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Biologic degeneration of vein grafts after thrombotic occlusion: Thrombectomy within 3 days results in better indices of viability

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Objectives: **To clarify the mechanism for poor patency of vein grafts after thrombectomy and the time limit for successful salvage operation, we investigated the time course of biologic degenerative changes in thrombosed vein grafts.**

Materials and Methods: **The right femoral artery was replaced with a femoral vein graft in 25 mongrel dogs. After 3 months, grafts were explanted in 5 dogs (control grafts), and the remaining 20 dogs underwent femoral artery ligation to create a thrombosed graft. Of the 20 grafts, 5 were explanted at 3 days after ligation (group I-3) and 5 were explanted at 5 days after ligation (group I-5). Of the remaining 10 grafts, 5 underwent thrombectomy at 3 days after ligation (group II-3) and 5 underwent thrombectomy at 5 days after ligation, and were reimplanted into the left femoral artery, then explanted 28 days after reimplantation. The grafts were assessed with immunohistochemistry and prostaglandin (PG) I_2 assay (6-keto-$PGI_{1\alpha}$).**

Results: **Of the 25 grafts, occlusion recurred in 3 in group II-5 within 28 days after reimplantation. There were significant differences between group I-5 and group I-3 or control grafts for percentage of areas positive for α-actin, total number of cells per field, and proliferating cell nuclear antigen (PCNA)–positive cells in layer of thickened intima and atrophied media (I/M), and for total cell and PCNA- positive cell numbers per field in the adventitia. Mean 6-keto$PGF_{1\alpha}$ was 40 ± 14.1 pg/mg/min in control dogs, 84 ± 18.9 pg/mg/min in group I-3, and 15.4 ± 7.7 pg/mg/min in group I-5, demonstrating a significant reduction in group I-5 (P = .009).**

Conclusion: **Graft wall cell viability and PGI_2 production in thrombosed vein grafts are well preserved for up to 3 days. Therefore graft salvage operations no later than 3 days after thrombotic occlusion may provide acceptable long-term patency of salvaged grafts. (J Vasc Surg 2003;38:305-12.)**

Vein grafts have been widely used for bypass grafting in small-caliber arteries, and their excellent patency is attributed to the antithrombogenic function of live cells in the graft wall. To preserve this cell function, various techniques for graft preparation have been introduced; however, vein graft patency rate for infrainguinal or coronary artery bypass grafts is only about 70% at 5 years.[1-3] The main cause of graft failure is graft stenosis due to progressive intimal hyperplasia, which frequently results in thrombotic occlusion despite intensive graft surveillance.[4] Patency of thrombosed vein grafts frequently can be restored with thrombectomy or direct thrombolysis, with or without revision procedures; however, the patency rate of the salvaged grafts is poor, as low as 19% to 31% at 2 years after salvage operation.[5-7] Nackman et al[8] found that older grafts achieved longer patency after direct graft thrombolysis, likely because of reflection of their qualitative adequacy as conduits. In vein grafts older than 1 year selected for this therapy, a 2-year primary patency rate of 49% was predicted. Nevertheless, this improved patency rate for the selected graft group is still insufficient to explain the poor results of grafts after thrombectomy, suggesting the presence of more relevant factors. Graft thrombosis may induce inflammatory reactions and subsequent degeneration of viable components in the graft wall, and the level of degenerative changes may affect graft patency after salvage operation.

To clarify the mechanism for poor patency and the time limit for successful salvage operation in thrombosed grafts, we investigated the time course of biologic degenerative changes in thrombosed vein grafts and their influence on graft patency after salvage operation.

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Competition of interest: none.

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MATERIALS AND METHODS

Twenty-five randomly selected mongrel dogs of both sexes, weighing 12 to 15 kg, were used in the study. Their care and use complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and with the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council.

All 25 animals were anesthetized with intravenous thiamylal sodium. Endotracheal tubes were inserted, and the lungs were ventilated with 33% oxygen. With sterile surgical technique, the right femoral artery and vein were dissected. After intravenous administration of heparin (100

Table I. Quantitative cell analysis in vein grafts after thrombotic occlusion

	Femoral veins (n = 5)	*Control (n = 5)*	*Group I-3 (n = 5)*	*Group I-5 (n = 5)*	*Group II-3 (n = 5)*	*Group II-5 (n = 2)*	P*
Intima/media							
Thickness (μm)	13.6 ± 4.4	51.9 ± 11.6	51.3 ± 10.4	59.8 ± 22.5	233.8 ± 51.8	382.5 ± 46.0	.602
%-α-Actin (%)		54.2 ± 20.0	40.6 ± 21.9	6.6 ± 2.9	47.5 ± 21.8	3.8 ± 0.7	.009
Total cells	45.8 ± 5.3	32.8 ± 9.3	39.9 ± 3.1	11.4 ± 7.9	29.2 ± 8.6	26.5 ± 10.6	.009
Proliferating cells		17.9 ± 10.0	29.2 ± 11.5	5.8 ± 3.9	14.2 ± 6.9	10.5 ± 2.1	.016
Apoptotic cells		9.0 ± 5.3	9.6 ± 3.2	8.3 ± 6.2	7.8 ± 4.4	17.5 ± 0.7	.602
Macrophages		0	2.7 ± 2.4	0.4 ± 0.3	0	0	.028
Adventitia							
Total cells	20.2 ± 6.5	20.3 ± 3.6	23.8 ± 9.7	10.7 ± 7.4	27.0 ± 10.7	17.5 ± 9.2	.021
Proliferating cells		11.9 ± 4.8	12.6 ± 5.6	4.0 ± 2.5	10.2 ± 4.6	9.0 ± 5.7	.028
Apoptotic cells		8.1 ± 2.8	7.6 ± 2.0	8.4 ± 6.2	7.2 ± 1.8	18.0 ± 1.4	.754
Macrophages		0	2.7 ± 1.8	0.4 ± 0.2	0	0	.076
Surrounding tissue							
Macrophages		0	2.5 ± 1.0	2.1 ± 1.1	0.8 ± 1.8	5.5 ± 0.7	.465

