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Endothelial Implants Provide Long-Term Control of Vascular Repair in a Porcine Model of Arterial Injury[1]

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Cell culture and animal data support the role of endothelial cells and endothelial-based compounds in regulating vascular repair after injury. We describe a long-term study in pigs in which the biological and immunological responses to endothelial cell implants were investigated 3 months after angioplasty, approximately 2 months after the implants have degraded. Confluent porcine or bovine endothelial cells grown in polymer matrices were implanted adjacent to 28 injured porcine carotid arteries. Porcine and bovine endothelial cell implants significantly reduced experimental restenosis compared to control by 56 and 31%, respectively. Host humoral responses were investigated by detection of an increase in serum antibodies that bind to the bovine or porcine cell strains used for implantation. A significant increase in titer of circulating antibodies to the bovine cells was observed after 4 days in all animals implanted with xenogeneic cells. Detected antibodies returned to presurgery levels after Day 40. No significant increase in titer of antibodies to the porcine cells was observed during the time course of the experiment in animals implanted with porcine endothelial cells. No implanted cells, Gelfoam, or focal inflammatory reaction could be detected histologically at any of the implant sites at 90 days. These data suggest that tissue-engineered endothelial cell implants may provide long-term control of vascular repair after injury, rather than simply delaying lesion formation and that allogeneic implants are able to provide a greater benefit than xenogeneic implants. © 2001 Academic Press

Key Words: **vascular injury; perivascular; endothelium; intimal hyperplasia; tissue engineering.**

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INTRODUCTION

The endothelium is critical to preserving vascular homeostasis, serving not only as a physical barrier but also as a source of a balanced array of biologically active compounds [1–5]. Endothelial denudation or injury occurs after vascular interventions such as angioplasty, bypass grafts, and organ transplantation [6–8], disrupting vascular homeostasis and eliciting a cascade of events that culminates in tissue hyperplasia and lumenal obstructions [9, 10]. Perivascular endothelial cell implants allow for the transplantation of a large number of confluent endothelial cells possessing the desired secretory functions at a site distant from the lumen but adjacent to the site of vascular injury, thus separating boundary properties from biochemical regulation. In this position, xenogeneic and allogeneic endothelial implants reduced intimal hyperplasia and thrombotic occlusion to the same extent 4 weeks after balloon injury of pig carotid arteries [11, 12].

In the present study, we addressed the biological and immunological effects of perivascular xenogeneic and allogeneic implants in a long-term model of porcine carotid artery injury. We hypothesized that perivascular endothelial implants would inhibit intimal hyperplasia and thrombotic occlusion by enabling vascular healing and restoring homeostasis such that a permanent implant would not be necessary to observe lasting benefits. To directly test this hypothesis, we determined the inhibitory potential of perivascular endothelial implants at a time point after evidence of implant degradation or destruction. The answer to this query could determine not only whether such therapies could provide benefits clinically, but is also at the heart of important and fundamental issues related to the biology of tissue repair. We examined whether perivascular endothelial implants could maintain control of vas-

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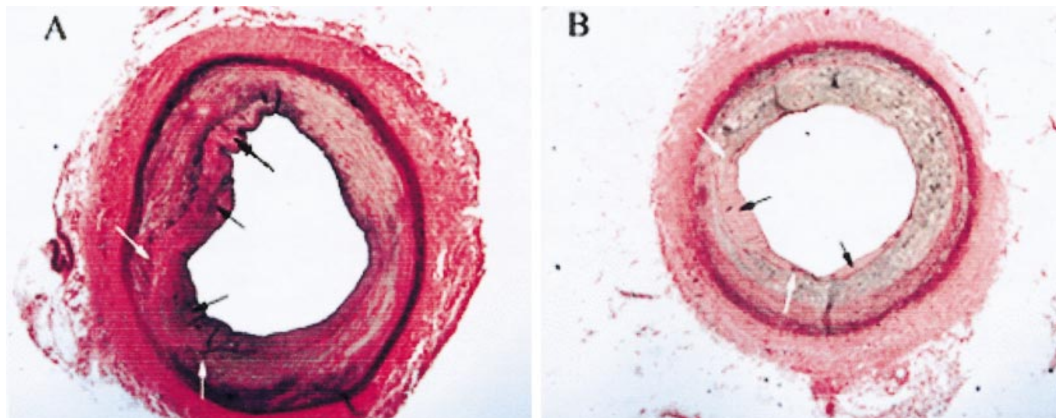

