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Overexpression of transforming growth factor β1 in arterial endothelium causes hyperplasia, apoptosis, and cartilaginous metaplasia

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ABSTRACT **Uninjured rat arteries transduced with an adenoviral vector expressing an active form of transforming growth factor β1 (TGF-β1) developed a cellular and matrix-rich neointima, with cartilaginous metaplasia of the vascular media. Explant cultures of transduced arteries showed that secretion of active TGF-β1 ceased by 4 weeks, the time of maximal intimal thickening. Between 4 and 8 weeks, the cartilaginous metaplasia resolved and the intimal lesions regressed almost completely, in large part because of massive apoptosis. Thus, locally expressed TGF-β1 promotes intimal growth and appears to cause transdifferentiation of vascular smooth muscle cells into chondrocytes. Moreover, TGF-β1 withdrawal is associated with regression of vascular lesions. These data suggest an unexpected plasticity of the adult vascular smooth muscle cell phenotype and provide an etiology for cartilaginous metaplasia of the arterial wall. Our observations may help to reconcile divergent views of the role of TGF-β1 in vascular disease.**

Transforming growth factor β1 (TGF-β1) is a pleiotropic growth factor expressed in diverse adult mammalian tissues, including the arterial wall (1–4). Several studies associate TGF-β1 expression with the development of arterial disease (5–9), whereas others suggest that TGF-β1 expression prevents arterial lesion formation (10–13). Still other investigations describe a role for TGF-β1 in the differentiation and transdifferentiation of adult cell types, including vascular endothelial cells (1, 2, 14). The processes by which arterial lesions form or regress and the signals according to which the differentiated state of vascular cells is maintained or modulated remain poorly understood. TGF-β1 may play a major role in regulating these important biological processes, and therefore its role in vascular biology merits further definition.

We recently developed an animal model of endothelium-specific *in vivo* gene delivery and hypothesized that this model would be useful in defining the role of biologically active gene products in an otherwise normal artery (15). Here we report the use of this model to investigate the role of TGF-β1 in the artery wall. Overexpression of TGF-β1 from arterial endothelium caused dramatic and unanticipated changes in the arterial phenotype, including pronounced effects on cellularity, matrix content, and the differentiated state of cells in the vascular media.

MATERIALS AND METHODS

Construction of Adenoviral Vectors. The adenoviral vectors AV1LacZ4 (gift of B. Trapnell, Genetic Therapy Incorporated, Gaithersburg, MD) and AdRSVTGF-β1 have been described previously (16, 17). These vectors express a nuclear-targeted *Escherichia coli* β-galactosidase (β-gal) transgene and a constitutively active form of porcine TGF-β1 (18), respectively. In both vectors, the transgene expression cassette is driven by the Rous sarcoma virus long-terminal repeat promoter. Vectors were prepared and titered as described (16).

***In Vivo* Gene Delivery and Detection of Transgene Expression.** Endothelium-specific gene delivery (15) to the left common carotid arteries was performed in 350- to 400-g Sprague–Dawley rats (Taconic Farms). Expression of TGF-β1 from transduced arteries was assayed by ELISA (Genzyme) of conditioned medium of explant cultures; the assay is specific for the active form of TGF-β1. Latent TGF-β1 is detected only after acid activation. To collect samples for the TGF-β1 assay, a 1-cm section of each transduced artery was removed and placed in 250 μl DMEM with 10% fetal calf serum. Media were collected after 24 hr and assayed both without acid activation (active TGF-β1) and after acid activation (total TGF-β1).

Morphometric Analysis. *In situ* perfusion fixation, tissue processing, embedding, staining, and morphometric analysis were performed essentially as described (15). Care was taken to embed only the central 1 cm of excised arteries to ensure that morphometric and histologic analyses were limited to areas exposed to recombinant gene products. Planimetry was performed on hematoxylin- and eosin-stained sections (two per artery) by an observer blinded to the treatment group, by using a computer-assisted image-analysis system (IPLAB SPECTRUM, Signal Analytics).