No. of cells expressed as cell number per field of 50×200 μm^2.
*Group I-3 vs group I-5.

IU/kg), a 5 cm length of femoral vein was harvested for use as a graft. Both ends of the graft were removed for histologic analysis, and the femoral artery was replaced with the vein graft in reverse fashion, using continuous 7-0 polypropylene sutures. After 3 months, 5 of the 25 grafts were explanted for use as control grafts; the remaining 20 dogs underwent femoral artery ligation distal to the graft to thrombose the grafts. Of the 20 thrombosed grafts, 5 were explanted at 3 days after ligation (group I-3) and 5 were explanted at 5 days after ligation (group I-5), and were subjected to histologic analysis, immunohistochemistry, and prostaglandin I_2 (PGI_2) assay. Of the 10 remaining grafts, at 3 days (group II-3) or 5 days (group II-5) after thrombotic occlusion, thrombectomy was performed three times in each graft via arteriotomy distal to the graft with a 3F balloon catheter (Fogarty arterial embolectomy catheter; Baxter Healthcare, Deerfield, Ill). The thrombectomized grafts were immediately harvested for sampling for scanning electron microscopy, and autogeneously reimplanted into the contralateral femoral artery with end-to-end anastomosis. These grafts were explanted 28 days after reimplantation and subjected to histologic analysis and scanning electron microscopy.

Neither antiplatelet agents nor anticoagulants were given during the observation period. At graft retrieval, the animals were again anesthetized, and graft patency was assessed with palpation of the distal femoral arteries. After heparinization, a euthanasia solution was administered intravenously. The grafts, including both anastomoses, were carefully harvested, and two 1 cm long segments were cut from the graft. One segment was fixed with 10% buffered formalin; the other segment was longitudinally opened, rinsed with heparinized saline solution for removal of thrombi, and, after excision of excessive surrouding tissue, prepared for PGI_2 assay.

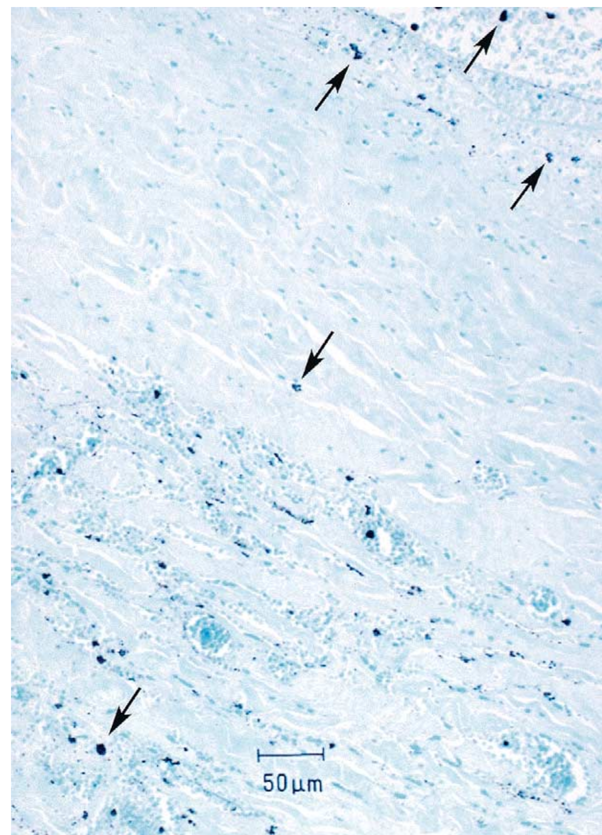

Fig 1. Immunohistochemistry for macrophages in thrombosed grafts. Note moderate infiltration of macrophages *(arrows)* both inside and outside of 3-day grafts. (Mouse anti-human CD68 staining method; original magnification, ×30.)