FIG. 1. Representative photomicrographs of arterial cross sections show the effects of perivascular endothelial cell implants on neointimal formation 90 days after balloon injury of porcine carotid arteries (Verhoeff's elastin stain, magnification, ×20). Perivascular endothelial cell implants (B, PAE implant) reduced intimal thickening when compared to control arteries (A). Dark arrows point to the neointimal lesions; white arrows point to IEL and medial disruption.

cular repair 3 months after injury and whether xenotransplantation was more, or less, effective than allotransplantation at controlling intimal hyperplasia in a long-term experiment. We also investigated whether differential antibody responses to the allogeneic and xenogeneic cells could be detected during the time course of a 3-month study and if there was a correlation to the biological effects. The results of this study provide additional insight into endothelial regulation of vascular repair and are a further step toward identifying potential clinical therapies for reducing restenosis and thrombosis that occur after vascular interventions.

METHODS

In vitro biologic activity of endothelial cells in Gelfoam. Endothelial cells were cultured in Gelfoam as previously described [11]. Blocks (2.5 × 1.0 × 0.3 cm^3) of sterile Gelfoam (Pharmacia & Upjohn, Kalamazoo, MI) were seeded with 0.8–1 × 10^5 cells per sponge. The cell-loaded Gelfoam blocks were placed in 17 × 100-mm polypropylene tubes containing 2 mL DMEM with 10% calf serum (Life Technologies, Inc., Grand Island, NY) and incubated for up to 2 weeks. The number of cells attached to the Gelfoam was determined after digestion with collagenase (Type I, Worthington Biochemical Corp., Freehold, NJ). Cell viability was checked by trypan blue exclusion as well as a LIVE/DEAD viability/cytotoxicity kit supplied by Molecular Probes (Eugene, OR). Endothelial cells were grown to confluence before implantation. Control Gelfoam matrices were incubated in DMEM containing 10% calf serum prior to implantation.

The production of heparan sulfate proteoglycans by endothelial cells has been shown to play a critical role in regulating vascular response to injury *in vivo* [12]. Therefore, the amount of heparan sulfate in conditioned medium produced by BAE and PAE cultured in Gelfoam was measured [13]. Total sulfated glycosaminoglycan was determined using dimethylmethylene blue (DMB) [14] and the amount of heparan sulfate was assessed after enzymatic digestion. Because transforming growth factor-beta (TGF-β) is also an inhibitor of smooth muscle cell proliferation *in vitro* [15], conditioned media were assayed using a standard ELISA kit (R&D Systems, Minneapolis, MN) to quantify levels of TGF-β. Endothelial integrity was determined by uptake of acetylated low-density lipoprotein (DiI-Ac-LDL, Biomedical Technologies, Stoughton, MA).

In vivo biologic activity of endothelial cells cultured on Gelfoam. The ability of the endothelial implants to reduce intimal hyperplasia and thrombosis when wrapped around balloon-injured porcine carotid arteries was assessed as previously described [11, 12]. This study conformed to the guidelines specified in the National Institutes of Health *Guide for Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee of the Veterans Association Medical Center (West Roxbury, MA). Fourteen male domestic pigs, 32.4 kg ± 1.2 kg, were obtained from Animal Biotech, Inc. (Danboro, PA). Anesthesia was induced with intramuscular ketamine (1000 mg), xylazine (150 mg), and atropine (0.6 mg) and maintained with inhaled isofluorane (0.5–1.5%) via an endotracheal tube. All animals also received intravenous cefazolin (500 mg, pre- and postsurgery) to prevent infection. The intraarterial pressure

