	26838 ± 1660	52284 ± 1041	35679 ± 938	38638 ± 1326	§($P \leq .05$),†

VC, Vein cuff; *ES,* end-to-side; *PC,* polytetrafluoroethylene.
Data are expressed as mean ± standard error of the mean. All values are significant at $P \leq .001$, unless otherwise stated.
*Control group versus all groups.
†PC group versus VC and ES groups.
‡VC group versus ES group.
§Control group versus ES and PC groups only.

RESULTS

Graft patency. Forty-two grafts were placed in 23 domestic Yorkshire pigs. Five graft occlusions were noted and discarded from the analysis. One ES, one VC, and three PC grafts occluded during the study period.

Histology and morphometric analysis. IH was identified by the thickness of the intima layer, defined as the tissue that extends between the lumen and the internal elastic lamina. Both the internal and the external elastic laminas were well stained and demarcated as dark wavy lines with the van Gieson's counterstain method. The intima and media layers were evaluated for thickness and area at several points in the heel, base, and toe sections of the recipient artery at the predetermined timepoints. The results of the morphometric evaluations are presented in Tables I to VI, which are arranged by anastomotic configuration and period of observation. Fig 2 is a montage of the IH responses observed with each graft configuration after transverse sectioning of the middle of the anastomosis. A progression in the IH response was observed in the VC, ES, and PC groups as compared with the controls at 4 weeks. A modest increment in intima thickness was detected in the artery that received a vein cuff, with the worst response noted in the artery that received a PTFE-cuffed anastomosis.

Fig 3 graphically illustrates the distribution and relative magnitude of the IH responses associated with each anastomosis that was evaluated, with particular emphasis on the effect at the heel, base, and toe of the distal anastomosis.

We qualitatively analyzed serial vein cuff sections

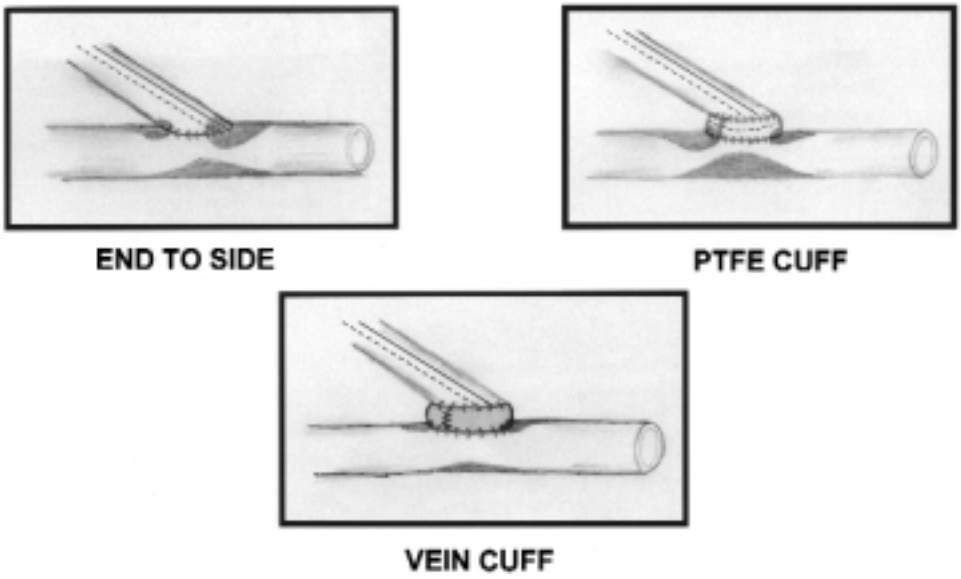

Fig 3. Magnitude of intimal hyperplasia (IH) responses at heel, toe, and mid-anastomosis within each study group at 4 weeks. End-to-side grafts showed pronounced IH at the toe compared with polytetrafluoroethylene *(PTFE)* and vein cuff grafts. PTFE grafts primarily affected the base and heel of anastomosis, whereas vein cuff grafts showed minimal IH at any site.

Table II. Morphologic data at the anastomotic heel at 4 weeks

	Control (n = 4)	*VC (n = 6)*	*ES (n = 6)*	*PC (n = 9)*	*P value*
Intima thickness (μm)	12.8 ± 0.6	12.6 ± 0.334	234 ± 18	390 ± 13	*,†,‡
Media thickness (μm)	268 ± 8	351 ± 5.8	364 ± 9	804 ± 13	*,†,‡
Intima area (μm^2)	1248 ± 123	1306 ± 61	23524 ± 3570	38324 ± 2492	§,†,‖
Media area (μm^2)	26838 ± 1660	34404 ± 1155	35607 ± 1803	80051 ± 2680	¶,†

VC, Vein cuff; *ES*, end-to-side; *PC*, polytetrafluoroethylene.
Data are expressed as mean ± standard error of the mean. All values are significant at $P \leq .001$, unless otherwise stated.
*Control group versus all groups.
†PC group versus VC and ES groups.
‡VC group versus ES group.
§Control group versus ES and PC groups only.
‖ES group versus VC group.
¶Control group versus PC group only.

to assess the morphologic alterations that occur within vein cuffs and between the artery vein and vein-PTFE interface. Fig 4 shows the progression of IH in the artery as it approaches the vein cuff artery anastomosis. The intima of the outflow artery increases as it approaches the vein cuff. The internal elastic lamina and external elastic lamina of the artery and vein cuff are clearly identified. Intimal thickening of the vein cuff is clearly shown, indicating a shift of the IH response away from the target artery and to the cuff-graft interface.