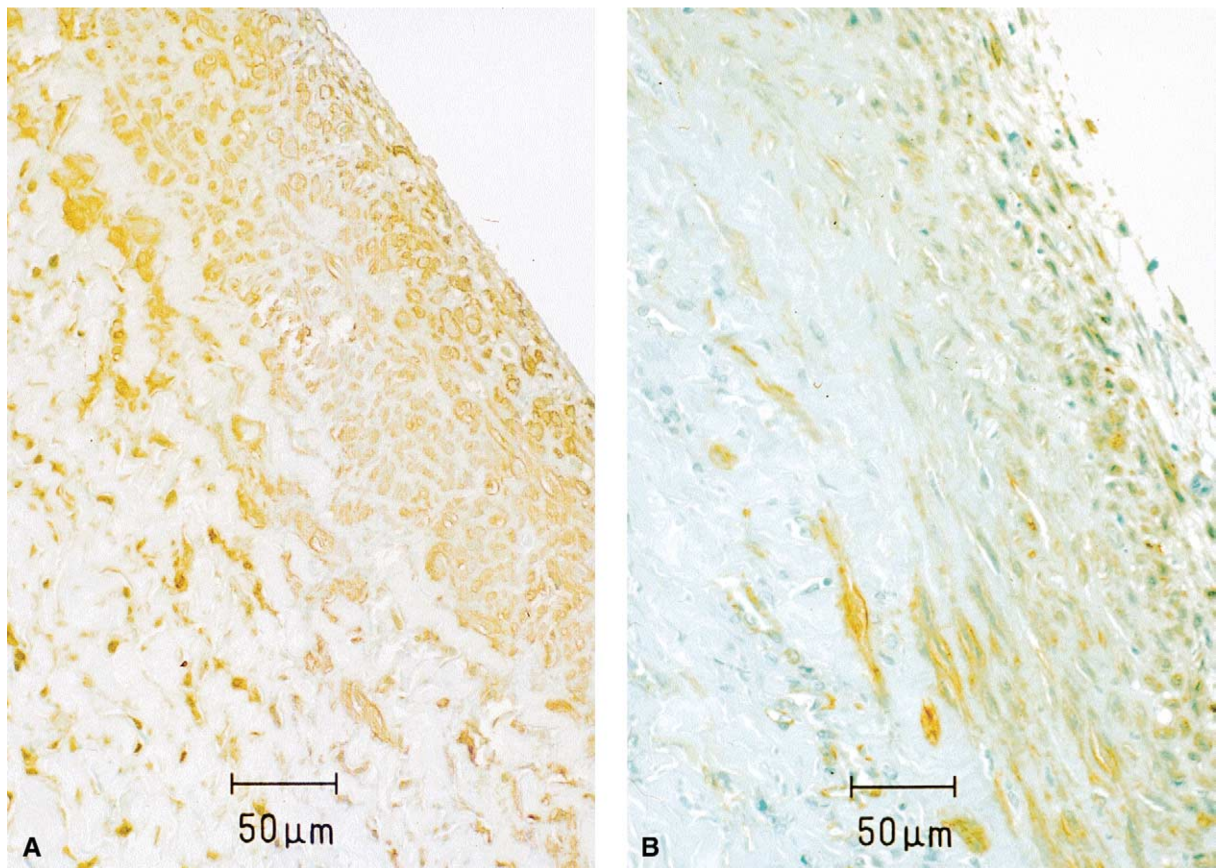

Fig 2. Immunohistochemistry results for α-actin positive cells in thrombosed grafts. **A,** Three-day graft shows no significant decrease in positive cell area when compared with control graft. **B,** Note obvious decrease in positive cell area in 5-day graft.

Histologic analysis, immunohistochemistry, and scanning electron microscopy. Specimens for histologic anaysis and immunohistochemistry were cross-sectioned and embedded in paraffin. Hematoxylin-eosin, Verhoeff–van Gieson, and Masson trichrome staining methods were used to identify the three vessel layers; vascular smooth muscle cells, proliferating cells, apoptotic cells, and macrophages were identified at immunohistochemistry with monoclonal mouse anti-human smooth muscle actin antibody 1A4 (α-SM actin, 1:100; DAKO, Carpinteria, Calif),[9] monoclonal mouse anti-proliferating cell nuclear antigen (PCNA)(PC-10, 1:200; DAKO),[10] single-stranded DNA (ssDNA) staining with goat anti-rabbit immunogloblin (DAKO ENVISION+, 1:200; DAKO),[11] and mouse anti-human CD68 (KP1, prediluted; DAKO),[12] respectively. Canine inguinal lymph nodes and femoral arteries 24 hours after harvesting were used as negative and positive controls for ssDNA, PCNA, and CD68, or α-SM actin. In ssDNA staining, hematoxylin was used as counterstain to detect ssDNA-negative cells.

The specimens for scanning electron microscopy were fixed with 1% glutaraldehyde, dehydrated in a graded *t*-butyl alcohol series, freeze-dried, and coated with platinum before examination.

Histologic and morphometric quantitative analysis. Numbers of cell nuclei were microscopically counted in a complex layer of thickened intima and atrophied media (I/M) and in the adventitia. Degeneration of graft wall cells was assessed with PCNA-positive cell numbers, apoptotic cell numbers, and ratio of α-SM actin–positive area to total area (%–α-actin positive area), which was measured with planimetry with a computerized morphometric analysis system (Apple, Cupertino, Calif), with the public domain program Image (Research Service Branch, National Institutes of Health, Bethesda, Md). All measurements were performed in one visual field with 50×200 μm within the I/M along the luminal surface or within the adventitia along the external elastic lamina. These quantitative data were represented by an average of three randomly selected microscopic visual fields in each section.