Histochemistry, Immunohistochemistry, and Transmission Electron Microscopy. Histologic sections of arteries were stained with hematoxylin and eosin, Movat's pentachrome, Alcian blue (for proteoglycans), and von Kossa stain (for mineralization). The tissue was characterized pathologically with the assistance of Lent Johnson, Distinguished Scientist, Division of Bone Pathology, Armed Forces Institute of Pathology, Washington, DC. Specific cell types were identified by immunostaining for von Willebrand's factor (endothelial cells), smooth muscle actin, and S-100 protein [a useful marker for chondrocytes (19, 20)]. Additional immunostaining was performed for type II collagen (Biodesign). Antibodies were from Dako (von Willebrand's factor and S-100) and from Sigma (smooth muscle actin). Bound antibodies were detected by immunoperoxidase staining with the avidin–biotin technique. Transmission electron microscopy was performed on

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Abbreviations: β-gal, β-galactosidase; TUNEL, transferase-mediated dUTP-biotin nick end labeling; TGF-β1, transforming growth factor β1.
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ultra-thin sections of two TGF-β1-transduced arteries, with techniques and instruments we have described (21).

Measurement of Cellular Proliferation and Apoptosis. To assess rates of cellular proliferation, rats were injected with BrdUrd (30 mg/kg body wt per dose, subcutaneously) at 17, 9, and 1 hr before death. BrdUrd incorporation was detected immunohistochemically, as described (15). Overall proliferation data for the intima and media of an artery were obtained by counting all (intimal and medial) cells as well as all (intimal and medial) BrdUrd-positive cells in sections. Additional proliferation data on subsets of cells (e.g., for chondrocytes or for intimal cells adjacent to chondrocytes) were obtained by counting all cells in these specific areas. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) (16). Labeling with dUTP-biotin and detection of incorporated label in histologic sections were performed essentially as described (22), except that streptavidin–alkaline phosphatase and new fuchsin substrate (Dako) were used for biotin-dUTP detection. Sections were counterstained with hematoxylin.

Statistical Analysis. All data are expressed as mean ± SEM. All statistical analyses were performed with two-tailed, unpaired *t* tests.

RESULTS

Expression of TGF-β1. At 3 days after gene delivery, both immunohistochemistry for active TGF-β1 (23) and *in situ* hybridization with a rat TGF-β1 cDNA probe (24) localized TGF-β1 expression predominantly to the luminal endothelium of arteries transduced with the TGF-β1 vector ("TGF-β arteries"; data not shown). No significant TGF-β1 expression was detected in arteries transduced with the AV1LacZ4 vector (β-gal arteries). To measure the level of TGF-β1 secretion, TGF-β and β-gal arteries were placed in explant culture, and the conditioned media were assayed for active and total TGF-β1. At 3 days after gene transfer, TGF-β arteries ($n = 3$) secreted 120 ± 18 pg/24 hr of active TGF-β1 and 2,400 ± 692 pg/24 hr of total (active + latent) TGF-β1. Arteries transduced with the AV1LacZ4 vector ("β-gal arteries"; $n = 4$) secreted no detectable active TGF-β1 and 230 ± 135 pg/24 hr of total TGF-β1. The increased expression of latent TGF-β1 in the TGF-β arteries might result from a strong up-regulation of endogenous rat TGF-β1 expression via autoinductive pathways (25, 26), or may indicate that even this mutant ($Cys^{223, 225} \rightarrow Ser$) TGF-β1 protein can exist in an acid-activatable (i.e., latent) form. By 4 weeks after gene transfer, TGF-β arteries ($n = 3$) secreted no active TGF-β1 and 660 ± 144 pg/24 hr of total TGF-β1. Four-week β-gal arteries ($n = 3$) secreted no detectable TGF-β1. Based on these measurements, the total TGF-β1 protein produced by the artery wall during the 4 weeks after TGF-β1 gene transfer was less than 100 ng, more than 4 orders of magnitude below the systemic dose of TGF-β1 delivered in a related rat model of arterial injury (6).

Morphometry of Transduced Arteries. Microscopic examination and planimetry of histologic sections of perfusion-fixed arteries harvested at 3 days and at 1, 2, 4, and 8 weeks after gene transfer revealed a pattern of intimal and medial growth and regression in the TGF-β arteries (Fig. 1). At 4 weeks, the intimal area was significantly greater in the TGF-β arteries (Fig. 1*A*) than in the β-gal arteries (0.058 ± 0.010 versus 0.0075 ± 0.0050 mm^2; $P = 0.005$). Surprisingly, by 8 weeks the intima in the TGF-β arteries had nearly completely regressed (Fig. 1*A*) and there was no longer any difference between the two groups (0.0067 ± 0.012 versus 0.0067 ± 0.012 mm^2; $P = 1.00$). The medial area of the TGF-β arteries also was maximal at 4 weeks (Fig. 1*B*), at which point it was significantly greater than that of the β-gal arteries (0.19 ± 0.019 versus 0.12 ± 0.0065 mm^2; $P = 0.028$). By 8 weeks, the medial areas of the TGF-β and β-gal arteries were no longer different (0.16 ± 0.0087 versus 0.14 ± 0.0056 mm^2; $P = 0.19$). Intimal/medial area ratios at 4 weeks (Fig. 1*C*) were also greater in the TGF-β arteries than in the β-gal arteries (0.30 ± 0.030 versus 0.061 ± 0.038 mm^2, respectively; $P = 0.001$).