To standardize the comparisons, we calculated the intima / media area ratios at the heel, base, and toe of the anastomosis for each study group. The statistical analysis results are presented in Figs 5 to 7. Fig 5 depicts the results for intima / media area ratios obtained at the heel of the anastomoses. Only modest changes in intima / media area ratios were measured after 1 week. PC and ES grafts showed intima / media area ratios that were significantly greater as compared with those of the controls ($P \leq .05$), and VC grafts did not differ from control arteries. The intima / media area ratio increased in the ES and PC groups at 4 weeks, relative to their ratio at 1 week after the grafting procedure ($P \leq .001$). At 4 weeks, large increases were identified in ES and PC grafts as compared with

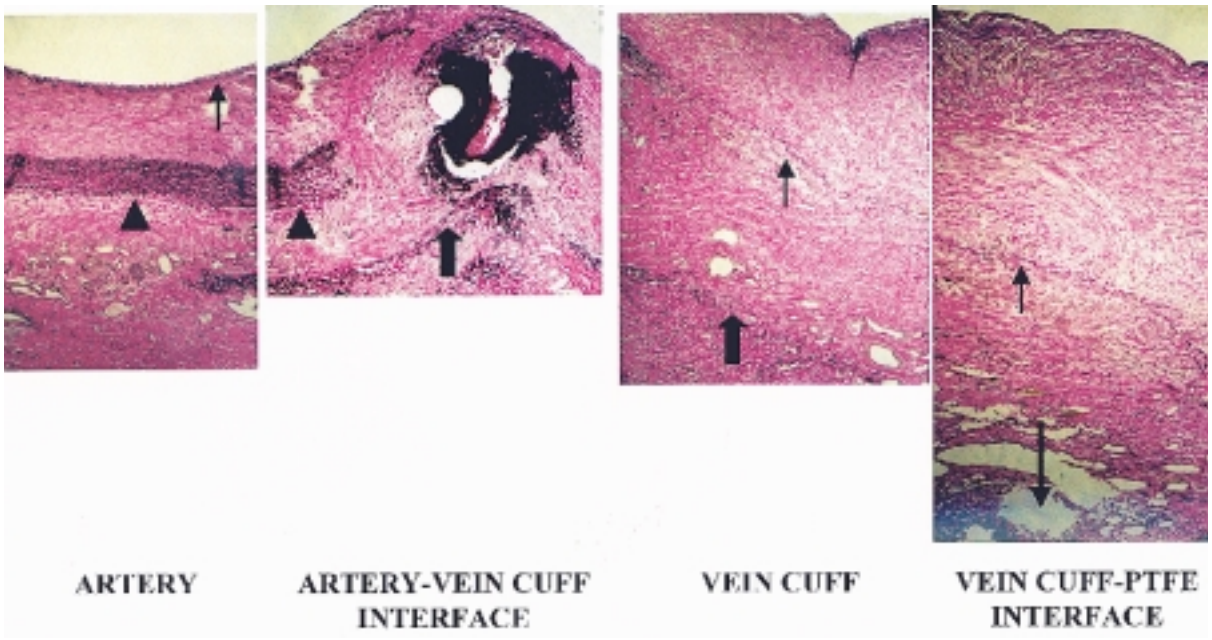

Fig 4. Verhoeff's elastic stain and van Gieson's counterstain results of vein cuff anastomosis showing shift of intimal hyperplasia response from artery-vein cuff interface to vein cuff–prosthesis interface. *Arrows* indicate internal elastic lamina of artery and vein cuff. *Triangles* indicate external elastic lamina of recipient artery. *Thick arrows* indicate external elastic lamina of vein cuff. *Long downward arrow* points to location of suture hole of underlying vein graft (magnification, 74×). *PTFE,* Polytetrafluoroethylene.

Table III. Morphologic data at mid-anastomosis at 1 week

	Control (n = 4)	*VC (n = 6)*	*ES (n = 5)*	*PC (n = 5)*	*P value*
Intima thickness (μm)	13 ± 0.6	28.8 ± 1.5	29.3 ± 0.75	55 ± 1.9	*,†,‡
Media thickness (μm)	268 ± 8	302 ± 6.5	283 ± 4.2	373 ± 4.6	*,†,‡
Intima area (μm^2)	1248 ± 123	2743 ± 307	2555 ± 133	5160 ± 392	*($P \leq .05$),†
Media area (μm^2)	26838 ± 1660	30549 ± 1311	29340 ± 888	37232 ± 914	§,†

VC, Vein cuff; *ES,* end-to-side; *PC,* polytetrafluoroethylene.
Data are expressed as mean ± standard error of the mean. All values are significant at $P \leq .001$, unless otherwise stated.
*Control group versus all groups.
†PC group versus VC and ES groups.
‡VC group versus ES group.
§Control group versus PC group only.