Radioimmunoassay of PGI_2 production. PGI_2 production of vein graft specimens was measured with radioimmunoassay as its stable metabolite, 6-keto-prosta-

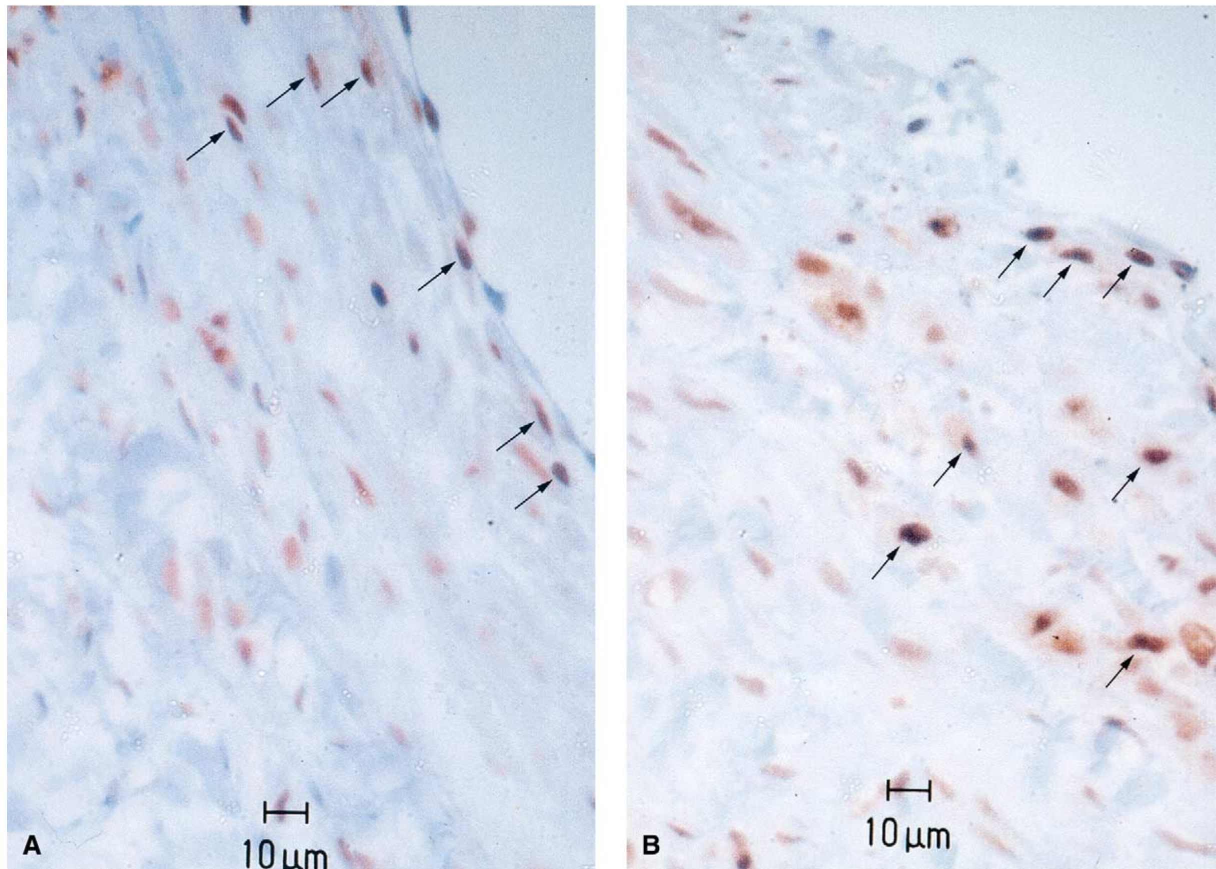

Fig 3. Immunohistochemistry results for proliferating cell nuclear antigen (PCNA)–positive cells *(arrows)* in thrombosed grafts. **A,** Control graft. **B,** Note significant increase in PCNA-positive cells in 3-day graft. **C,** There is obvious decrease in PCNA cells in 5-day graft. (PCNA staining method; original magnification, ×60.) *Continued.*

glandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), (^{125}I-6-keto-$PGF_{1\alpha}$ kit; New England Nuclear Research Products, Boston, Mass) in an unstimulated condition. Each specimen was placed in an incubation well containing 1.0 mL of Tris sodium chloride buffer (pH 7.4) and incubated in a shaker for 5 minutes at 37°C. After incubation, each specimen was quickly removed from the incubation well, and indomethacin (200 µg/mL) was added. The remaining fluid was pipetted into a polypropylene tube and frozen at −80°C for later assay; and the specimen removed was placed in a heat chamber for 24 hours at 100°C, and the dry weight was obtained. Values for 6-keto-$PGF_{1\alpha}$ were expressed as picograms of immunoreactive 6-keto-$PGF_{1\alpha}$ per milligram of dried vein tissue per minute.