To begin to identify the cellular and molecular elements that contributed to the intimal and medial growth observed in the TGF-β arteries, we examined sections from arteries harvested 3 days as well as 1, 2, 4, and 8 weeks after gene transfer and stained with hematoxylin and eosin, Movat's pentachrome, Alcian blue, and von Kossa stains. Cell types were identified with the aid of immunohistochemical stains for von Willebrand's factor, smooth muscle actin, and S-100 protein (a marker for chondrocytes) (19, 20).

At 3 days and 1 week, the TGF-β arteries were morphologically indistinguishable from the β-gal arteries (not shown). At 2 weeks, the TGF-β arteries had occasional, focal accumulations of collagen and proteoglycans in the media (Fig. 2*A*), whereas the β-gal arteries appeared unchanged from earlier time points (not shown). The abnormal-appearing areas of the media of the TGF-β arteries were associated with a thicker overlying neointima than was present either along the more normal-appearing areas of the same arteries or along the entire circumference of the β-gal arteries. At 4 weeks the TGF-β arteries contained a substantial cellular neointima (Fig. 2*B*); whereas the β-gal arteries appeared essentially normal (Fig. 2*C*). In the 4-week TGF-β arteries, the neointima usually was present only focally and was thickest along the posterior surface of the artery (Fig. 2 *B* and *E*), which is consistent with the tendency for transduction to occur in dependent areas in this model (i.e., posterior in the supine rat; data not shown). In the media and to a lesser extent in the neointima, vascular

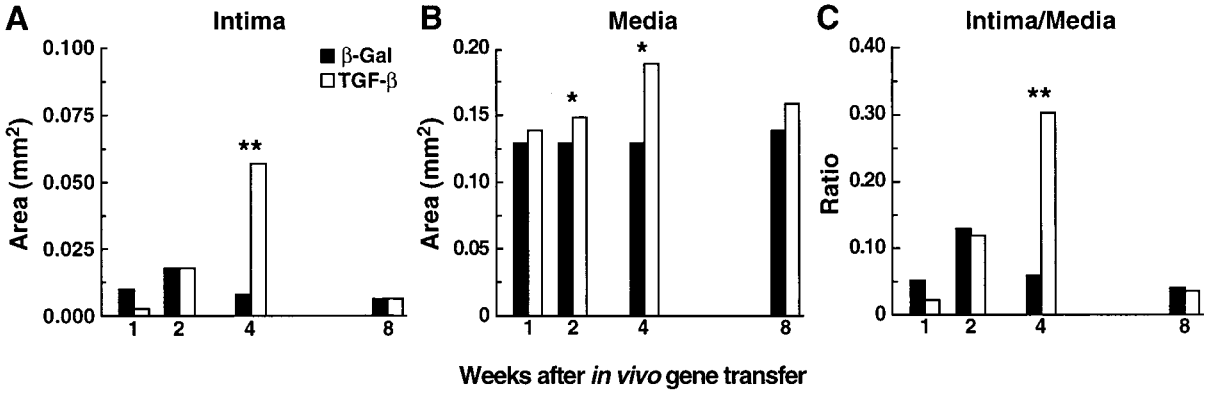

FIG. 1. Morphometric findings in TGF-β and β-gal arteries. Intimal area (*A*), medial area (*B*), and intima/media area ratio (*C*) were measured and calculated by using computer-assisted planimetry. Bars represent means of three to five arteries per group; *, $P < 0.05$; **, $P < 0.01$ for comparison of TGF-β and β-gal arteries at indicated time points.