VC grafts and controls ($P \leq .001$). Of note, a regression in the intima / media area ratio occurred in the VC group, such that the intima / media ratio for the VC group at 4 weeks was significantly less than the corresponding ratio at 1 week after the grafting procedure. Similar regressions were noted at the base and toe of the anastomosis.

The base or the mid-anastomotic region, with one notable exception, did not exhibit significant changes in intima / media area ratio at either 1 week or at 4 weeks after anastomosis (Fig 6). The only remarkable change at these time periods was a significant increase in intima / media area ratios in the PC grafts. These ratios were significantly elevated as compared with the VC, ES, and control ratios ($P \leq .001$). Also, the change in intima / media area ratio in PC grafts at 4 weeks was significantly greater than the change in PC intima / media area ratio at 1 week ($P \leq .001$). In general, the intima / media area ratio changes that were observed in PC grafts at the mid-anastomotic region were slightly smaller than those observed at the heel of the anastomosis.

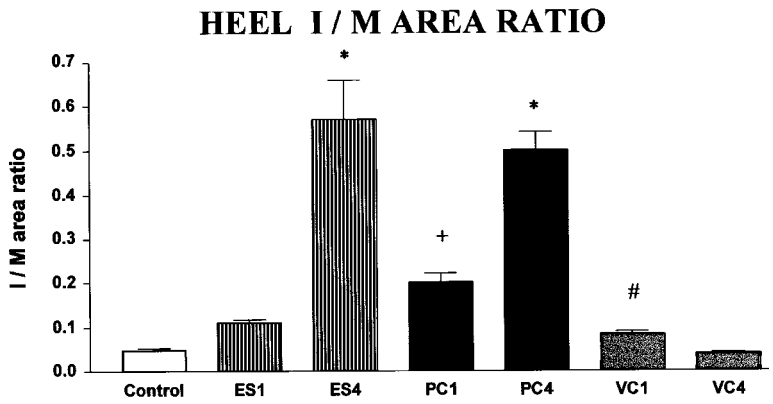

Fig 5. Bar graph of intima / media *(I/M)* area ratios from heel of each study group at 1 and 4 weeks. Within each group, there were significant differences between 1 and 4 weeks ($P \leq .001$). *ES,* End-to-side; *PC,* polytetrafluoroethylene cuff; *VC,* vein cuff.
*VC4 versus ES4 and PC4 ($P \leq .001$).
#VC4 versus VC1 ($P \leq .001$).
+VC1 versus PC1 ($P \leq .001$).

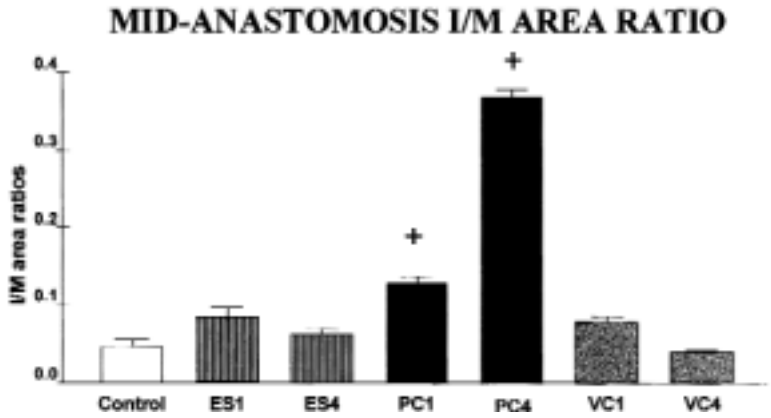

Fig 6. Bar graph of intima / media *(I/M)* area ratios from mid-anastomosis of each study group at 1 and 4 weeks. Within each group, there were significant differences between 1 and 4 weeks ($P \leq .001$). Vein cuff *(VC)* and end-to-side *(ES)* grafts did not differ at 1 and 4 weeks. *PC,* Polytetrafluoroethylene cuff.
+PC1 and PC4 were significantly elevated compared with VC and ES grafts ($P \leq .001$).

Table IV. Morphologic data at mid-anastomosis at 4 weeks

	Control (n = 4)	*VC (n = 6)*	*ES (n = 6)*	*PC (n = 9)*	*P value*
Intima thickness (μm)	13 ± 0.6	10 ± 0.3	29 ± 1.7	257 ± 13	*,†,‡
Media thickness (μm)	268 ± 8	234 ± 4.9	350 ± 8.82	493 ± 11	*,†,‡
Intima area (μm^2)	1248 ± 123	905 ± 64	2516 ± 323	24093 ± 2426	§
Media area (μm^2)	26838 ± 1660	23582 ± 989	34095 ± 1704	48557 ± 2044	§,‡

VC, Vein cuff; *ES,* end-to-side; *PC,* polytetrafluoroethylene.
Data are expressed as mean ± standard error of the mean. All values are significant at $P \leq .001$, unless otherwise stated.
*Control group versus all groups.
†PC group versus VC and ES groups.
‡VC group versus ES group.
§PC group versus all groups.