TABLE 1

Histopathological Characteristics of Porcine Carotid Arteries after Balloon Injury

Characteristics	Control Gelfoam	BAE	PAE
Number of vessels, n	11	5	7
Occluded vessels, %	18	0	0
EEL area (mm^2)	11.62 ± 0.86	10.63 ± 1.53	10.77 ± 1.10
IEL length (mm)	7.99 ± .45	8.07 ± 0.91	7.52 ± 0.84
Fracture length (mm)	1.54 ± 0.11	1.34 ± 0.23	1.59 ± 0.09
Intima area (mm^2)	1.61 ± 0.20	0.76 ± 0.13*	0.67 ± 0.12*
Media area (mm^2)	5.16 ± 0.18	4.52 ± 0.45	4.97 ± 0.30
Lumen area (mm^2)	3.25 ± 0.57	4.15 ± 1.0	3.67 ± 0.81
Residual lumen (ratio)	0.66 ± 0.02	0.82 ± 0.04*	0.80 ± 0.06*
Injury index[a]	0.19 ± 0.01	0.17 ± 0.02	0.22 ± 0.02
Restenosis index[b]	1.22 ± 0.13	0.85 ± 0.07*	0.53 ± 0.07*,†

[a] Injury index = IEL fracture length/IEL circumference.
[b] Restenosis index = $[I/(I + M)]/(F/\text{IEL})$.
* $P < 0.05$ compared to control arteries.
† $P < 0.05$ compared to BAE.

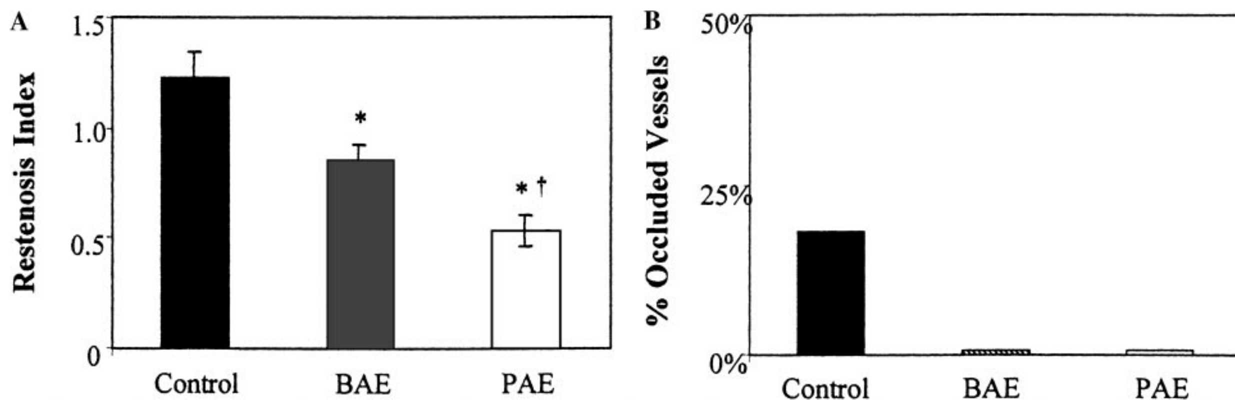

FIG. 2. Effects of endothelial cell implants on the restenosis index and thrombosis of balloon-injured porcine carotid arteries. Restenosis index = $[I/(I + M)]/(F/\text{IEL})$. (A) Bar graph shows that endothelial cell implants decreased the restenosis index by 56 and 31% for porcine and bovine cells, respectively. $*P < 0.05$ vs control arteries, $\dagger P < 0.05$ vs BAE arteries. ANOVA, $P < 0.0004$. (B) Bar graph shows a decrease in occlusive thrombosis for arteries wrapped with endothelial cell grafts compared to control arteries.

and electrocardiogram were continuously monitored throughout the procedure.