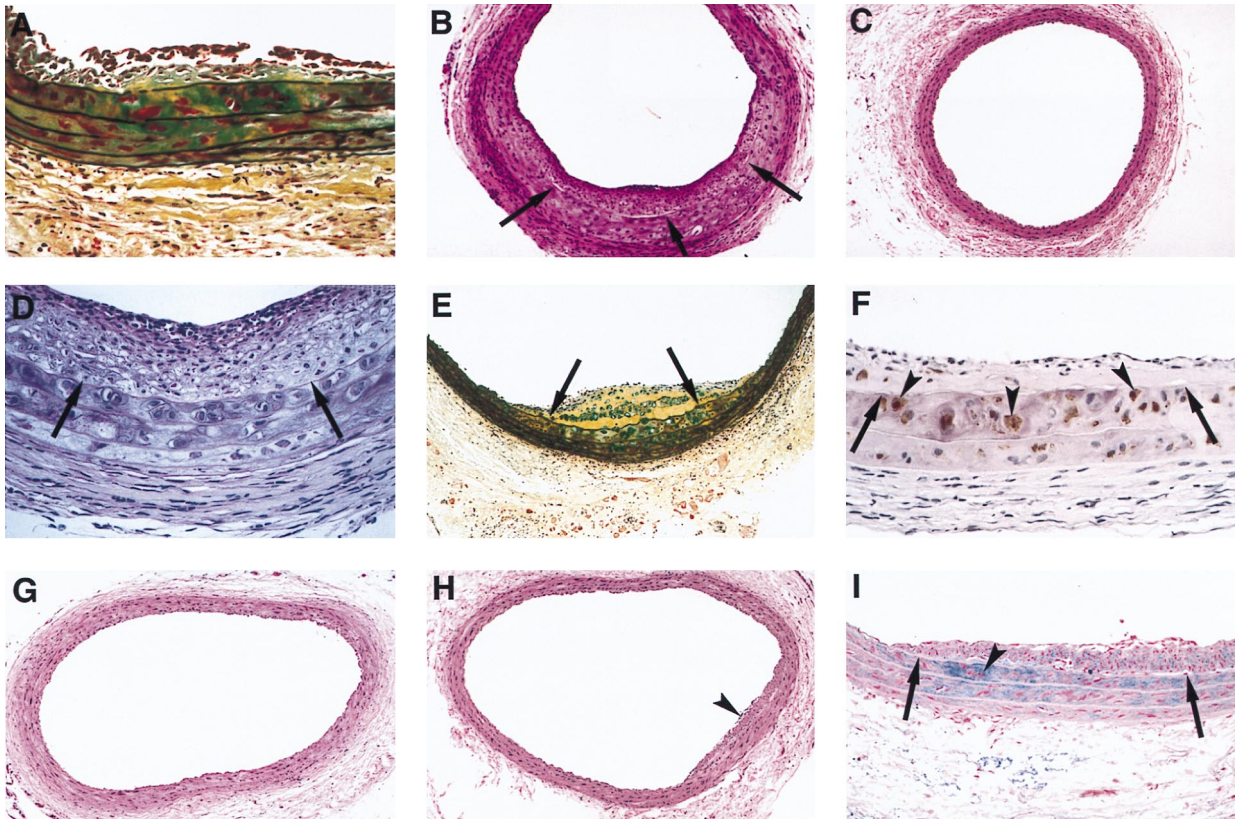

FIG. 2. Histologic appearance of TGF-β and β-gal arteries. (*A*) In a 2-week TGF-β artery, increases in medial proteoglycans (green) and collagen (yellow) are apparent. (*B*) In a 4-week TGF-β artery, asymmetric increases in intimal and medial thickness are seen. (*C*) In a 4-week β-gal artery, the intima and media appear essentially normal. (*D–F*) In 4-week TGF-β arteries, a cellular and matrix-rich intima is present with both medial and intimal cartilaginous metaplasia. Numerous cells appear in lacunae, surrounded by a proteoglycan and collagen-rich matrix. In *F*, S-100 immunostain identifies chondrocytes. (*G*) In an 8-week β-gal artery, the intima and media appear essentially normal. (*H–I*) In an 8-week TGF-β artery, a small area of neointima is present. Arrows in *B, D, E, F*, and *I* indicate internal elastic lamina; arrowheads in *F* indicate some of the cells staining for S-100 antigen; arrowheads in *H* and *I* indicate small remaining neointima and an area of proteoglycan accumulation, respectively. Sections were stained with hematoxylin and eosin (*B–D, G, H*), Movat's pentachrome (*A* and *E*), S-100 immunostain (*F*), or Alcian Blue (*I*). Original magnifications: (*E*) $\times$20; (*B, C, G,* and *H*) $\times$25; (*A, F,* and *I*) $\times$65; (*D*) $\times$80.