Statistical analysis

Data were analyzed with StatView software (Avacus Concepts, Berkeley, Calif) and are presented as mean ± 1 SD. The Kruskal-Wallis test (nonparametric analysis of variance) and subsequent multiple comparisons with the Mann-Whitney *U* test or X^2 test were used to determine differences of quantitative data between groups. $P < .05$ was considered statistically significant.

RESULTS

Graft patency Patency of the 25 grafts was assessed with palpation at the groin. All 5 control grafts, all 10 grafts in group I before ligation, and all 5 grafts in group II-3 were patent, whereas 3 of 5 grafts in group II-5 had occluded again within 28 days after reimplantation.

Histologic analysis, immunohistochemistry, and morphometric analysis

At histologic analysis, before implantation the femoral vein grafts had an endothelial lining, a thin medial layer with average thickness of 13.6 ± 4.4 µm, and a relatively thick adventitia with a thickness of 50 to 150 µm. Average number of cells in the media and adventitia was 45.8 ± 5.3 and 20.2 ± 6.5 per field, respectively.

Control grafts. Graft reendothelialization was complete within 3 months. Thickened intima associated with atrophied media, with average thickness of 51.9 ± 11.6 µm

(range, 33-64 μm), was a common sequela. The %–α-actin positive area in the I/M and the number per field of total cells, PCNA-positive cells, and apoptotic cells in the I/M and the adventitia are given in Table I. Neither macrophages nor other types of white blood cells were recognized at CD68 and hematoxylin-eosin staining.

Group I. There were no obvious differences in graft connective tissue when compared with control grafts. At microscopy, inflammatory cells, more than 80% of which were macrophages, moderately infiltrated around the graft area and luminal thrombus in the 5-day and 3-day grafts (Fig 1). Significant decreases in α-actin positive area and in number of total cells and PCNA- positive cells were recognized in the 5-day grafts compared with the 3-day grafts or control grafts (Figs 2 and 3). Significant numbers of apoptotic cells were constantly recognized in all grafts, including control grafts (Fig 4). The %–α-actin positive area of the I/M and the number per field of total cells, PCNA-positive cells, apoptotic cells, and macrophages for both the I/M and adventitia in groups I-3 and I-5 are given in Table I. All values showed significant differences between group I-5 and group I-3 or control grafts.

Group II. The %–α-actin positive area of the I/M and the number per field of total cells, PCNA-positive cells, apoptotic cells, and macrophages for both the I/M and adventitia in groups II-3 and II-5 are given in Table I. At scanning electron microscopy the thrombectomized grafts before reimplantation showed a rough fibrous denuded luminal surface, whereas the surfaces of all of the 5 grafts in group II-3 were covered with a confluent endothelial cell lining (Fig 5). The 2 patent grafts in group II-5 also had endothelial cells; however, coverage was sparse and incomplete, and histologic analysis revealed severe intimal hyperplasia, compared with the grafts in group II-3.

Prostacyclin production

Mean values for 6-keto-$PGF_{1\alpha}$ in the control grafts and groups I-3 and I-5 are given Table II. These values reveal dominant PGI_2 hyperproduction in the 3-day grafts and significant reduction in the 5-day grafts (P = .009). The dominant PGI_2 production in the 3-day grafts was significantly higher, even when compared with that in control grafts (P = .016), testifying to satisfactory reimplantation results in grafts that underwent thrombectomy 3 days after occlusion.

DISCUSSION

Cultured human arterial smooth muscle cells synthesize PGI_2;[13] therefore the medial vascular smooth muscle cells may have an important role in maintaining patency of vein grafts by their significant production of PGI_2.[14,15] Degenerative changes in the graft wall after thrombotic occlusion should be evaluated in each vessel layer; however, the medial layer of the canine femoral

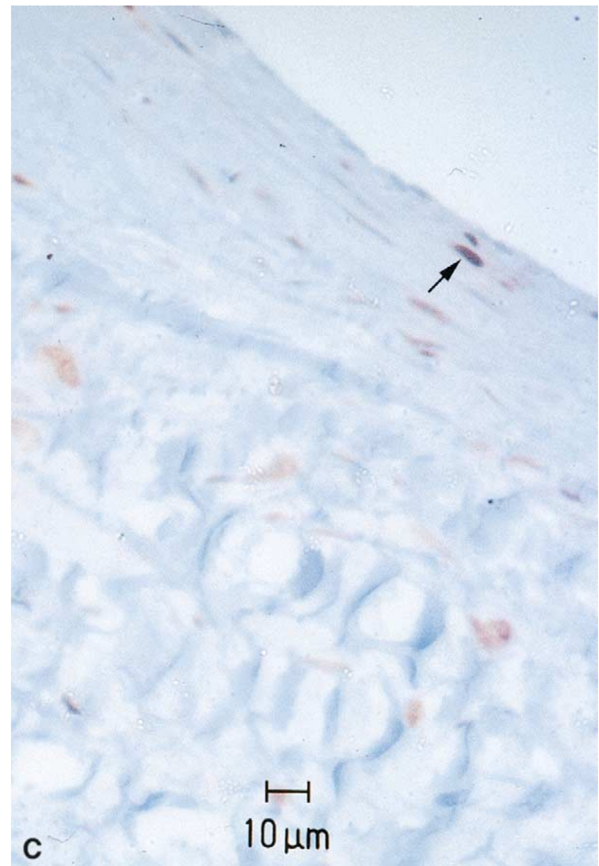

Fig 3. (*Continued.*)

vein is very thin compared with human saphenous vein, and various levels of intimal thickening were associated with marked medial atrophy in the grafts 3 months after implantation. Thus quantitative assessment was performed for the I/M complex layer and the adventitia.

