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Evidence for Apoptosis in Human Atherogenesis and in a Rat Vascular Injury Model

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Apoptosis is a physiological cell death process important for normal development and involved in many pathological conditions. In atherosclerosis, pathological accumulation of cells in the intima has been attributed to the migration and proliferation of smooth muscle cells, macrophages, and lymphocytes. In this report, we explored the possibility that apoptosis may also contribute to the pathogenesis of this disease. We examined 35 human atherosclerotic lesion samples and identified a substantial number of cells undergoing apoptosis in 25 of the samples. Furthermore, in a rat vascular injury model, apoptotic cells were specifically identified in the neointima. The presence of apoptotic cells was demonstrated by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling, nuclear staining with propidium iodide, and electron microscopy. Immunostaining with cell-type-specific markers and subsequent terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling analysis on the same sample revealed that the majority of the apoptotic cells were modulated smooth muscle cells as well as macrophages. These results indicate that apoptosis occurs in cells of the injured blood vessel as well as the advanced atherosclerotic lesion and that physiological cell death may have an important role in determining the course of atherogenesis. (Am J Pathol 1995, 147:267–277)

It is now well established that apoptosis is a vital aspect of normal development and dysregulated apoptosis may have an important role in the pathogenesis and progression of a variety of diseases including cancer, acquired immune deficiency syndrome, heart disease, and neurological disorders.[1–9] The process of apoptosis differs from accidental cell death in that it proceeds via a series of discrete, morphologically distinguishable steps.[6,7,10] Although the mechanism of apoptosis is still not well understood, a genetic basis for this process is now apparent, indicating that apoptosis is an important aspect of general cell behavior.[10–12]

In atherosclerosis, excessive accumulation of cells in the intima is believed to be the major cause of disease progression.[13,14] This accumulation has been attributed to increased migration and/or proliferation of smooth muscle cells (SMCs), monocytes/macrophages, and T lymphocytes.[13,15] We have observed that within the sclerotic region of the advanced human atheromatous plaque there was a low density of cells with little appearance of cellular debris. The process by which a cellular lesion evolves into an acellular, sclerotic lesion is poorly understood but presumably involves extracellular matrix synthesis and cell deletion. These observations propelled us to examine the possibility that vascular disease progression could potentially be influenced by the apoptotic process. In this study we examined human atherectomy samples for the presence of apoptotic cells using several established biochemical assays and electron microscopy. In addition, we utilized a well established rat vascular injury model to determine whether apoptosis is an aspect of cellular response to injury in the blood vessel. Our results suggest that apoptosis has a significant role in the early vascular injury response and in influencing progression of the human atherosclerotic disease.

Materials and Methods

Tissue Collection and Preparation

Atherectomy specimens were flushed out of the instrument chamber with saline solution and fixed im-

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mediately with 4% buffered formaldehyde. For storage and transportation, the specimens were immersed in 70% ethanol. After dehydration, the tissue fragments were embedded in paraffin that allowed sectioning in the plane of their largest dimension (usually longitudinal). Routine stains included hematoxylin and eosin (H&E), trichrome-fibrin, and Van Gieson-elastin. For animal studies, balloon injury was performed on rat iliac arteries according to established procedures.[16,17] At 9, 14, and 28 days after injury, the entire vasculature was perfused with 4% buffered formaldehyde via the ascending aorta, and multiple vascular rings were embedded and stained as described above.

Immunohistochemistry

The indirect avidin-biotin horseradish peroxidase or alkaline phosphatase visualization methods (ABC standard and elite, Vector Red, Vector Laboratories, Burlingame, CA) were used. Primary antibodies utilized included HAM56 (Dako Corp., Carpinteria, CA) for the identification of monocytes/macrophages, anti-α-smooth muscle (α-SM) actin (Sigma Chemical Co., St. Louis, MO) for the detection of SMCs, anti-proliferating cell nuclear antigen (anti-PCNA; Dako) for nonquiescent cells, anti-CD45RO (Dako) for mature activated T lymphocytes and granulocytes, anti-CD20 (Dako) for B lymphocytes, and anti-CD15 (BPS, Indianapolis, IN)for neutrophils.

Apoptosis Assays

Terminal Deoxynucleotidyl Transferase (TdT)-Mediated dUTP-Biotin Nick End Labeling (TUNEL) Assay

Paraffin sections were dewaxed, rehydrated, treated with proteinase K (20 μg/ml), and blocked for endogenous peroxidase activity with 3% H_2O_2. Subsequent end-labeling with TdT (0.3 U/μl) in TdT buffer together with 2 μmol/L biotin 16-UTP was carried out for 1 hour at 37°C in a humidifying chamber. After end-labeling, sections were washed in phosphate-buffered saline (PBS), and incubated with avidin and biotin-horseradish peroxidase complex, rinsed in PBS, and stained with diaminobenzidine.[18,19] Apoptotic nuclei were identified by the presence of dark brown staining. For colocalization of apoptotic cells with cell-type-specific markers, the samples were first immunostained with the monoclonal antibodies (HAM56, α-SM actin, or CD45RO) with the horseradish peroxidase substrates for color development. The TUNEL assay was then performed on the same sections with the alkaline phosphatase substrate (Vector Red™) for color development. This dye is fluorescent red when analyzed at the rhodamine excitation wavelength.