Surgical procedure. Right femoral arterial access with an 8F sheath was obtained via cut down, and an 8.0-mm-diameter angioplasty balloon (Cordis, Inc., Miami, FL) was advanced to the common carotid artery under fluoroscopic guidance. Angiography was performed and recorded by cineradiography. The right and left carotid arteries were injured by 30-s balloon inflations at 8 atmospheres pressure (five inflations per side, in overlapping segments). The balloon/artery ratio (1.28 ± 0.05) did not vary significantly between treatment groups. After final angiography to assess vessel patency, a mid-line neck incision was made and both left and right common carotid arteries were isolated and gently wrapped with Gelfoam containing BAE ($n = 6$ arteries, 2 arteries per animal), PAE ($n = 10$ arteries, 2 arteries per animal), or no cells ($n = 12$ arteries, 2 arteries per animal). The carotid sheath was closed to immobilize the device and sutured to facilitate the location of the implant site at sacrifice.

Tissue processing. On the 90th postoperative day, animals were euthanized with intravenous potassium chloride (40 meq). The carotid arteries were perfused at 100 mm Hg for 10 min with 4% paraformaldehyde in 0.1 mol/L PBS (pH 7.4) to fix the arteries *in situ.* The arteries were isolated and the vessel was divided into three 10-mm-long segments: proximal to the suture, at the suture (middle), and distal to the suture. The segments were paraffin-embedded. Five-micrometer sections were obtained from the proximal, middle, and distal segments and stained with VerHoeff's elastin stain. Morphometric analysis was performed on all segments. The intimal (I), medial (M), lumen (L), and external elastic lamina (EEL) areas as well as the internal elastic lamina (IEL) circumference and IEL fracture length (F) were measured using computerized digital planimetry with a video microscope and customized software. Morphometric measurements were made by an observer blinded to the treatment groups. The extent of injury was represented by the fracture length of the IEL, normalized for the size of the artery by the circumference of the IEL: injury index = F/IEL. Intimal hyperplasia was also normalized by the total artery wall area: $I/(I + M)$. The restenosis index was then defined as $[I/(I + M)]/(F/\text{IEL})$. The residual lumen was also measured, which reflected the change in vessel geometry after injury and repair. The residual lumen was defined as $L/(L + I)$. Dissected arteries, as determined by frank rupture of the EEL, and uninjured arteries with an intact IEL were excluded from all analyses ($n = 5$ arteries). Artery sections were also stained with mouse anti-porcine CD31 (PECAM-1, 1:10 dilution, Serotec Inc., Raleigh, NC) followed by an avidin-biotin peroxidase complex method to assess reendothelialization of the carotid arteries. Porcine spleen was used as a positive control and mouse IgG was used as a negative control.

Fluorescence immunocytochemistry. Sera were collected from pigs before surgery and at 4 days, 2 weeks, 1 month, 40 days, 60 days, and 90 days postsurgery and tested for an antibody response to the same strain of endothelial cells as those used for implantation. BAE or PAE were grown to confluence on glass coverslips and fixed in 3% paraformaldehyde. All samples were incubated with 100 μg/mL RNase (Worthington Biochem Corp.) at 37°C for 30 min, blocked with rabbit serum (Life Technologies, Inc.) followed by nonfat dry milk (5.0% in PBS, blotting grade, BioRad Laboratories, Hercules, CA) for 30 min at room temperature. After washing with PBS, samples were incubated with undiluted porcine sera for 2 h at 4°C followed by a 1-h incubation with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-pig IgG (diluted 1:20, Sigma Chemical Co.), and propidium iodide (10 mg/mL in 1:100 dilution, Calbiochem, San Diego, CA). Controls for nonspecific staining were stained only with the secondary antibody. The samples were examined with a confocal laser-scanning microscope. Six fields were selected for each sample by locating confluent areas of cells with propidium iodide nuclear staining. The intensity of immunofluorescence was measured using customized software.

Cytotoxic antibodies. PAE and BAE were incubated at 37°C with porcine serum from experimental animals at dilutions of 1:20 and 1:100 as preliminary experiments had determined that preformed antibodies cytotoxic for BAE or PAE were not detected at these dilutions. After 2 h the total, viable, and nonviable cell numbers were determined.