cells with the histologic appearance of chondrocytes were present, apparently in lacunae (Fig. 2 *B* and *D–F*). These chondrocyte-like cells were present in 5 of 5 (100%) 4-week TGF-β arteries and constituted 10–25% of all intimal and medial cells in these arteries. Identification of these cells as chondrocytes was further supported by the presence of immunoreactive S-100 protein (Fig. 2*F*) and by the absence of immunoreactive smooth muscle actin (not shown). Large amounts of collagen and proteoglycan were present adjacent to the chondrocytes (Fig. 2*E*). To investigate further the composition of the cartilaginous matrix, we performed immunostaining for type II collagen, which is a characteristic component of cartilaginous rather than vascular matrix. Immunostaining for type II collagen was negative, although this was potentially because of a negative effect of TGF-β1 on type II collagen expression (27). The von Kossa stain did not reveal mineralization in the 4-week TGF-β arteries, suggesting that osteogenesis does not occur in this model. Transmission electron microscopy of sections from these arteries revealed rounded cells with a high nuclear/cytoplasmic ratio, surrounded by lacunae. A loose extracellular matrix was present, appearing more cartilaginous than vascular, with collagen fibers, abundant proteoglycans, and little elastin (not shown). These histological and ultrastructural features permit identification of these areas as sites of cartilaginous metaplasia, defined as "the presence of chondrocytes in lacunae within a collagenous mucopolysaccharide-rich matrix" (28). The appearance of chondrocytes in the arterial wall was not caused by migration of cells from cartilage in the adjacent upper airway, because we noted the same findings in arteries wrapped circumferentially in polytetrafluoroethylene vascular graft material (not shown).

Because chondrogenesis often is followed by osteogenesis, we tested whether arteries harvested 8 weeks after gene transfer might exhibit evidence of calcification and/or osteogenesis. As expected, β-gal arteries appeared largely normal, similar to those harvested at earlier time points (Fig. 2*G*). Surprisingly, 8-week TGF-β arteries were almost indistinguishable from the β-gal arteries, containing only focal areas of neointima and an essentially normal media (Fig. 2*H*). There were rare, focal areas of increased collagen and proteoglycan in the intima and media (Fig. 2*I*), but neither cartilage nor calcification was present. Thus, the dramatic phenotypic changes found at 4 weeks in TGF-β arteries were nearly completely reversed by 8 weeks.

Cellular Proliferation and Apoptosis. To investigate mechanisms underlying the development and regression of the neointima, we measured the proliferative indices in arteries harvested at 1, 2, 4, and 8 weeks. In the 4-week TGF-β arteries, we noted striking local variability in proliferative rates. For this reason, separate proliferative indices were calculated both for the chondrocytes and for intimal nonchondrocytic cells adjacent to the medial chondrocytes. One week after gene transfer, the proliferative indices in the β-gal arteries—20 $\pm$ 6.6% in the intima (Fig. 3*A*) and 7.4 $\pm$ 3.6% in the media (Fig. 3*B*)—were elevated above those reported for quiescent arteries [$<$1% (29, 30)]. These increases, which we observed previously (15), are due both to surgical manipulation and to an effect of adeno-