Propidium Iodide (PI) Assay

Paraffin-embedded tissues were dewaxed, rehydrated, and stained with PI (4 μg/ml) solution in minimal essential medium/HEPES containing 100 μg/ml DNAse-free RNAse A for 30 minutes at 37°C.[20] Slides were washed three times in PBS, dehydrated in gradient alcohol, then in xylenes, mounted on coverslips, and examined under the fluorescent microscope. Condensed and/or fragmented apoptotic nuclei were identified by the presence of bright yellow/orange fluorescence.

Quantitation

With a Zeiss light microscope, a CCD video camera, and Image-1 image analysis software, selected tissue areas within the atherectomy specimens were traced manually, and the positive and negative nuclei within these areas were counted automatically according to a threshold of contrast that was determined visually once for the entire series. From the atherectomy specimens, comparable areas were selected by cell type and cell number, distinguishing (1) macrophage/monocyte-enriched areas displaying predominantly HAM56-positive cells, foam cells, other inflammatory cells, and blood vessels (Figure 1a); (2) myxomatous areas containing predominantly α-SM actin-positive cells with a characteristic, stellate, altered phenotype and abundant lucent matrix (Figure 2a); and (3) sclerotic areas of low cellular density enriched with dense, sclera-like collagenous matrix (Figure 2g). All other tissue types, especially outright necrotic, atheromatous, or thrombotic areas, as well as areas with intermediate cellularity that could not be classified unequivocally, were omitted from the measurements.

In the ballooned rat carotid arteries, the entire media and the entire neointima were traced manually and assessed for positive and negative nuclei automatically using the Image-1 software. The tunica media, containing only an occasional proliferating or apoptotic cell at the time points chosen, served as a negative control; human tonsil tissue, neonatal brain sections, and DNAase I treated vascular tissues served as additional controls.

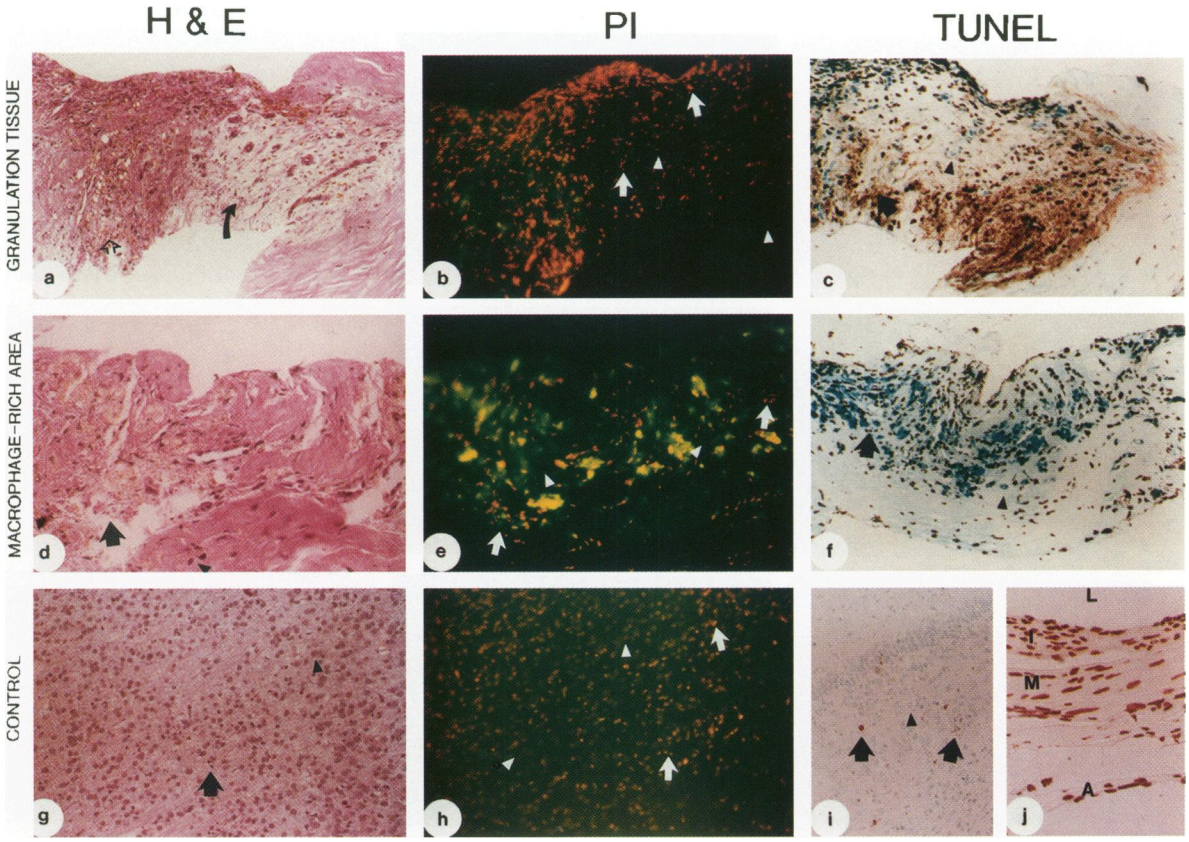

Figure 1. *Detection of apoptosis in human atherosclerotic lesions. Serial sections from human lesions* (a–f) *show extensive apoptosis by both PI and TUNEL.* a: *Typical appearance of a granulation tissue with a macrophage/foam-cell-rich area* (*open arrow*) *and blood vessel-enriched area* (*curved arrow*). *Staining with PI* (b *and* e) *and TUNEL* (c *and* f) *demonstrates the presence of fragmented nuclei* (*arrows*) *and normal nuclei* (*arrowheads*). *Apoptotic nuclei are identified by the presence of bright orange fluorescence in PI assays and dark brown staining in TUNEL assays. Control panels of rabbit newborn brain* (g–i) *demonstrate apoptotic neurons* (*arrows*) *and normal neurons* (*arrowheads*). *TUNEL assay on balloon-injured rat carotid artery* (j) *shows that DNAse I treatment causes positive TUNEL staining in the normally negative medial layer. L, lumen; I, intima; M, media; A, adventitia. Magnification,* ×*60.*