Statistical analysis. All data are presented as means ± SE. Statistical analysis comparing treatment groups used a single-factor ANOVA and a nonpaired Student's t test. Values of $P < 0.05$ were considered significant.

RESULTS

Growth kinetics and biochemical activity of engrafted cells. BAE and PAE cultured within polymer matrices (Gelfoam) lined the interstices and grew well within the three-dimensional matrix with cell doublings observed approximately every 36 h and a saturation density of $\approx 1 \times 10^6$ and 0.7×10^6 cells/cm^3 Gelfoam for BAE and PAE, respectively. Cell viability remained at 95% over the 2-week culture course. Bo-

vine and porcine endothelial cells cultured in Gelfoam matrices retained their identity and normal postconfluent biosecretory ability. Endothelial cells cultured in Gelfoam produced similar amounts of heparan sulfate, an inhibitor of smooth muscle cell proliferation *in vitro,* as cells grown in tissue culture dishes [13]. In addition, BAE and PAE cultured in Gelfoam produced statistically similar amounts of glycosaminoglycans ($3.51 \pm 0.53\ \mu g/10^6$ cells and $2.91 \pm 0.24\ \mu g/10^6$ cells, respectively), heparan sulfate ($1.43 \pm 0.13\ \mu g/10^6$ cells and $1.52 \pm 0.02\ \mu g/10^6$ cells, respectively), and TGF-β (809.6 ± 148 pg/10^6 cells and 894.4 ± 135 pg/10^6 cells, respectively). Both BAE and PAE retained their ability to take up Ac-LDL when cultured in Gelfoam.

Endothelial cell implant inhibition of intimal thickening and thrombosis. The pigs used in this study were randomly selected to receive one of the following treatments after balloon injury: Gelfoam-BAE, Gelfoam-PAE implants, or Gelfoam implants without cells. All neck incisions healed well and all animals gained weight throughout the 12-week postoperative period. Morphometric analysis of the proximal, middle, and distal segments of each artery revealed no significant differences in the injury response between the three segments for any of the treatment groups. Therefore, measurements made from three sites on each vessel were averaged so that each vessel resulted in one data point.

Twelve weeks after injury to the carotid arteries, extensive neointimal proliferation or occlusive organized thrombus was observed at the site of vessel injury in control animals. The restenosis index of control animals receiving Gelfoam alone (1.22 ± 0.13) did not differ significantly from that observed at 1-month for either balloon injury alone or balloon injury treated with empty Gelfoam [11]. Arteries wrapped with Gelfoam containing either BAE or PAE showed a significant decrease in both the restenosis index and thrombosis (Fig. 1, Table 1). The restenosis index in arterial segments treated with BAE or PAE implants was reduced by 31% to 0.85 ± 0.07 ($P < 0.05$) or by 56% to 0.53 ± 0.07 ($P < 0.05$), respectively (Fig. 2), compared to control animals (ANOVA, $P < 0.0004$). In addition, there was a significant difference between the restenosis index of arteries treated with PAE compared to arteries treated with BAE ($P < 0.05$). Gelfoam matrices containing endothelial cells also reduced thrombosis (Fig. 2). Extensive occlusive organized thrombosis was observed in two arteries (18%) of the control Gelfoam group and in none of the arteries treated with endothelial implants (0%). CD31 (PECAM-1, a cell surface protein expressed by endothelial cells) staining of sectioned arteries from each of the treatment groups revealed complete reendothelialization in all sections 3 months after injury (Fig. 3).