After thrombotic occlusion, moderate numbers of macrophages surrounded the grafts, both from outside and from the luminal thrombus, and a few macrophages infiltrated the wall. Viability of the graft wall cells seemed to be well preserved in the 3-day grafts; however, a marked decrease in cell numbers occurred between day 3 and day 5 after thrombotic occlusion. In the thrombosed grafts, two types of cell death were observed: apoptosis and necrosis or karyolysis. To assess apoptosis we used the ssDNA method, which has great value for identification of apoptosis and is useful for differentiation of apoptosis and necrosis.[16] However, there are no specific measures for direct identification of necrosis; therefore we estimated the occurrence of necrosis by decrease in total cell number at hematoxylin-eosin staining. In the 5-day grafts, apoptotic cell numbers showed no changes compared with the 3-day or control grafts, whereas not

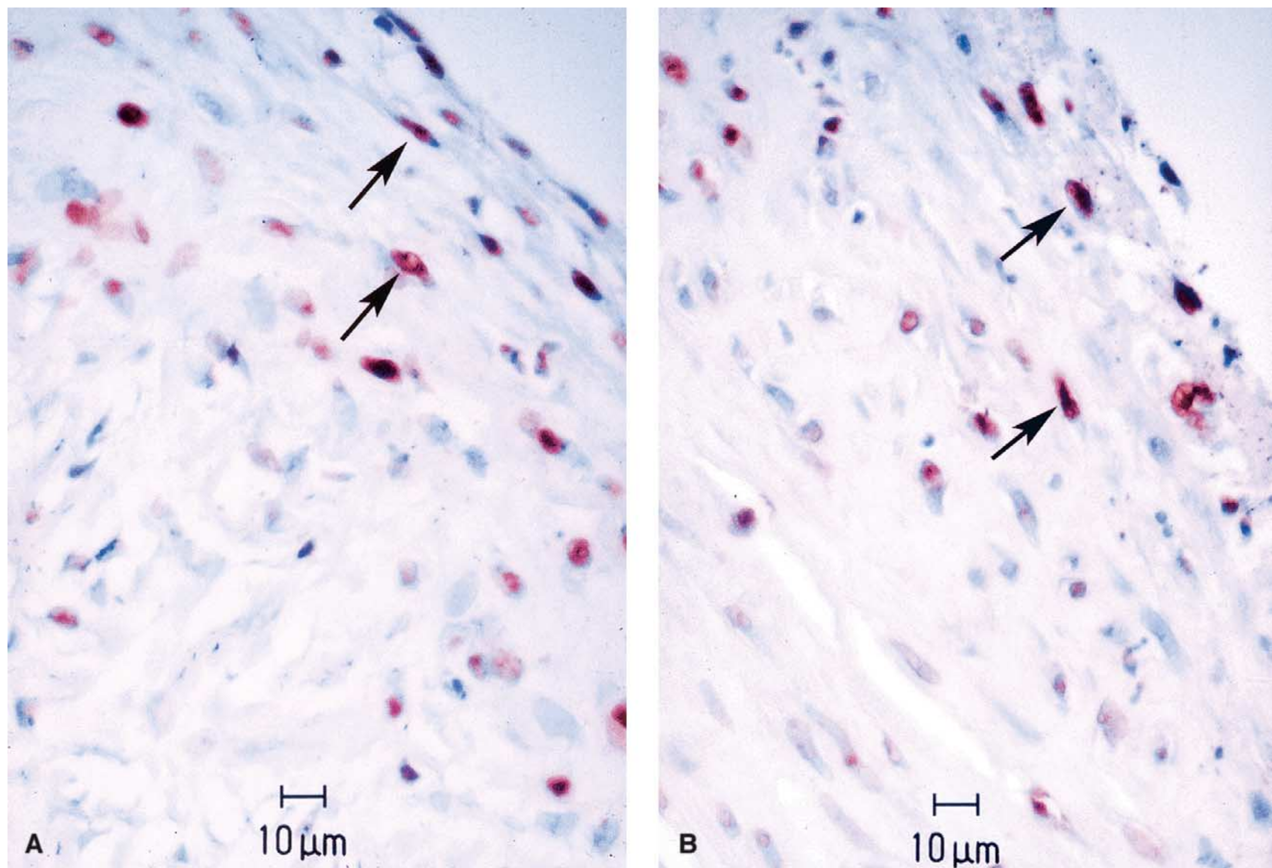

Fig 4. Immunohistochemistry results for apoptotic cells in 3-day grafts. There are no significant changes in apoptotic cell numbers *(arrows)* between control grafts **(A)**, 3-day grafts **(B)**, or 5-day grafts **(C)**. (ssDNA staining method; original magnification, ×60.) *Continued.*

only total cells but also PCNA-positive and α-actin positive cells markedly decreased in number compared with the 3-day grafts. These findings suggest that necrosis is the probable main mechanism of cell death in the thrombosed graft wall and that most of the killed cells were α-actin positive cells, because of their marked reduction in the 5-day grafts.