Electron Microscopy

Human tissues were immersion fixed in 1% formaldehyde and 2.5% glutaraldehyde and rinsed with PBS. The tissues were rinsed and stored in PBS containing 5% sucrose, postfixed in 1% aqueous OsO_4, and stained *en bloc* with 0.2% uranyl acetate. After dehydration, embedding in epon, sectioning and staining with uranyl acetate and lead citrate, the tissues were examined with a Phillips CM 12 transmission electron microscope.

Results

Apoptosis in Advanced Human Coronary Atherosclerotic Lesions

Atherosclerotic lesions from 35 patients (age, 42 to 83 years; mean, 63 years) were obtained by directional atherectomy of critically narrowed coronary arteries and analyzed for the presence of apoptosis. Some of the distinguishing features of apoptosis are chromatin condensation and internucleosomal DNA fragmentation.[6,21,22] Results were essentially the same when we examined the atherosclerotic lesions by two different established techniques that detect compacted and fragmented DNA *in situ*: TUNEL,[18,19] and nuclear staining with PI.[20,23,24] TUNEL and PI staining allow the identification of a small population of cells undergoing apoptosis and the correlation of apoptotic events with specific cell types or morphologically distinct regions of a tissue. In addition, these *in situ* assays allow the detection of apoptotic cells before the DNA is extensively cleaved.[18]

A survey of these 35 human atherosclerotic samples revealed that 25 samples contained a substantial number of apoptotic cells. Because atherosclerotic lesions are extremely heterogeneous in nature, we selected for comparative analysis only nonnecrotic and nonthrombotic cellular regions that