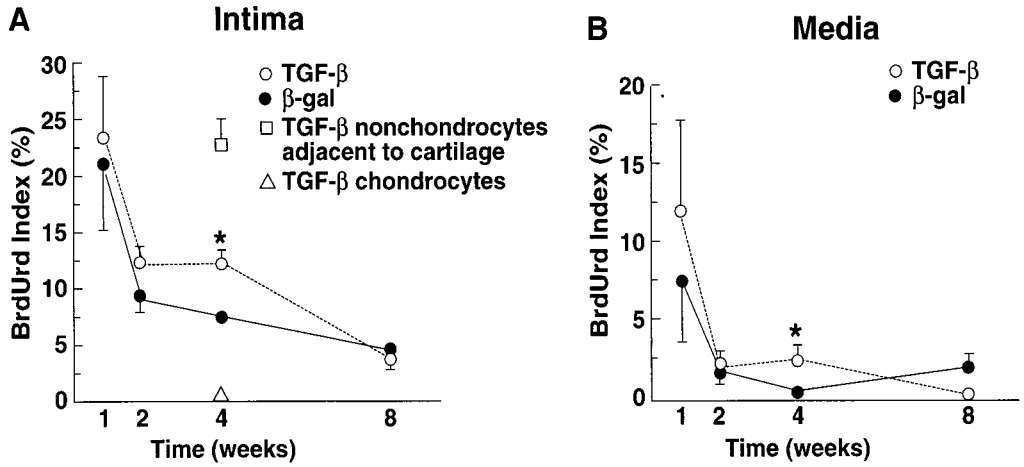

FIG. 3. Cell proliferation of TGF-β and β-gal arteries measured by incorporation of BrdUrd. (*A*) Proliferation in intima. At 4 weeks (but not at other time points), the difference in proliferative indices between TGF-β and β-gal arteries is significant (*, $P < 0.05$). In the TGF-β arteries at 4 weeks, the proliferative index was elevated in nonchondrocytes adjacent to cartilage but was low in chondrocytes. (*B*) Proliferation in media. At 4 weeks (but not at other time points), proliferative indices in the TGF-β1 and β-gal arteries were significantly different (*, $P < 0.05$). Data are mean ± SEM of three to five arteries/group.

virus. By 2 weeks, the proliferative index in the media had returned to background levels (1.6 ± 0.70%), whereas the intimal proliferative index remained elevated (9.0 ± 1.9%). At 1 and 2 weeks after gene delivery, the proliferative indices of TGF-β arteries were similar to those of the β-gal arteries (Fig. 3). At 4 weeks, however, both intimal and medial proliferation were significantly greater in the TGF-β arteries than in the β-gal controls. Intimal proliferation in the TGF-β arteries was increased modestly (1.7-fold) over the relatively high level in the β-gal arteries (12 ± 1.3% versus 7.2 ± 0.55%; $P = 0.011$). The relative increase in medial proliferation of TGF-β1 versus β-gal arteries was greater than in the intima (7-fold; 2.5 ± 0.76% versus 0.35 ± 0.20%; $P = 0.046$).

The chondrocytes (present only in the 4-week TGF-β arteries) virtually never stained for BrdUrd incorporation. The chondrocyte proliferative index was essentially zero (0.46 ± 0.30% in the intima and 0.49 ± 0.30% in the media). These low rates contrast with the overall BrdUrd indices in the intima (12 ± 1.3%) and the media (2.5 ± 0.75%) of the 4-week TGF-β arteries. The proliferating cells in these arteries were concentrated in the intima, overlying the areas of cartilaginous metaplasia (Fig. 3*A* and 4*A*). The proliferative index was significantly higher in the nonchondrocytic cells in these areas than in the cells (entirely nonchondrocytic) in the remainder of the intima (22 ± 1.7% versus 4.4 ± 1.9%; $P = 0.0002$). The BrdUrd data suggest that TGF-β1 expression leads to increased focal vascular cell proliferation, which is confined essentially to the regions of highest TGF-β1 expression (i.e., adjacent to the areas of cartilaginous metaplasia). Cells in these regions that have not been transformed into chondrocytes are the most proliferative.

We hypothesized that apoptotic cell death might explain the dramatic regression of the cellular component of the neointima between 4 and 8 weeks after gene transfer. Sections from 2-, 4-, and 8-week β-gal and TGF-β arteries were stained with the TUNEL technique for evidence of DNA fragmentation (as an indication of apoptosis) (31). We found a surprisingly high prevalence of TUNEL-positive smooth muscle cell nuclei in both the intima and media of all sections examined, as have others using vascular tissue obtained under different conditions (22, 32). To avoid false positivity in our assessment of apoptosis, we considered TUNEL-positive cells to be apoptotic only if they also demonstrated pyknosis, chromatin margination, or chromatin fragmentation. These features were by far most abundant in the 4-week TGF-β arteries, where they were most pronounced in intimal and medial chondrocytes in areas of cartilaginous metaplasia (Fig. 4 *B* and *C*). Approximately 60% of intimal and medial chondrocytes were apoptotic. No similar areas of apoptosis were observed either in the 2- and 8-week TGF-β arteries or in any of the β-gal arteries. Transmission electron microscopy performed on sections taken from these arteries revealed pyknotic, fragmented nuclei with chromatin condensation, features characteristic of apoptosis (not shown). These data indicate that TGF-β1 withdrawal is associated with increased apoptosis and that apoptosis contributes to the neointimal regression.