PGI_2 assay revealed that hyperproduction of PGI_2 occurred in the 3-day grafts. Thrombosed grafts sustained a moderate level of macrophage infiltration. Macrophages produce interleukin (IL)–1[17] and IL-6,[18] and IL-1 is a potent vasodilator, causing prolonged induction of PGI_2 and cyclic adenosine monophosphate synthesis in human vascular smooth muscle cells.[19] In the 3-day grafts, more than 70% of the graft wall cells still lived, and the graft wall was surrounded with significant numbers of macrophages. The IL-1 produced by macrophages may stimulate these live graft wall cells, resulting in hyperproduction of PGI_2. Macrophage infiltration still continued in the 5-day grafts; however, PGI_2 production was significantly reduced because of the marked decrease in viable cells. This appears to explain the presence of a substantial gap in biologic function between the 3-day and 5-day grafts.

At scanning electron microscopy no endothelial cells were found in the luminal surfaces of the grafts immediately after thrombectomy; however, after reimplantation all of the 3-day grafts and 2 patent 5-day grafts were covered with endothelial cells, though coverage was incomplete in the 5-day grafts. At histologic analysis the level of intimal thickening in these 2 patent 5-day grafts was higher than that in the 3-day grafts, probably because of the incomplete endothelial coverage. The complete endothelial cell lining in the 3-day grafts suggests that vein grafts thrombectomized within 3 days after thrombotic occlusion may achieve a long-term patency rate comparable to that of primary vein grafts.

CONCLUSION

PGI_2 production of vein grafts is still well preserved 3 days after thrombotic occlusion; however, marked cell degeneration occurs thereafter, and production declines as a result of cell death. Therefore, to obtain acceptable long-

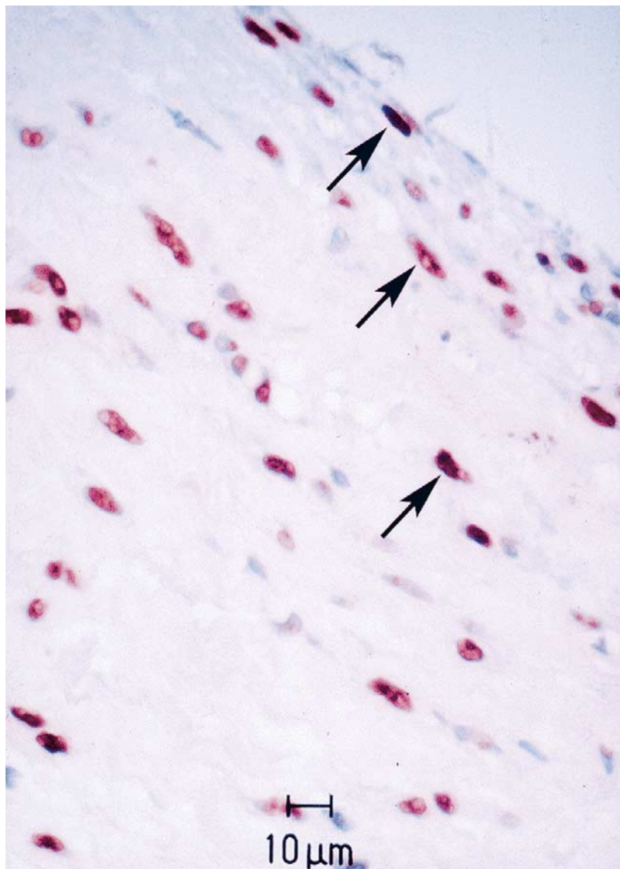

Fig 4. (*Continued.*)

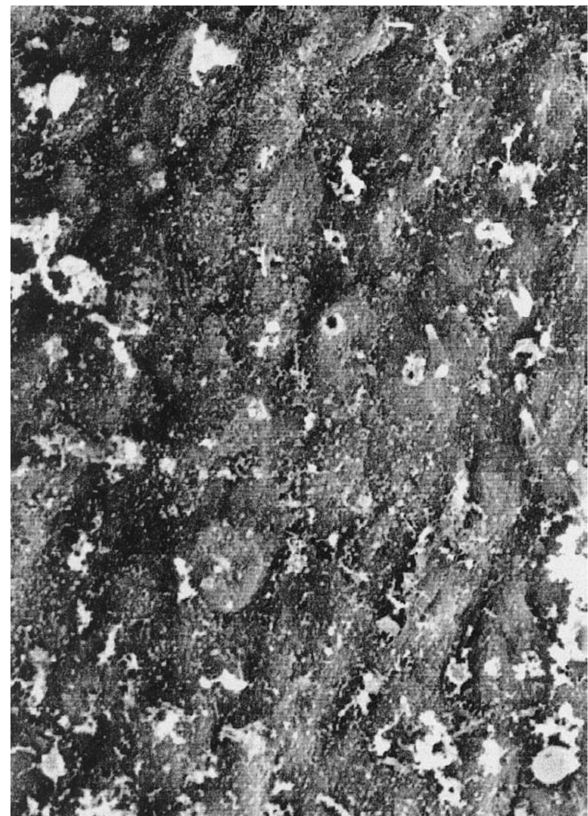

Fig 5. Scanning electron micrograph of luminal surface of salvaged graft 28 days after thrombectomy reveals a tight endothelial cell lining. (Original magnification, ×1000.)