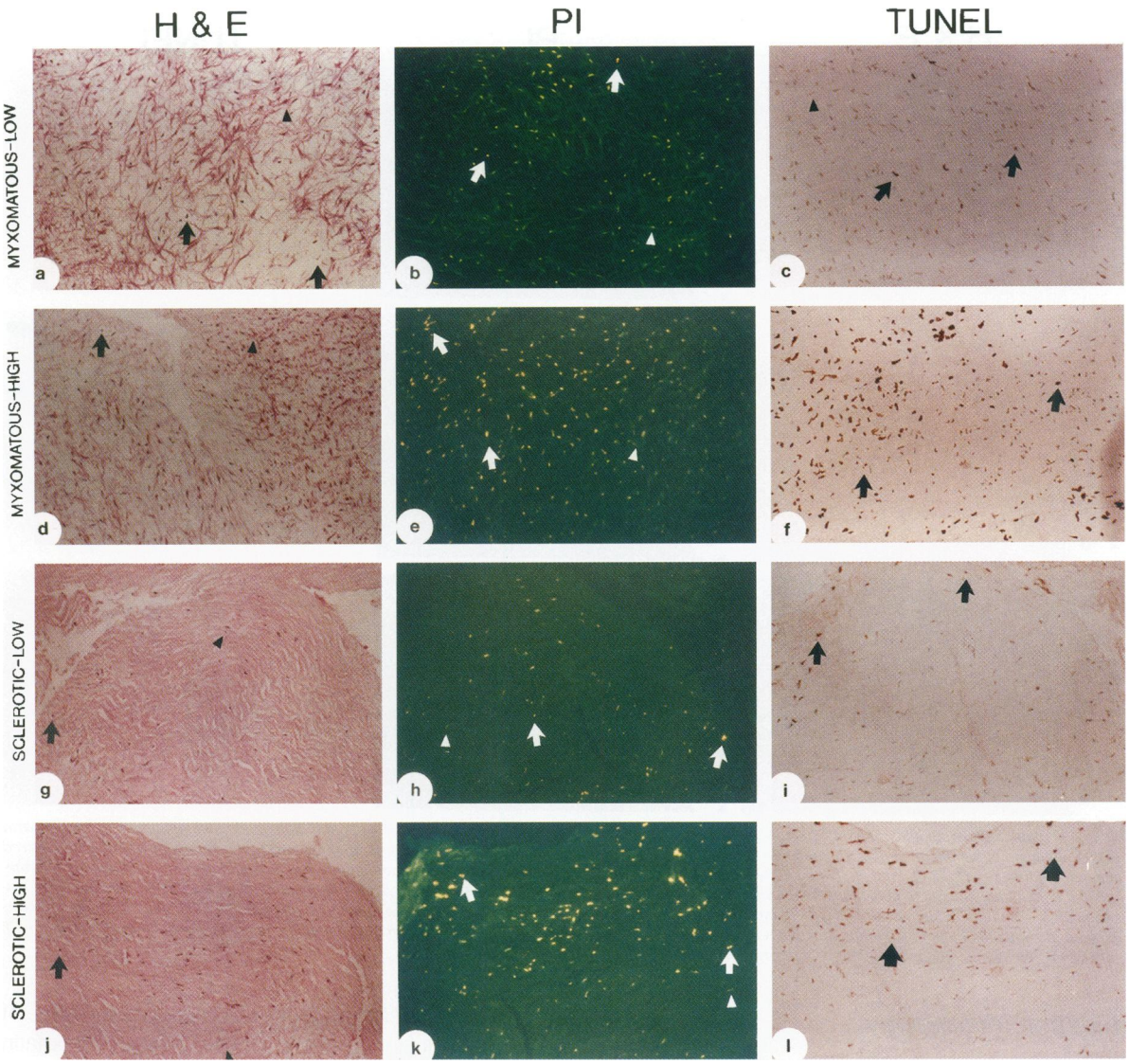

Figure 2. *Variability in the number of apoptotic cells in atherosclerotic lesions. Human lesions from myxomatous and sclerotic regions with high and low levels of apoptosis are shown. Serial sections were compared using both PI and TUNEL. Variability in the myxomatous lesions was demonstrated by two areas: one showing a low number of apoptotic cells in the region* (a–c) *and the other showing a high number of apoptotic cells* (d–f). *Arrows indicate selected apoptotic cells and arrowheads indicate the non-apoptotic cells for comparison. Similarly, variation in the number of apoptotic cells from sclerotic lesions was demonstrated in the following panels:* g–i, *low, and* j–l, *high. Magnification, ×60.*

could be identified unequivocally by morphological and immunohistochemical criteria. These include (1) a macrophage-enriched area, (2) a stellate-shaped SMC-enriched region defined as the myxomatous area, and (3) a collagen-enriched, sclerotic area (Figures 1 and 2). The macrophage-enriched and myxomatous area each represented approximately 10% of the total lesion whereas the sclerotic area was approximately 50%. We found that apoptosis was most prominent in the macrophage-enriched area with lower levels in the other two regions and that the amount of apoptosis varied considerably even within the same lesion type (Figures 1 and 2). Quantitative analysis revealed that the sclerotic area, which represents approximately one-half of the lesion area, was 10% positive for apoptotic cells, whereas the myxomatous and the macrophage-enriched regions were approximately 15 and 40% positive, respectively (Figure 3a). In contrast, only a few apoptotic cells were detected in the underlying normal smooth muscle layer, in uninjured rat blood vessels, as well as in 10 other human atherectomy samples (Figure 3 and results not shown). We also examined the degree of proliferation in these atherosclerotic specimens as assayed by immunostaining for PCNA.[25] From 10 samples that were positive for apoptosis, only 3

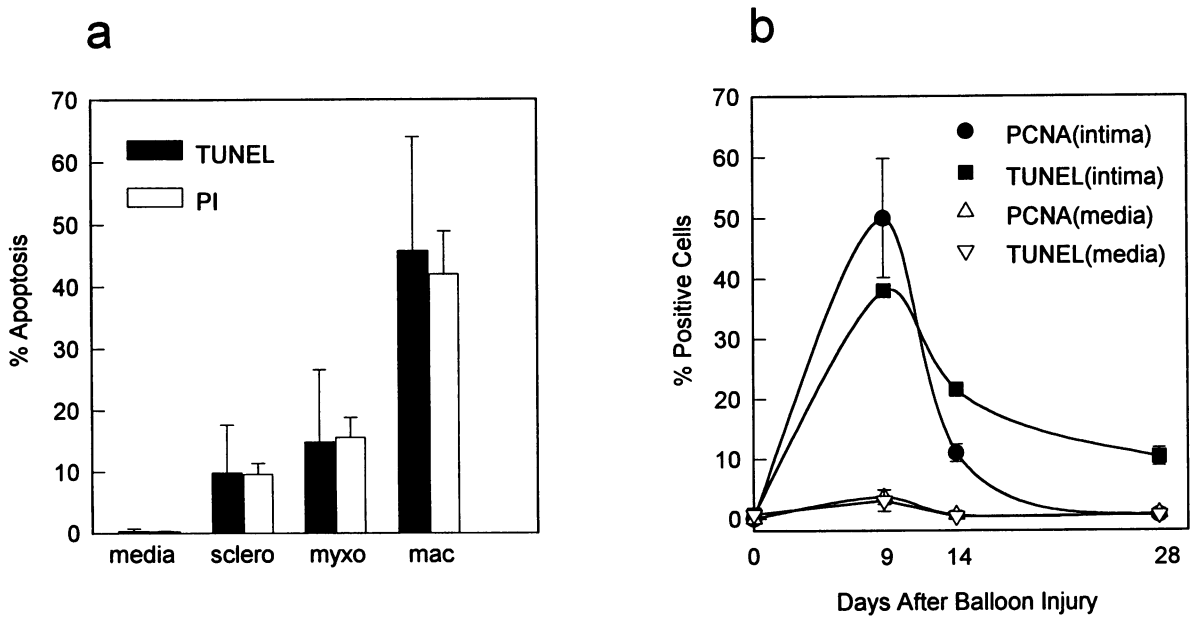

Figure 3. *Quantitation of apoptosis in human coronary atherosclerosis and in a rat injury model.* a: *The levels of apoptosis in the selected human lesions were determined by both TUNEL and PI. Percentage of apoptotic nuclei was determined by counting the number of labeled cells divided by total cells, using the microscope-based video image analysis system (Image 1). The normal media, sclerotic, myxomatous, and macrophage-enriched regions are 11, 58, 12, and 13% of the total area, respectively. Standard error bars are shown (n = 25 human lesions). Immunohistochemical analyses of lesions with antibody to α-SM actin identified most of the cells from the normal media layer and myxomatous and sclerotic lesions as smooth muscle in origin. The macrophage-rich region was identified by monoclonal antibody HAM56.* b: *Analysis of rat balloon-injured arteries for apoptosis (TUNEL) and cellular proliferation (PCNA); n = 3 sections from animals at each time point. The PCNA and TUNEL analyses were always performed together with human tonsil and neonatal brain sections, respectively, as positive controls.*