DISCUSSION

The major findings of this study are: (*i*) endothelium-specific delivery of a constitutively active TGF-β1 cDNA increases

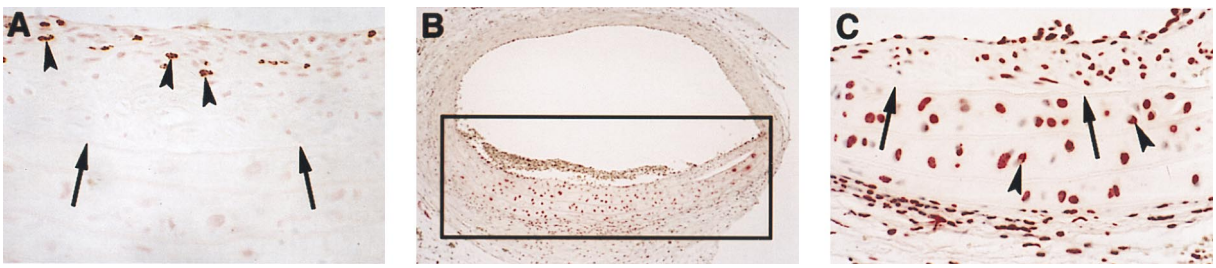

FIG. 4. Proliferation (BrdUrd incorporation) and apoptosis (TUNEL staining) in 4-week TGF-β arteries. (*A*) Proliferating cells (arrowheads and elsewhere) are confined to upper intima. No proliferation is detected in chondrocytic portions of intima or media. (*B*) Low-power view shows TUNEL-positive nuclei (red dots) confined to the area of cartilaginous metaplasia (box). (*C*) High-power view shows widespread pyknosis and occasional chromatin margination (arrowheads). Arrows in *A* and *C* indicate internal elastic lamina. TUNEL stains of β-gal arteries (not shown) appeared essentially as the top (unboxed) area of *B*. Original magnifications: (*A* and *C*) ×65; (*B*) ×20.

arterial wall TGF-β1 expression for at least 4 weeks, potentially due at least in part to up-regulation of endogenous TGF-β1 expression; (*ii*) overexpression of TGF-β1 in an uninjured artery leads to formation of a cellular and matrix-rich neointima that regresses almost completely in association with TGF-β1 withdrawal; (*iii*) apoptosis of intimal cells results in significant changes in arterial morphology, including neointimal regression; (*iv*) vascular smooth muscle cells appear to be capable of transdifferentiation into chondrocytes; and (*v*) cartilaginous metaplasia may result from increased local expression of TGF-β1. Although transfer of a TGF-β1 cDNA to the arterial wall has been reported previously (7), virtually all of the above observations are novel. Our experimental system, in which high levels of TGF-β1 are produced and experimental end points are followed for a more prolonged period of time, has provided additional, unanticipated insights into the potential roles of TGF-β1 in the arterial wall.

The remarkable regression of the complex cellular and matrix-rich arterial lesions between weeks 4 and 8 is intriguing. In animal studies, only the earliest, simplest lesion of experimental atherosclerosis (the fatty streak) is reversible. In nonhuman primates, interventions that promote regression of more advanced arterial lesions deplete the lipid component and have far less impact on the cellular and matrix elements (33, 34). In diseased human arteries, therapies intended to cause lesion regression result in only slight ($<10\%$) increases in luminal diameter (35, 36). Notably, at the 8-week time point in this study (i.e., after lesion regression), the luminal area of the TGF-β arteries was 50% greater than that of the β-gal controls: 0.54 ± 0.023 versus 0.36 ± 0.067 mm^2; $P = 0.062$. The nearly complete resolution of intimal thickening (Fig. 2*H*) and the apparent enlargement of the lumen in the setting of TGF-β1 withdrawal suggest that further definition of the mechanisms of lesion regression in this model may reveal strategies for promoting the regression of intimal lesions. More specifically, our observations suggest that substantial regression of intimal lesions might be achieved by therapeutic strategies that promote vascular cell apoptosis. Apoptotic cells have been identified in the arterial wall (16); however, apoptosis has not been associated previously with such significant alterations in the intimal mass and luminal area of adult arteries.

In addition to the apparent increase in lumen caliber at 8 weeks, we noted other evidence of arterial wall remodeling in the TGF-β arteries. Specifically, the overall cross-sectional area of the TGF-β arteries, measured as the area within the external elastic lamina, was larger than the cross-sectional area of the β-gal arteries at both 4 and 8 weeks after gene transfer: 0.63 ± 0.11 versus 0.46 ± 0.047 mm^2, $P < 0.005$, and 0.70 ± 0.057 versus 0.50 ± 0.122 mm^2, $P < 0.01$, respectively. The increase in vessel size at 4 weeks may result from remodeling to minimize lumen loss resulting from intimal growth, as described in human arteries (37). As the intima of the TGF-β arteries regresses between 4 and 8 weeks, the artery does not appear to constrict or remodel in response, leaving both a larger artery and a larger lumen. We are cautious about these conclusions regarding lumen size and overall cross-sectional area. Our measurements, made by blinded observers using sections from perfusion-fixed arteries, revealed consistent and significant differences between the TGF-β and β-gal arteries. Nevertheless, lumen diameter and arterial size are best measured by casting techniques, and our results should be confirmed in a larger, prospective study in which these techniques are applied.