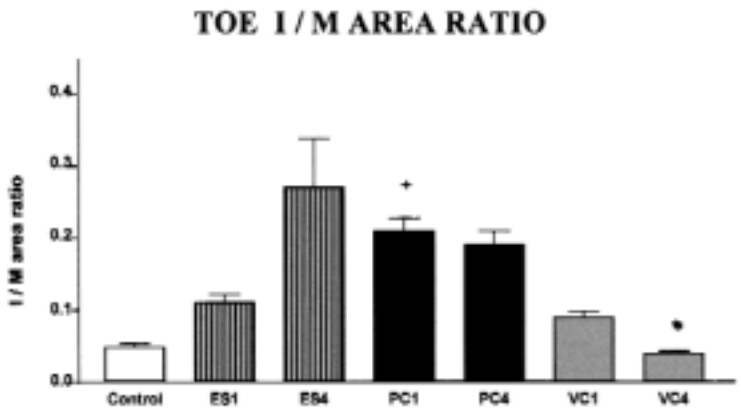

Fig 7. Bar graph of intima / media *(I/M)* area ratios from toe of each study group at 1 and 4 weeks. Within each group, there were significant differences between 1 and 4 weeks ($P \leq .001$). *ES,* End-to-side; *PC,* polytetrafluoroethylene cuff; *VC,* vein cuff.
*VC4 versus ES4 and PC4 ($P \leq .001$).
+PC1 was increased compared with ES1 and VC1 ($P \leq .001$).
No differences were noted between PC4 and ES4.

Table V. Morphologic data at the anastomotic toe at 1 week

	Control (n = 4)	*VC (n = 6)*	*ES (n = 5)*	*PC (n = 5)*	*P value*
Intima thickness (μm)	13 ± 0.6	25 ± 1.3	32 ± 2	64 ± 2.8	*,†,‡
Media thickness (μm)	268 ± 8	289 ± 5.7	239 ± 5.1	306 ± 5.2	*,†,‡
Intima area (μm^2)	1248 ± 123	2484 ± 244	3022 ± 414	6340 ± 552	§,†
Media area (μm^2)	26838 ± 1660	28272 ± 1124	23985 ± 1018	30310 ± 1042	‖

VC, Vein cuff; *ES,* end-to-side; *PC,* polytetrafluoroethylene.
Data are expressed as mean ± standard error of the mean. All values are significant at $P \leq .001$, unless otherwise stated.
*Control group versus all groups.
†PC group versus VC and ES groups.
‡VC group versus ES group.
§Control group versus PC group only.
‖ES group versus VC and PC groups.

Table VI. Morphologic data at the anastomotic toe at 4 weeks

	Control (n = 4)	*VC (n = 5)*	*ES (n = 6)*	*PC (n = 9)*	*P value*
Intima thickness (μm)	13 ± 0.6	9 ± 0.22	116 ± 14.4	58 ± 3.7	*,†,‡
Media thickness (μm)	268 ± 8	321 ± 4.9	325 ± 7.26	317 ± 5.8	*,†,‡
Intima area (μm^2)	1248 ± 123	1078 ± 48	11711 ± 2974	5860 ± 728	§($P \leq .05$), ‖($P \leq .01$)
Media area (μm^2)	26838 ± 1660	32003 ± 976	32652 ± 1487	31218 ± 1145	NS

VC, Vein cuff; *ES,* end-to-side; *PC,* polytetrafluoroethylene.
Data are expressed as mean ± standard error of the mean. All values are significant at $P \leq .001$, unless otherwise stated.
*Control group versus all groups.
†PC group versus VC and ES groups.
‡VC group versus ES group.
§Control group versus PC group only.
‖ES group versus VC and PC groups.

Fig 7 displays the analysis of the intima / media area ratios in the toe region of the anastomosis. Significant increases in intima / media area ratio occurred within a given group between weeks 1 and 4 ($P \leq .05$), except for the PC group. At 1 week, a significant increase in the intima / media area ratio for the PC grafts was identified ($P \leq .001$), and no significant differences were present in VC and ES grafts relative to control