samples contained a few clusters of cells that were PCNA positive. These results indicate that, within the selected, cellular regions of many of the atherosclerotic lesions, there is marked apoptosis when compared with the underlying normal SMC layer and that this can occur virtually in the absence of cellular proliferation.

Smooth Muscle Cells and Macrophages Colocalized with Cells Undergoing Apoptosis

SMCs, T and B lymphocytes, and macrophages are the major cellular components of human atherosclerotic lesions.[13,14] To begin to understand the possible implication of apoptotic cell death in vascular lesions, we next attempted to determine the cell types that are undergoing apoptosis. Given the positive correlation of apoptosis with macrophage-enriched areas within the lesions, we first performed immunohistochemistry using a monoclonal antibody specific for human monocytes/macrophages (HAM56) and subsequently TUNEL on the same tissue section (10 samples). Consistent with the previous result, a majority of the HAM56-positive cells were also TUNEL positive. However, as illustrated in Figure 4, a and b, both apoptotic and non-apoptotic macrophages could be observed. Similar analyses were also performed with a monoclonal antibody directed against α-SM actin, a marker for vascular SMCs, and TUNEL. As shown in Figure 4, c and d, many TUNEL-positive cells also stained intensely for α-SM actin, indicating that SMCs are one of the major cell types undergoing apoptosis within atherosclerotic lesions. Analyses for mature activated T lymphocytes and granulocytes (CD45RO), predominant types of B lymphocytes (CD20), and neutrophils (CD15 and peroxidase activity) were also carried out. We were unable to detect neutrophils within the lesions but were able to identify a small number of non-apoptotic T and B lymphocytes (Figure 4, e and f, and results not shown). These results indicate that phenotypically altered SMCs as well as macrophages make up the bulk of the apoptotic cells.

Apoptosis in an Animal Model of Vascular Injury

Having demonstrated the presence of apoptotic SMCs in advanced human atherosclerotic lesions, we asked whether apoptosis is restricted to the late stages of lesion progression or whether it is also an integral aspect of earlier cellular responses after vascular injury. To address this, a well established rat