Xenogeneic and allogeneic humoral immune response. The results of sera immunofluorescence after endothelial transplantation are shown in Fig. 4. The serum was tested against the same strain of BAE or PAE used for implantation. Sera from animals treated with BAE implants tested against cultured BAE showed a significant increase in fluorescent intensity at the 2-week and 1-month time points (86.35 ± 3.53 and 87.05 ± 4.05, respectively) compared to presurgery fluorescence (46.08 ± 2.62, $P < 0.05$). Further analysis revealed the presence of increased levels of cytotoxic anti-BAE antibodies at these time points. However, sera from these same animals did not show a significant increase in either total or cytotoxic anti-BAE antibodies at 4 days (45.94 ± 6.84), 40 days (58.99 ± 4.69), 60 days (50.45 ± 3.73), or 90 days (40.99 ± 1.53) when compared to the presurgery levels. This reaction indicates that these pigs produced an increased amount of antibodies against implanted BAE cell surface antigens after the 4th postoperative day. The detectable serum antibodies returned to presurgery levels ≈40 days postimplantation. Sera from pigs that received PAE implants were tested by an identical set of experiments (Fig. 4). Sera from animals treated with PAE implants showed an insignificant increase in fluorescent intensity at 1 month (63.08 ± 7.44) compared to presurgery fluorescence (53.86 ± 2.54, $P = NS$) and an insignificant increase in cytotoxic antibodies at 14 days. No increase in total or cytotoxic anti-PAE titer was detected at any other time point during the course of the experiment. No significant increase in fluorescence was detected in sera from control animals at any of the time points tested. Histological examination of the implant sites at 3 months revealed no evidence of remaining Gelfoam or the allogeneic or xenogeneic endothelial cells. The typical degradation rate of Gelfoam is approximately 4–6 weeks postimplant. Moreover, leukocyte infiltration at the implant site, evident at the 1-month time point [11, 12], was not detected histologically in any animals at 3 months.

DISCUSSION

It has long been known that the intact endothelium provides control over a wide range of vascular biology including thrombosis, vasomotor tone, smooth muscle cell proliferation and migration, lipid infiltration, and leukocyte adhesion, transmigration, and transformation [16–19]. Endothelial loss or damage disrupts vascular homeostasis and can result in proliferative neointimal lesions [10]. We have demonstrated that transplanted allogeneic or xenogeneic endothelial cells can regulate vascular repair even when placed at a distance from the lumen [11–13]. The ability of perivascular endothelial implants to influence intimal hyperplasia and thrombosis is an interesting phenom-

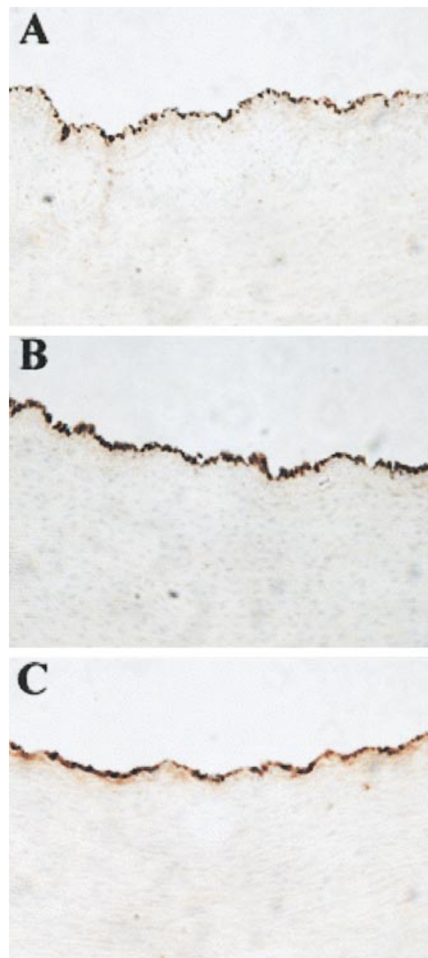

FIG. 3. Results of CD31 (PECAM-1) staining of carotid arterial segments 90 days after balloon injury. On the 90th postoperative day, a complete endothelial lining was evident in segments from all treatment groups. Brown cells are CD31-positive cells. Representative cross sections from (A) Control, (B) PAE, and (C) BAE groups, magnification ×200.