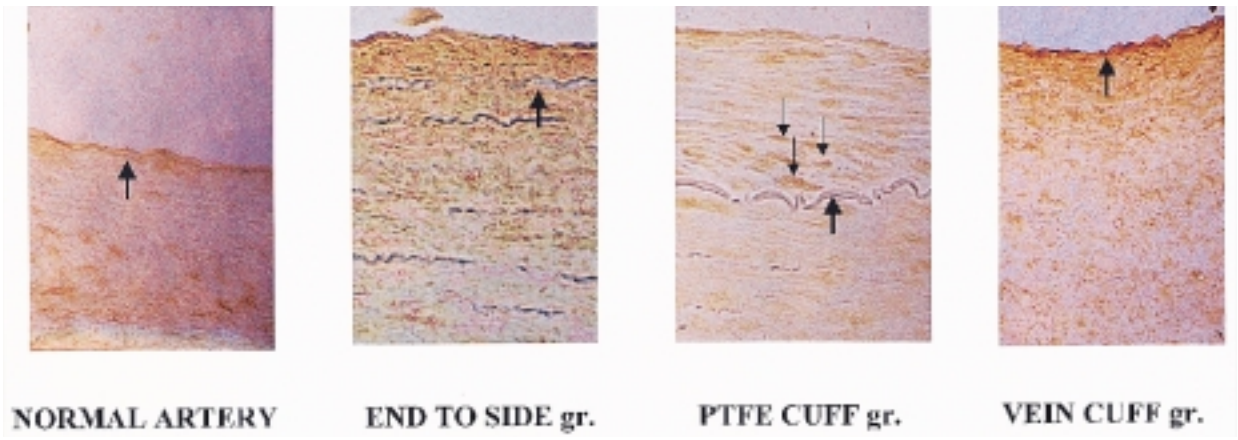

Fig 8. Immunohistochemistry results for transforming growth factor–β_1 showing staining of elliptical cells in intima of polytetrafluoroethylene *(PTFE)* cuff group *(inverted arrows)*. Diffuse staining is observed in intima of other study groups. *Thick arrows* indicate location of internal elastic lamina (magnification, 400×). *gr,* Graft.

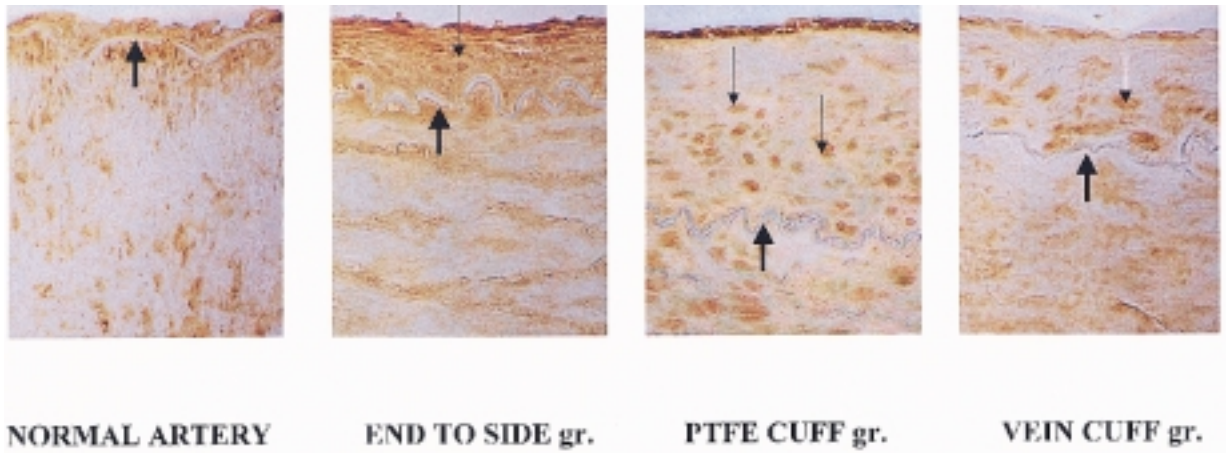

Fig 9. Immunohistochemistry results for platelet-derived growth factor–BB showing positive staining of elliptical cells in intima *(inverted arrows)*. *Thick arrows* indicate location of internal elastic lamina (magnification, 400×).

and to each other. By 4 weeks, the increment in intima / media area ratio of the ES group became similar to that achieved by the PC group. Both the ES and PC groups displayed an intima / media area ratio greater than the VC group at 4 weeks ($P \leq .001$).

Immunocytochemistry. IH is one example of tissue remodeling in response to stimuli. TGF-β_1 and PDGF-BB have been identified as major factors in hyperplasia and tissue remodeling. To test whether or not these factors play a role in prosthetic graft–induced IH, we assessed their presence with immunohistochemistry.

Fig 8 displays the presence of TGF-β_1 immunoreactive products in the four study groups. Mild TGF-β_1 staining is shown in the intima of the control artery. The PC group shows positive staining of elliptical cells in the intima. Diffuse non-specific intimal staining is observed in the other study groups.

Fig 9 shows the distribution of PDGF-BB in the intima of the study groups. PDGF-BB staining was identified in elliptical cells of the intima in all the study groups. To determine the nature of these elliptical cells, we probed the tissue with antibodies to smooth muscle α-actin. Fig 10 shows positive α-actin reaction