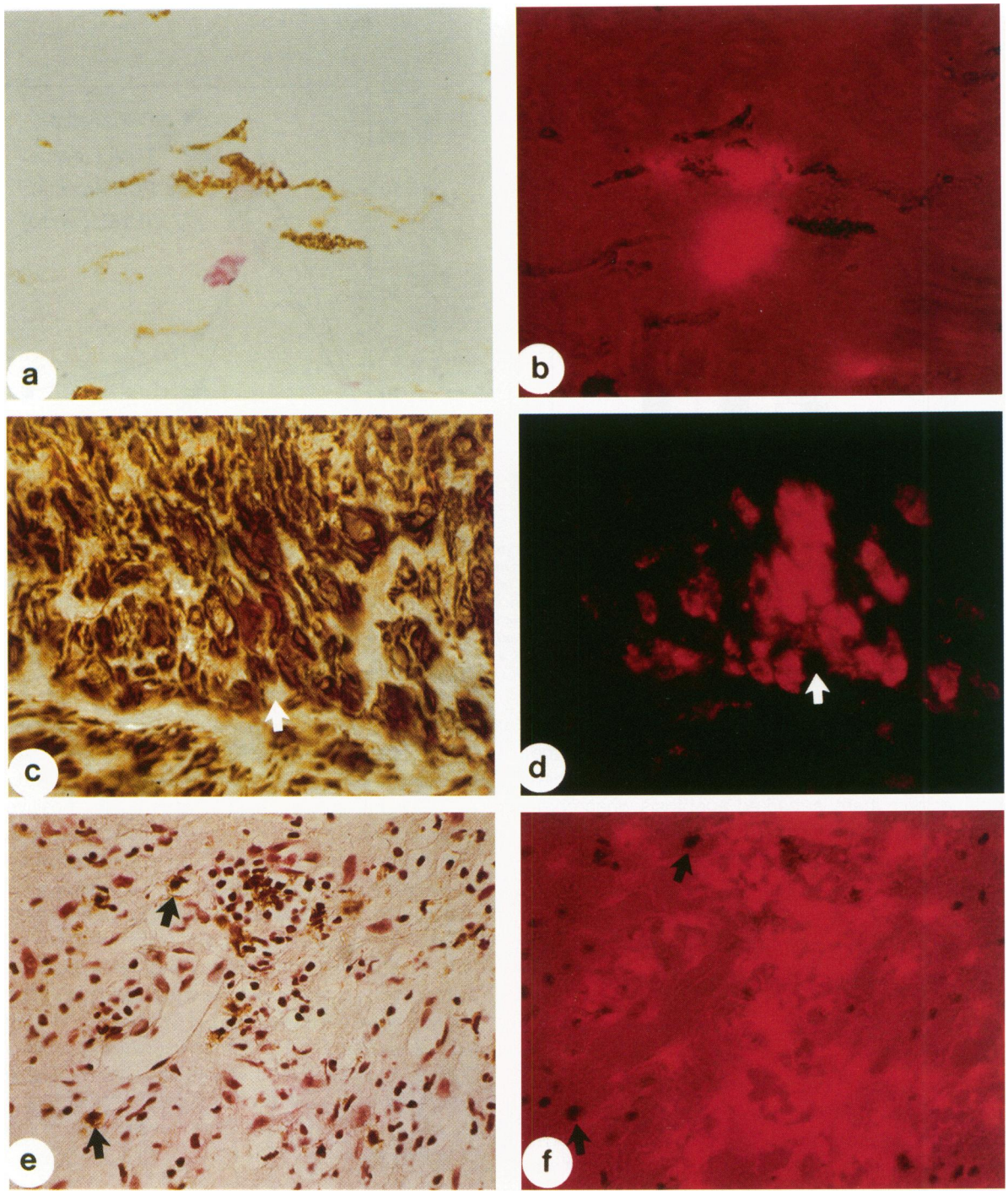

Figure 4. *Smooth muscle cells and macrophages colocalized with apoptotic cells.* a: *Human coronary atherectomy lesions were first immunostained with the monoclonal antibody against a human macrophage-specific marker (HAM 56) using the horseradish peroxidase substrates for color development (brown).* b: *The TUNEL assay was then performed on the same sections using the alkaline phosphatase substrate (Vector Red) for color development. This red dye is fluorescent at the rhodamine excitation wavelength, and analysis under the fluorescent scope revealed that some macrophages (brown) colocalized with apoptotic cells (red). Similar analyses with α-SM actin antibody and TUNEL (*c *and* d*) in a region that contained mostly SMCs revealed the presence of apoptotic cells (arrow). Immunohistochemistry using the antibody against the majority of T lymphocytes (CD45RO) demonstrated non-apoptotic T lymphocytes (*e *and* f*, arrows) in the atherosclerotic lesions. Magnification, ×120.*

vascular injury model was used.[16,17] In this model, injury of the artery with a balloon catheter induces maximal SMC proliferation within the 1st week and proliferation declines to basal levels by 8 weeks. Furthermore, total SMC number is maximal at 2 weeks despite continued proliferation.[16] We performed

TUNEL and PI at three time points and found that, by 9 days after injury, approximately 40% of the cells in the neointima were undergoing apoptosis (Figure 3b). The number of apoptotic cells declined to approximately 20% by 14 days and was still considerably above basal level (10%) by 28 days after the initial injury (Figure 3b). In contrast, the number of cells undergoing apoptosis in the media was very low, with detectable levels only at 9 days after injury (3.0%). The peak of both apoptosis and proliferation was maximal at day 9 and the number of TUNEL-positive cells was approximately 75% of the PCNA-positive cells at this time (Figure 3b). However, in the late period after injury, the number of TUNEL-positive cells was considerably greater than the PCNA-positive cells (Figure 3b). Our analysis indicates that injury to the vessel wall initiates a profound apoptotic response in neointimal SMCs that generally parallels SMC proliferation.

Electron Microscopic Demonstration of the Presence of Apoptotic Smooth Muscle Cells

Apoptosis has been defined by a number of ultrastructural criteria, including nuclear and cytoplasmic condensation, membrane budding, cell fragmentation, and phagocytosis of the apoptotic bodies.[10,26,27] To provide morphological evidence that SMCs undergo apoptosis, we performed electron microscopic studies on human atherectomy samples. We were able to identify a number of cells containing dense cytoplasmic actin fibers that showed different stages of typical apoptotic deteriorations, including early chromatin condensation (Figure 5, a, b, e, and f), membrane budding (Figure 5, c and d), and fragmentation of cells into apoptotic bodies (Figure 5c). Additionally, normal cells with open chromatin were observed to engulf neighboring apoptotic bodies and some of these apoptotic bodies were positive for α-SM actin as demonstrated by immunogold electron microscopy (Figure 5f and data not shown). These results further demonstrate that intimal SMCs are undergoing apoptosis in atherosclerotic lesions.

Discussion

It is well documented that injury to the vessel wall triggers excessive cell migration and proliferation and these events are believed to be a major cause of vascular diseases.[10,13] However, the accumulation of cells in the intima is theoretically the sum of cell migration and cell growth as well as cell death, and alterations in any of these events could affect the disease process. We investigated the possibility that apoptosis has a role in the development of the human atheromatous lesion and detected levels of apoptosis that ranged from 10% in sclerotic regions to 40% in macrophage-rich regions. It should be emphasized that a significant level of apoptosis was observed in just 25 of the 35 human samples; the remaining 10 samples contained only a few apoptotic cells. Because the atherotome removes only a portion of the obstructing lesion, these results do not allow us to draw conclusions about the apoptotic activity of the entire plaque in these patients. However, given that we quantitated apoptotic cells in plaque areas with comparable morphology and cellularity, the difference in the level of apoptosis between samples is likely a significant one. The presence of apoptosis was demonstrated by several biochemical and morphological criteria, including cell shrinkage, chromatin condensation, fragmentation of DNA, budding of cytosolic and nuclear components into membrane-bound apoptotic bodies, and phagocytosis by neighboring cells. These features differ significantly from other forms of cell death in which swelling of cytoplasmic organelles and bursting of the cytoplasmic and nuclear materials are typically seen.[10] Overall, these results indicate that apoptosis may be an important component of atherosclerotic lesion progression.