enon. While the specific mechanism is not known at this time, we have previously shown that endothelial-derived compounds and other macromolecules rapidly diffuse throughout the blood vessel wall and have effects at a distance from their site of release [20–24]. The experiments presented here allowed us to determine whether these effects were transient or could be observed at a time after implant survival was no longer detected. Three months postimplant neither Gelfoam nor the transplanted endothelial cells could be detected at the implant site, and yet both allogeneic and xenogeneic implants inhibited intimal hyperplasia. However, an interesting divergence in the biological effect was observed for the arteries treated with xenogeneic implants. Implants containing xenogeneic cells reduced the restenosis index by 31%, while implants containing allogeneic cells were almost twofold more effective in this regard.

Temporary vs long-term effects. A critical issue in developing potential therapies for the accelerated arteriopathies that occur after vascular interventions is related to the duration of therapeutic effects. Many agents that halt disease progression seemingly lose effect when the therapy is discontinued. For example, when animals were made transiently thrombocytopenic after balloon denudation, intimal proliferation was inhibited during platelet suppression but returned in full with platelet recovery [25]. The intimal lesion that developed after balloon denudation followed sequentially by 14 days of thrombocytopenia and 14 days of platelet recovery was identical to the lesion observed in animals with intact platelet function 14 days after injury [25]. Thus, removing critical elements and stimuli necessary for neointima formation temporarily prevented lesion formation. However, if blood vessel homeostasis is not reestablished by the time critical elements return, neointimal formation is only delayed rather than permanently inhibited or reduced. Both xenogeneic and allogeneic implants reduced intimal hyperplasia long after evidence of the Gelfoam or transplanted cells could be detected at the implant site. A local leukocytic response was observed at the xenogeneic, and to a lesser degree at the allogeneic implant sites, at 28 days [11]. However, leukocyte infiltration at the implant sites was not detected in any animals at 3 months, providing evidence that this response had resolved. CD31 staining at 90 days revealed that arteries in all treatment groups were completely reendothelialized, further supporting that the effects of the implants are permanent since substantial intimal proliferation would not be expected to occur after the endothelium is restored.

Xenotransplantation vs allotransplantation. The differential effect of xenotransplantation and allotransplantation on experimental restenosis at 90 days suggests a relationship between the biological and immunological responses. The difference in effects on intimal thickening by transplanted BAE or PAE does not appear related to variable production of vasoregulatory factors. *In vitro* secretion of heparan sulfate and TGF-β by BAE or PAE cultured in Gelfoam were identical. However, one cannot entirely dismiss the effects of different levels of other inhibitory or stimulatory compounds. Endothelial cells produce a myriad of factors that maintain vascular homeostasis [16–19]. Yet, the variable secretion of unidentified vasoregulatory compounds seems an unlikely explanation for the observed difference. A more likely interpretation is the difference in host response that occurs during the time course of the experiment. Serial serum samples from animals implanted with bovine cells over the 3-month duration of the experiment demonstrated a significant increase in titer of cytotoxic antibodies to the bovine cells beginning 4 days after injury and resolving at ≈40