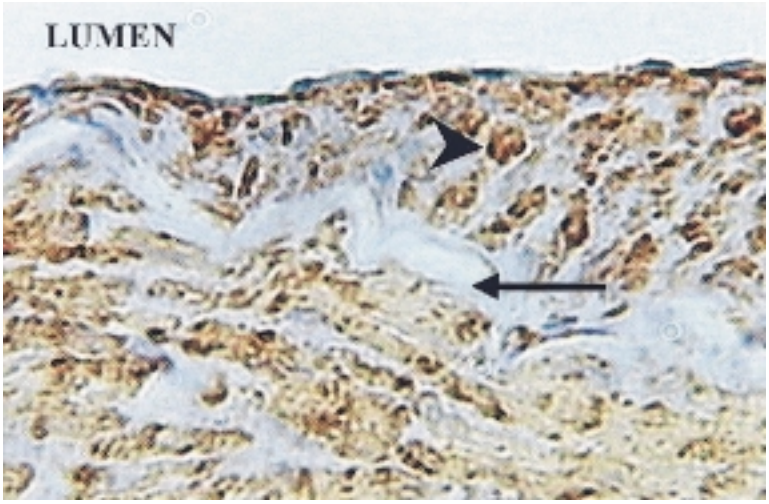

Fig 10. Immunohistochemistry results for smooth muscle α-actin showing staining of elliptical cells within intima and media *(arrowhead)*. Morphologically similar cells in intima similarly showed positive stain results for transforming growth factor–β_1 and platelet-derived growth factor–BB in adjacent serial sections. *Long arrow* points to internal elastic lamina (magnification, 1400×).

product results in the intimal elliptical cells. The presence of α-actin reaction products is consistent with the these elliptical cells being smooth muscle cells immersed in the intima of arteries undergoing IH.

DISCUSSION

Several investigators have proposed various hypotheses to explain the possible mechanisms associated with increased prosthetic graft patency and adjunctive vein cuffs at the distal anastomosis. The prominent theories are that vein cuffs influence IH development by promoting high shear stress flow patterns via the cuff's unique geometric configuration, reduction of compliance mismatches at the distal anastomosis, and inherent biologic properties of autogenous tissue.[14,27-29] The goal of the current investigation was to directly and indirectly determine the degree to which each of these variables is affected by the presence of a vein cuff. Flow patterns for VC and PC grafts were kept similar with the construction of distal anastomoses with identical angles and geometric configurations. The differences in distal anastomotic IH development between these two groups should therefore reflect the biologic properties of the material used to construct the cuff. Similarly, ES and PC grafts were in direct contact with the target outflow artery and differed only in anastomotic graft angle and geometric configuration. The differences in IH between these two groups should reflect the influence of graft angulation, and VC grafts should control for geometic and biologic effects.

Our study results show a marked reduction in the development of IH at the distal anastomosis of prosthetic bypass grafts that use VCs as compared with grafts with PTFE cuff interposition and standard ES anastomosis. Because VC and PC grafts exhibited comparable anastomotic geometric configurations and angulation, the superiority of VC must be caused by the biologic properties of autogenous tissue. Our results are consistent with the concept that the vein wall absorbs the multifactorial impact of a prosthetic graft and effectively transfers responses of reactive IH from the host artery to the arterializing vein.

One possible explanation for this observation is a reduction in compliance mismatch. Tyrrell et al[14] have demonstrated decreased compliance mismatches of vein cuffs and Taylor patches in an in vitro graft model. These authors state that "…for any cylinder under a given distending presssure, the stress applied to the circumference is double that applied to the length." With the longitudinal cutting of a piece of saphenous vein and the reorientation of the vein's axis, the cuff becomes anisotropic (more compliant) longitudinally rather than transversely.[14] However, the direct influence of mechanical properties of veins has been challenged by experiments that compared vein cuffs with vein cuffs whose mobility was limited by PTFE wrapping.[20] Norberto et al[20] reported no differences in IH development between these two groups and concluded that the mechanical properties of vein cuffs are not the primary variable modu-

lating IH development. The data from the current study do not support this position. The only difference between PC and VC grafts was in the material used to construct the cuff. This observation strongly suggests that compliance differences between the stiffer PTFE cuff and vein cuff may influence IH development at the distal anastomosis.

Shear stress alterations that result from anastomotic configurations and material properties influence the development of IH.[30-33] The graft angle at the distal anastomosis is one of the determinants of shear stress and flow pattern. The degree of angulation contributes to turbulent flow vortices and areas of low shear stress within the anastomosis.[28,32-34] Because of these important considerations, we constructed the VC and PC with the same configuration, so that the influence of geometry and flow patterns would be accounted for in the experimental design. The pronounced IH response of PCs as compared with VCs strongly implies that stable flow vortices are not as important as the presence of autogenous tissue.[28] Thus, our data show that the biologic properties of the material used to create the cuff are responsible for the improved outcome in our study.

The proliferation and migration of smooth muscle cells from the media to the intima and the accumulation of extracellular matrix in response to injury are the hallmarks of IH.[30] To assess the biologic properties that regulate these events in the presence of VCs, we investigated the distribution and sites of various cytokines known to modulate anastomotic IH development. TGF-β_1 and PDGF-BB are recognized as two cytokines that play a major role in tissue remodeling and IH. Our results confirmed the presence and apparent upregulation of TGF-β_1 and PDGF-BB in the intima and media of the host artery and in the vein cuff. These cytokines are in the same location as cells that stain positively to smooth muscle α-actin, which suggests that the suppression of smooth muscle cell cytokine production may limit the progression of IH in the VC. The VC may therefore be a potential site for gene therapy–directed IH inhibition.