The level of apoptosis in the positive lesions was surprisingly high. From the control analysis of rabbit neonatal brain (Figure 1, h and i) and the electron microscopy studies, we are confident that the TUNEL and PI assays are identifying apoptotic cells. Furthermore, as shown in Figure 3a, these two distinct assays independently identified a very similar level of apoptosis within the lesions. However, the issue of the true percentage of cells undergoing apoptosis *in vivo* in the atherosclerotic plaque is more difficult. For example, if additional cell death occurred after tissue removal there would be an overestimation of the level of apoptosis. Although we cannot rule out this possibility, it has been minimized because the tissues were fixed immediately after surgical removal. Nevertheless, given the complexity of the system, additional studies are warranted to firmly establish the level of apoptosis in the human atheromatous lesion. Regardless of the absolute value of apoptosis, it is considerably higher than the level of cell proliferation in these lesion samples. We are thus presented with the interesting dilemma that these lesions should be decreasing in size. Indeed, the advanced human lesion often contains sclerotic regions with a low density of cells; an observation that prompted us to examine

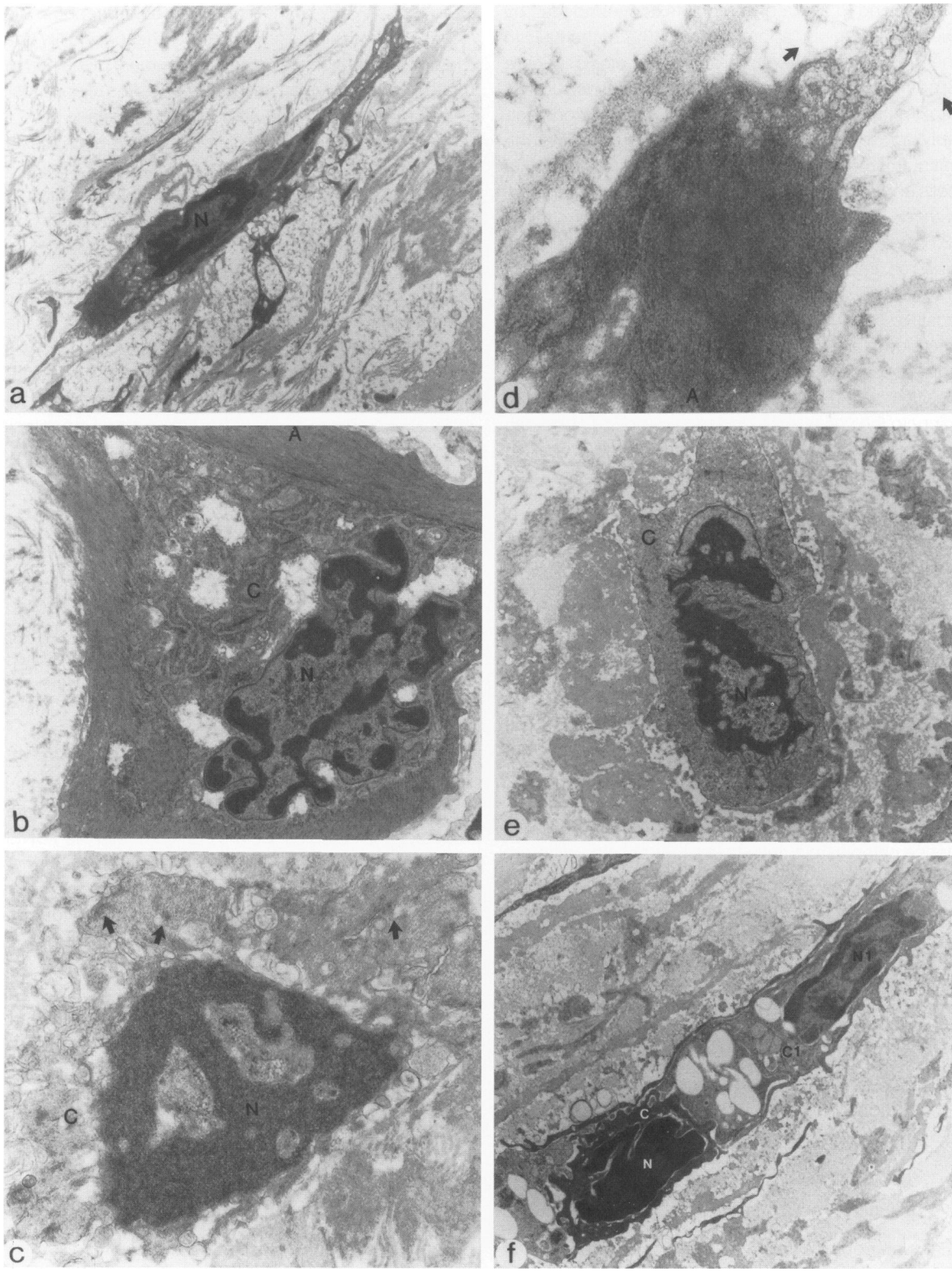

Figure 5. *Ultrastructure of apoptotic cells in human atherosclerotic lesions.* a: *A spindle-shaped SMC with pyknotic nucleus as well as vacuolated and condensed cytoplasm, characteristics of early apoptotic cell; magnification, ×6300.* b: *An apoptotic SMC with cytoplasmic actin fibers and pyknotic nucleus; ×10,000.* c: *A SMC in the late phase of apoptosis with condensed chromatin and fragmented cytoplasm. Arrows indicate the actin fibers; ×22,000.* d: *Membrane budding (arrows) in an apoptotic, actin-rich SMC before fragmentation; ×35,000.* e: *Fragmentation of nucleus into two condensed bodies; ×10,000.* f: *Comparison of an electron-dense apoptotic nucleus* (N) *and cytoplasm* (C) *with an adjacent macrophage/foam cell, possibly in the process of phagocytosis, with open chromatin* (N_1); *×6300.*

for the presence of apoptosis. However, these lesion regions are rich in fibrillar collagen and other extracellular matrix proteins that may prevent a regression in lesion size.[28–32] Finally, it should be emphasized that there currently is no information on the rate of apoptosis as well as on the ability of surrounding cells to phagocytize the apoptotic cells in the human atherosclerotic plaque. These parameters are vital for a true understanding of the role of apoptosis in the progression of this disease.

What role can apoptosis play in the pathogenesis of atherosclerosis? One possibility is that when the degree of apoptosis and phagocytosis of apoptotic bodies is well regulated, it may contribute toward vascular wound regression. Evidence supporting a role for apoptosis in normal wound repair has recently been reported in a rat skin incision model.[33] In this model, after the initial injury, there is a rapid burst of cell proliferation with very few cells undergoing apoptosis. However, in the wound regression phase between days 20 and 35, a high level of apoptosis in myofibroblasts was observed within the granulation tissue and this process was postulated to contribute toward wound regression.[33] Atherosclerotic lesions that contained a high level of apoptotic cells, such as the areas enriched in macrophages and, as illustrated in Figure 1, a-c, within granulation tissues may also represent regions of active vascular wound remodeling. Thus, it is possible that apoptosis is a part of normal vascular wound healing whereas dysregulated apoptosis and/or inefficient removal of apoptotic bodies may contribute to the progression of the atherosclerotic plaque and increase the severity of the disease. For example, excessive apoptosis without appropriate phagocytosis may contribute toward additional macrophage recruitment and secretion of inflammatory cytokines. This may, in turn, induce further rounds of episodic cellular migration, proliferation,and other disease-complicating factors such as the release of oxidized lipids that exacerbate the severity of atherosclerotic lesions.