One of the most notable and unanticipated findings was the occurrence of cartilaginous metaplasia in the TGF-β arteries. Members of the TGF-β family long have been associated with chondrogenesis in the skeletal system but not in cardiovascular organs (38, 39). For example, injection of TGF-β1 in the subperiosteum of a rat femur induces chondrogenesis, probably by inducing differentiation of precursor cells (40). Interestingly, cessation of TGF-β1 injection in this model leads to ossification, likely because of the effects of a factor that is present in the periosteum but absent in the artery wall. Cartilaginous metaplasia in the cardiovascular system (not previously associated with TGF-β1 expression) is found in human heart valves and in the aortas both of atherosclerotic mice and of mice lacking matrix GLA protein (28, 41, 42). In addition, we have observed cartilaginous metaplasia in an atherosclerotic lesion in the aorta of a Watanabe heritable hyperlipidemic rabbit (R.V., unpublished observation).

Cartilaginous metaplasia in the cardiovascular system as well as the related process of arterial calcification have been attributed to the activity of a small population of relatively undifferentiated vascular cells (28, 42, 43). We cannot exclude that the chondrocytes observed in this study arose from a very small number of chondrocytic precursors that reside within the vascular wall and that expand dramatically in response to TGF-β1. Alternatively, the chondrocytes might be derived from blood-born sources. However, we believe that these explanations are unlikely, because they both postulate a complete disappearance of normal vascular smooth muscle cells and their replacement by blood-born or rapidly dividing cells. However, in examining both proliferation and apoptosis at time points before the appearance of the chondrocytes in the artery wall (Fig. 3 and data not shown) we did not find significant cell death or proliferative bursts in the media of TGF-β arteries. Indeed, it appears that most if not all of the rat carotid artery smooth muscle cells are capable of transdifferentiation into a chondrocytic phenotype (see Fig. 2*D,* in which these cells populate the entire vascular media). This observation appears to contradict previous statements about the inability of differentiated vascular smooth muscle cells to transdifferentiate to other cell types (44) and suggests an unexpected plasticity of the adult vascular smooth muscle cell phenotype. This plasticity, rather than the existence of small numbers of undifferentiated cells, may provide a substrate for growth factors that promote both cartilaginous metaplasia and vascular calcification.

In previous studies of the effect of TGF-β1 protein and gene delivery on the arterial wall, only lesion growth was reported, never regression (6, 7, 9, 45). Notably, all of these previous studies were of a shorter duration than ours ($\leq$3 weeks); regression might have occurred at later time points. In addition, generation of the distinctive phenotype we observed in the 4 week TGF-β arteries appears dependent on achievement of high levels of TGF-β1 protein. Arteries transduced with a lower concentration of virus (1×10^{10} pfu/ml) secreted only 1/5 as much active and total TGF-β1 in explant cultures (i.e., 24 and 435 pg/24 hr, respectively). These arteries did not develop neointimal thickening or cartilaginous metaplasia. Similar to our data, a reversible proliferative and fibrotic reaction occurs after injection of TGF-β1 protein into mouse subcutaneous tissues (46). In this model, however, chondrogenesis was not observed and the presence of apoptosis has not been investigated.

In summary, our data suggest new and complex roles for TGF-β1 in the arterial wall. Alterations in the local abundance of TGF-β1 appear to promote vascular cell transdifferentiation, vascular wall remodeling, arterial lesion growth, and lesion regression. These observations may begin to reconcile the two divergent views of the role of TGF-β1 in arterial disease, which envision TGF-β1 as either promoting (5–7, 9, 45) or inhibiting (10–13) lesion development. In the present study, modulation of TGF-β1 expression was associated both with proliferation and apoptosis. Thus, TGF-β1 appears capable of playing a role in both lesion growth and lesion regression. Accordingly, the relatively low levels of plasma TGF-β1 in patients with severe coronary artery disease (13) may not be paradoxical but may instead reflect a systemic

deficiency in TGF-β1 production that precludes a role for TGF-β1 in limiting lesion growth through the triggering of vascular cell apoptosis. Additional studies in which local patterns of arterial wall TGF-β1 expression are defined and manipulated should further clarify the relationship between this pleiotropic growth factor and the biology of the arterial intima.

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