A prominent observation from this investigation is a regression of IH in the VC group at 4 weeks as compared with the PC and ES groups. We postulate that the biologic properties of the vein cuff (eg, ability to respond functionally by cytokine synthesis, release) are responsible for the improved results. Because the vein cuff is the first biologic material to interact as an interphase with the graft, it spares the host artery the burden of strong stimuli for hyperplasia. The vein cuff dissipates the continuous injury response that stimulates IH development in the recipient artery and transfers the site of ongoing injury to the graft-cuff interface. We believe that it is this continuous injury response at the graft-cuff interface that ultimately causes these grafts to fail. The pronounced intimal reaction in the vein cuff, as shown in Fig 4, supports this hypothesis.

In conclusion, prosthetic bypass grafts modified with VCs decrease the IH response at target outflow arteries as compared with traditional ES anastomoses and grafts modified with PCs. Grafts with PCs showed exaggerated IH responses. Because PCs and vein cuffs had identical configurations and similar anastomotic angles, we presume that flow patterns in the cuff were similar. The induction of IH with cuffed configurations is therefore primarily dependent on the material used and less affected by angulation and configuration. The redistribution of hyperplasia into the vein cuff and away from the cuff artery interface suggests that intrinsic biologic properties of the vein cuff are primarily responsible for its protective effect. Techniques that diminish the development of IH in vein cuffs may therefore offer potential therapies for extending prosthetic graft patency further. Furthermore, our data suggest that cuffed prosthetic grafts may promote IH development and lead to early graft failure.

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Submitted Jun 8, 1999; accepted Aug 9, 1999.

DISCUSSION

Dr Anton N. Sidaway (Washington, DC). I congratulate Dr Kissin and his coauthors on a well-planned and executed study aiming to shed more light on the protective mechanism of vein interposition cuff placed at the outflow anastomosis of prosthetic bypass grafts. We have become accustomed to the excellent work that your group consistently produces, and this paper is no different.

I submit the following three comments and questions to the authors.

First, the authors have shown, as we did, that the perpendicular angle of the vein cuff is not the factor that plays the protective role. Probably the presence of venous autogenous tissue interposed between the prosthetic graft and the artery provides an autogenous buffer zone, moving the location of intimal hyperplasia to the graft-vein cuff anastomoses. Because the diameter of this anastomosis is significantly larger than a graft-artery anastomosis, the intimal hyperplastic lesion plays less a role in the patency of the cuff anastomosis. Did you analyze your data to show that significant intimal hyperplastic lesions develop at the graft-vein cuff anastomosis? In a way, did you compare the thickness of the lesion encountered in this location with that lesion that takes place at the arterial outflow?

Second, although the authors commented on the better

compliance match when vein interposition cuff is added to the outflow anastomosis, they provided no data that measured compliance. In our previous study, we found that vein cuff distensibility (and we tried to avoid using the term compliance) plays no role in alleviating intimal hyperplasia in a comparable model of graft placement in the canine carotid artery. Do the authors have any data that implicate better compliance match for the protective effect of the vein cuff?

Lastly, growth factors are known to be produced during the process of intimal hyperplasia. The intimal hyperplasia produced with this model is no different. In addition, the fact that growth factors are produced in a lesion does not show cause and effect. The interaction between the growth factor and its receptor results in its mitogenic effect. Did you perform any receptor binding studies to detect receptor up regulation if any?

I would like to thank the Society for the privilege of discussing this important paper. Thank you.

Dr Peter J. Pappas. Thank you, Dr Sidawy, for those insightful comments.

Your first question was in regards to measurements in the vein cuff itself. That was an insightful observation on your part, because within our own group we discussed whether or not this was intimal hyperplasia versus medial hyperplasia or just overall hypertrophy. We are now going back, and we are relooking at some of those vein wall specimens to see whether we can more clearly identify the internal elastic lamina, because it is our suspicion that what we are really looking at is arterial wall remodeling of the vein cuff and not actually a hyperplastic response in the intima.

Your second question regarding compliance is also insightful. We too had difficulties in trying to construct methods to measure compliance. So, the best thing that we could think of was to have a polytetrafluoroethylene cuffed and a vein cuffed group, and we keept the configuration identical so that the flow patterns would be similar and so that the only difference would be that one anastomosis would be made of autogenous tissue and that the other would be made with prosthetic tissue. Although I will tell you that at 1 and 4 weeks, when we harvested these specimens, there was an intense reaction around the grafts. The fibrosis was so great that I doubt the vein cuffs were expanding with each pulsation, suggesting that any differences in compliance eventually dissipate with time.

Your third question was the role of receptors and growth factors in the observed hyperplastic responses. The reason we looked at transforming growth factor–β_1 and platelet-derived growth factor–BB is because we hoped that if the vein cuff did show efficacy that in the next series of experiments we might be able to use gene therapy approaches to try to modify the response in the vein cuff. The fact that we did observe these growth factors and the fact that it was reported in another series that these growth factors are involved in the hyperplastic response led us to confirm that perhaps there is an association. But I agree with you that the next series of experiments needs to look at the receptor function and also a cause and effect relationship.