The events that trigger apoptosis within atherosclerotic lesions are currently unknown. We have found that the concentration of apoptotic cells is highest in regions enriched in macrophages and it is possible that within these regions macrophages are initiating and/or propagating these apoptotic events. For example, it is known that macrophages secrete tumor necrosis factor-α, a factor that induces apoptosis in a variety of cells.[15,34] Additionally, macrophages are also capable of generating and internalizing oxidized low density lipoprotein.[35–38] As there is evidence that apoptosis may be triggered via an oxidation-mediated pathway,[39,40] macrophages could trigger apoptosis by the generation of reactive oxygen species or directly by the production of oxidized low density lipoprotein.[41] However, we have also identified apoptosis in other regions that consist mostly of SMCs (eg, myxomatous regions) with minimal macrophage infiltration. In these regions, it is unlikely that apoptosis is triggered by macrophages, and presumably other, unidentified factors contribute toward this process.

The detection of apoptosis in the rat balloon catheter injury model may provide additional insight into the mechanism of this physiological cell death process in vascular SMCs. In this animal model, the contribution of inflammatory mediators such as macrophages and T lymphocytes is minimal and the growth factors responsible for part of the proliferation and migration events have been clearly defined.[42,43] The time course of apoptosis roughly parallels cell proliferation after vascular injury, indicating that there may be a linkage between these events. It is possible that depending on the degree or type of injury-mediated stimulation, SMCs will undergo cell division or apoptosis. This possibility is consistent with *in vitro* studies implicating the growth control molecules c-*myc* and p53 in the apoptotic pathway.[44–46] In addition, it is tempting to postulate that SMC apoptosis could explain the previous finding that intimal thickening remained constant or declined in the late phase of injury despite the presence of continued proliferation.[16,17] However, it should be cautioned that, until information on the rate of apoptosis in the injured blood vessel becomes available, such a possibility remains highly speculative. Finally, it is intriguing that spontaneous death of intimal and medial SMCs was observed during normal rat postnatal development of the vasculature over 20 years ago.[47,48] Indeed, a recent study of arterial remodeling in the neonatal lamb found that apoptoic events contributed toward this postnatal remodeling process.[49] It is possible that apoptosis is an integral aspect of the normal developmental program of the blood vessel and that injury in the adult vessel results in the recapitulation of such a program.

We have demonstrated that apoptosis is present at significant but highly variable levels in human atherosclerotic lesions and that the major cell types undergoing apoptosis are apparently SMCs and macrophages. This strongly suggests that, in addition to cell migration and proliferation, apoptotic cell death can also influence the progression of atherosclerosis. The identification of factors that influence the level of apoptosis in the human atherosclerotic lesion should

contribute toward a better understanding of vascular disease progression.

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