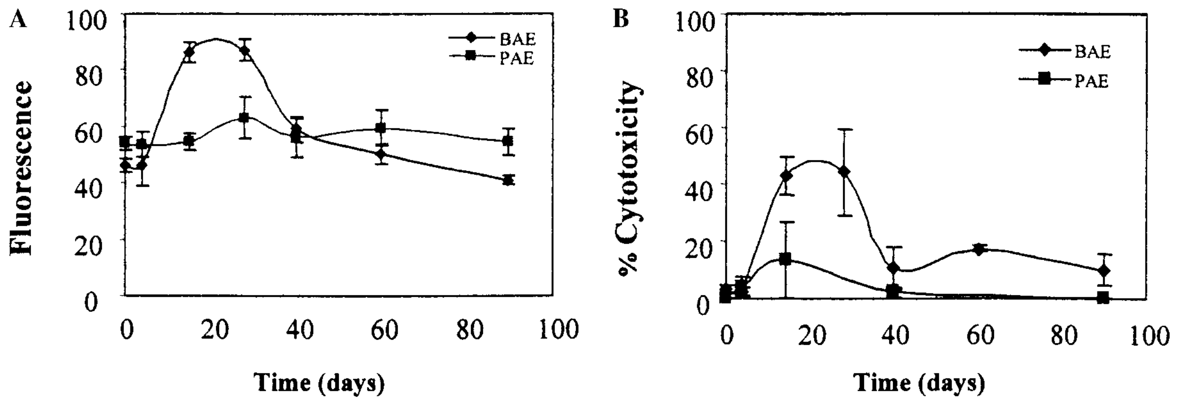

FIG. 4. Graph of xenogeneic and allogeneic humoral response. Sera obtained presurgery and 4 days, 2 weeks, 1 month, 40 days, 60 days, and 90 days after implantation from animals that received xenogeneic and allogeneic implants were tested against the same cells used for implantation. (A) Sera from animals that received BAE implants showed a significant increase in antibodies binding to BAE 2 weeks and 1 month postimplantation, when compared to presurgery levels ($P < 0.05$). No significant positive immunoreactivity was detected in sera from animals that received allogeneic PAE when compared to presurgery sera from the same animal. All samples were corrected for background by subtracting non-specific staining of the secondary antibody. (B) Sera (1:20 dilution) from animals treated with BAE implants showed a significant increase in cytotoxic anti-BAE antibodies at 14 and 28 days postimplantation. No significant increase was detected in sera obtained from animals treated with allogeneic PAE. Percentage cytotoxicity = (No. non-viable cells/No. total cells) * 100.

days. In contrast, no significant increase in titer of cytotoxic antibodies to the porcine cells was detected during the course of the experiment in serum from animals treated with allogeneic cells. The current postulated mechanisms of thrombosis and intimal hyperplasia consist of three temporal phases occurring at different times after injury [26]. An early phase, consisting of platelet activation and thrombus formation, occurs within minutes to hours after injury, an intermediate phase of cellular recruitment occurs hours to days after injury, and a late proliferative phase occurs days to months after injury. Therefore, an increase in cytotoxic antibodies to BAE 4–40 days postimplant would impact the implants' influence on late proliferation and produce an observable divergent effect on intimal hyperplasia in arteries treated with BAE implants compared to arteries treated with PAE implants at 90 days.

Thrombosis vs intimal proliferation. It must be appreciated that despite the different effects on intimal hyperplasia, both the xenogeneic and the allogeneic implants reduced occlusive thrombosis to a similar extent. This response highlights the different kinetics and biological mediation of thrombosis and smooth muscle cell proliferation. Inhibition of thrombosis does not eliminate smooth muscle cell proliferation or intimal hyperplasia, and that which controls the latter does not necessarily regulate the former [27–31]. For example, when implants containing endothelial cells with reduced expression of the antithrombotic and growth-inhibitory compound, perlecan (a secreted heparan sulfate proteoglycan), were placed around injured porcine carotid arteries, there was a complete loss of antithrombotic effects. However, the ability of these implants to inhibit intimal hyperplasia was diminished only in part [12]. Thrombosis peaks early after vascular injury, while neointima formation and remodeling occur later [32]. Thus, while the choice of cell type may be less important for thrombotic complications of vascular interventions, it is paramount for the inflammatory, proliferative, and remodeling effects that lead to intimal hyperplasia.

Tissue-engineered implants appear to provide a long-term benefit, rather than simply delaying lesion formation. The differential effects observed at the 3-month time point suggest that the allogeneic implants provided a greater benefit in controlling intimal thickening than xenogeneic implants in this model of experimental restenosis. Tissue-engineered endothelial cells may provide not only a tool by which to investigate the complex mechanisms of tissue repair and endothelial control over vascular injury, but also a novel means of inhibiting clinical neointimal formation in open surgical procedures. The potential therapeutic value, safety, and feasibility of such an approach as well as noninvasive methods of endothelial cell delivery merit further investigation.

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