Table II. 6-keto-$PGF_{1\alpha}$ production in vein grafts after thrombotic occlusion

	Control (n = 5)	*Group I-3 (n = 5)*	*Group I-5 (n = 5)*	P		
				Control vs. group I-3	*Control vs. group I-5*	*Group I-3 vs. group I-5*
6-keto-$PGF_{1\alpha}$ (pg/mg dry weight/min)	40.0 ± 14.1	84.1 ± 18.9	15.4 ± 7.7	.016	.009	.009

term patency of salvaged grafts, graft salvage operation should be performed within 3 days after occlusion.

REFERENCES

1. Green RM, McNamara J, Ouriel K, DeWeese JA. Comparison of infrainguinal graft surveillance techniques. J Vasc Surg 1990;11:207-15.
2. Lytle BW, Loop FD, Cosgrove DN, Ratliff NB, Easley K, Taylor PC. Long-term (5 to 12 years) serial studies of internal mammary artery and saphenous vein coronary bypass grafts. J Thorac Cardiovasc Surg 1985; 89:248-58.
3. Moran SV, Baeza R, Guranda E, Zalaquett R, Irarrazaval MJ, Marchant E, et al. Predictors of radial artery patency for coronary bypass operation. Ann Thorac Surg 2001;72:1552-6.
4. Olojugba DH, McCarthy MJ, Reid A, Carty K, Naylor AR, Bell PR, et al. Infrainguinal revascularisation in the era of vein-graft surveillance: do clinical factors influence long-term outcome? Eur J Vasc Endovasc Surg 1999;17:121-8.
5. Whittemore AD, Clowes AW, Couch NP, Mannick JA. Secondary femoro-popliteal reconstruction. Ann Surg 1981;193:35-42.
6. Brewster DC, Lasalle AJ, Robinson JG, Strayhorn EC, Darling RC. Factors affecting patency of femoropopliteal bypass grafts. Surg Gynecol Obstet 1983;157:432-42.
7. Cohen JR, Mannick JA, Couch NP, Whittemore AD. Recognition and management of impending vein-graft failure. Arch Surg 1986;121: 758-9.
8. Nackman GB, Walsh DB, Fillinger MF, Zwolak RM, Bech FR, Bettmann MA, et al. Thrombolysis of occluded infrainguinal vein grafts: predictors of outcome. J Vasc Surg 1997;25:1023-32.
9. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G. A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 1986;103:2787-96.
10. Casasco A, Giordano M, Daniva M, Casasco M, Icaro CA, et al. PC10 monoclonal antibody to proliferating cell nuclear antigen as probe for cycling cell detection in developing tissue: a combined immunocytochemical and flow cytometric study. Histochemistry 1993;99:191-9.

11. Vyverg M, Nielsen S. Dextran polymer conjugate two-step visualization system for immunohistochemistry. Immunohistochemistry 1998;6:3-10.
12. Pulford KA, Rigney EM, Micklem KJ, Jones M, Stross WP, et al. KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 1989;42:414.
13. Baenziger NL, Becherer PR, Majerus PW. Characterization of prostacyclin synthesis in cultured human arterial smooth muscle cells, venous endothelial cells and skin fibroblasts. Cell 1979;16:964-74.
14. Eldor A, Falcone DJ, Hajjar DP, Minick R, Weksler BB. Recovery of prostacyclin production by de-endothelialized rabbit aorta. J Clin Invest 1981;67:735-41.
15. Yatsuyanagi E, Sasajima T, Goh K, Inaba M, Kubo Y. Role of medial smooth muscle cell function in antithrombogenicity of vein grafts. Eur J Vasc Endovasc Surg 1998;15:350-6.
16. Su SB, Motoo Y, Iovanna JL, Berthezene P, Xie MJ, Sawabu N, et al. Overexpression of p8 is inversely correlated with apoptosis in pancreatic cancer. Clin Cancer Res 2001;7:1320-4.
17. Kunkel SL, Chensue SW, Phan SH. Prostaglandins as endogenous mediators of interleukin 1 production. J Immunol 1986;136:186-92.
18. Ohkawa F, Ikeda U, Kawasaki K, Kusano E, Igarashi M, Shimada K. Inhibitory effect of interleukin-6 on vascular smooth muscle contraction. Am J Physiol 1994;266:H898-902.
19. Beasly D. COX-2 and cytosolic PLA_2 mediate IL1-β-induced cAMP production in human vascular smooth muscle cells. Am J Physiol 1999;276:H1369-78.

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