Dr Michael S. Conte (Boston, Mass). I want to congratulate the authors both for an excellent presentation and, even more so, for taking a difficult clinical and extremely complex biologic problem to the animal laboratory.

I have just two questions. One is whether you looked, in addition to thickness, on what happens to lumen? That is, as time goes on, do you develop luminal narrowing in this model? Is it actually a model of prosthetic graft stenosis? It would be very interesting if that were true.

Second, can you hypothesize a little bit more on the mechanism? It appears that you have shown that the geometry is not the issue. Is there perhaps a direct toxic effect of the adjoining prosthetic graft, or what do you speculate is happening at the interface between the prosthetic graft and the autogenous tissue? Thank you.

Dr Pappas. Again, those were insightful questions. We tried to look at luminal narrowing when we were originally designing the study. The problem is that it is much easier to look at luminal narrowing when you are dealing with an angioplasty model in which the entire artery is circumferential. When you are analyzing an anastomosis and you are dealing with a hemisection of the wall of the artery, luminal narrowing becomes difficult to assess. So, even though we thought about it, we could not think of an accurate way in which to assess that, other than looking at the outflow artery to see whether there were any vein wall remodeling changes.

In regards to the mechanism, one of the things that we were curious about was that when you look at these prosthetic grafts in patients and when you perform your duplex scans on them, you see what is referred to in the literature as this stable vortex, which to me is just another name for turbulence. I have always assumed that turbulence will increase hyperplasia because of low shear stress zones. And I did not understand how the cuff, with this turbulent pattern, would decrease hyperplasia. I still do not think that it is flow related, and I still do not think that it is geometry related. My suspicion is that it has something to do with an inherent biologic property of the vein cuff itself. And one of the suspicions I have is that when you look at some of these sections, you see that the endothelial cells and the smooth muscle cells reorient themselves. So, there are obviously some biologic properties going on, and I am trying to dissect them out right now. But I think it has something to do with cytokine alterations and growth factors in the vein wall that affect remodeling of the cuff and outflow artery.

Dr Joseph L. Mills (Tucson, Ariz). I enjoyed that presentation very much. As you know, we have been interested in this same topic. We have studied the Taylor vein patch in a canine model and showed that intimal hyperplasia was reduced significantly with the vein patch. And so your paper supports our bias that it is not just geometry, it is a biologic effect.

What I would like to ask you is, because you used immunocytochemistry to look at smooth muscle cells with alpha smooth muscle cell actin, have you looked at proliferation rates in those smooth muscle cells? For example,

you could do proliferating cell nuclear antigen. And is smooth muscle cell proliferation reduced with the use of the vein cuff?

Thank you.

Dr Pappas. Originally when we designed the study, we were interested in that exact question, and we did give the animals 5′-bromo-3′-deoxyuridine before they were killed. However, we were unable to really get the 5′-bromo-3′-deoxyuridine staining to work. Proliferating cell nuclear antigen is another method in which you can use immunocytochemistry to look at the nuclei within the cells. And no, we have not done that as yet. But we have specimens that we have sectioned and not stained yet. We certainly could go back and reanalyze those specimens for proliferating cell nuclear antigen using the imaging analysis software.

Dr Edmund J. Harris, Jr (Stanford, Calif). This was a very nice study, but I ask whether you have duplicated one modification of this experiment, originally reported by Sidaway and his colleagues (Norberto JJ, Sidaway AN, Trad KS, Sidawy MK, DePalma RG. The protective effect of vein cuffed anastomoses is not mechanical in origin. J Vasc Surg 1995;21:558-66). This group wrapped a vein cuff with polytetrafluoroethylene to get at the question of compliance mismatch. Have you wrapped these vein cuffs with polytetrafluoroethylene in your model?

Dr Pappas. Although that is a good suggestion, I was more concerned about trying to bring things back to the clinical arena. And I am sure that you could wrap a piece of polytetrafluoroethylene around a vein cuff after you have done it in patients as well, but then that begs the question of why not just construct a prosthetic graft that has a hood anastomosis to it? I realize what you are saying about the added beneficial effects of the vein cuff, but no, we had not considered that as another limb.

Very briefly, to follow up on Dr Harris' question, we did use the wrap you suggested and we reported our results in the *Journal of Vascular Surgery* (Norberto JJ, Sidaway AN, Trad KS, Sidawy MK, DePalma RG. The protective effect of vein cuffed anastomoses is not mechanical in origin. J Vasc Surg 1995;21:558-66). In the canine carotid artery model, we wrapped the vein cuff on one side with a polytetrafluoroethylene jacket and we kept the contralateral side vein cuff unwrapped. There was no significant difference in the thickness of intimal hyperplasia between the two sides. So, we believed that it is not really distensibility or "compliance" that is protective, but that the presence of an autogenous tissue zone between the graft and the artery moved the location of the intimal hyperplasia from the graft-artery interface to the graft-venous cuff interface.