

# Tunica Adventitia of the Aorta is an Active Vascular Compartment

Julius A. Ogeng'o, Phillip Maseghe, Kevin Ongeti, Moses Obimbo, Beda Olabu

Correspondence to Dr. Phillip Maseghe Department of Human Anatomy, University of Nairobi P.O. Box 00100 – 30197 Nairobi, Kenya. **e-mail: jogengo@uonbi.ac.ke**

## ABSTRACT

The tunica adventitia has previously been regarded as a passive connective tissue covering that offers only nutritive and physical support to the arterial wall. Recently, however, emphasis has been given to its role in atherosclerosis. Although the normal structure may bear the anatomical basis of these functions, microscopic anatomy of the tunica adventitia in normal arteries is seldom reported. These data are important in understanding disease process and potential areas of intervention. The goat is a suitable model for studying cardiovascular disease and the aorta is frequently afflicted by atherosclerosis. This study, therefore, aimed at describing the structure of tunica adventitia of normal aorta in goat. Materials for the study were obtained from abdominal aorta of 6 healthy young adult male goats (*capra hircus*) age range 12 – 24 months. Fresh specimens from euthanized animals were fixed in 3% phosphate buffered glutaraldehyde, post fixed in 1% phosphate buffered osmium tetroxide then embedded in durcupan. Ultrathin sections were stained with uranyl acetate counterstained with lead citrate and examined with electron microscope. Some specimens were processed routinely for paraffin embedding and sectioning. They were stained with Mason's Trichrome and Weigert elastic/Van Gieson stains. The tunica adventitia was fibroelastic with numerous capillaries, arterioles and multiple cell types. The cells were active fibroblasts, phagocytic, perivascular and endothelial cells embedded in the fibrous stroma. These findings suggest that the tunica adventitia of the goat aorta is a metabolically active vascular compartment. These features namely microvasculature and multiple cell populations probably enable it to maintain structural and functional integrity and appropriately respond to vascular injury.

Key words: Tunica Adventitia, cells, Capillaries, Arterioles, Atherosclerosis

## INTRODUCTION

Tunica adventitia of arteries has previously been regarded as a passive compartment involved only in physical and nutritive support of the vessel wall. Recent data, however, suggest that it is actively involved in regulating the structure, function and disease processes of the vessel wall (Stenmark et al., 2012, 2013; Tang et al., 2013). Indeed, its removal leads to degeneration of the entire tunica media (Fugundes et al., 2012). Accordingly, there is renewed interest in the biology of the tunica adventitia, with many studies focusing on its role in atherosclerosis (Skilton et al., 2009, 2011, 2012; Simionescu and Sima, 2012;

Campbell et al., 2012). Indeed, we recently described features of atherosclerosis in tunica adventitia of coronary and carotid arteries (Ogeng'o et al., 2014). In spite of this, most studies on structure of arteries focus mainly on tunica intima and media with only peripheral reference to the tunica adventitia (Nowrozani, 2011; Popescu et al., 2013). Data on anatomical features of tunica adventitia in normal arteries are important in enhancing understanding of the disease processes and potential interventional strategies. Goat is a suitable model for studying vascular disease (Lemson et al., 1999; Zheng et al., 2000)

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because the structure of its vessels and physiological parameters resemble those of humans (Garcia et al., 1995; Prassinis et al., 2005). This study, therefore, aimed at

describing the structure of the normal tunica adventitia of the abdominal aorta, a common site of atherosclerosis, in young goats.

#### MATERIALS AND METHODS

Materials for this study were obtained from six young male goats (*capra hircus*) aged 12 – 24 months obtained from livestock farmers in Nairobi. The animals, verified to be healthy by qualified veterinary doctors, were euthanized with overdose of sodium pentobarbitone and fixed by perfusion using buffered 3% glutaraldehyde. 2 mm<sup>2</sup> sections were post fixed in 1% phosphate buffered osmium tetroxide. The post fixed specimens were rinsed in sodium phosphate buffer for 15 minutes then dehydrated by passing them through increasing concentrations of ethanol - 50%, 60%, 70%, 80%, 90%, 95% and 100% for 30 minutes each, and twice for one hour each in absolute ethanol. The sections were then cleared in propylene oxide for 30 minutes. Subsequently, the sections were infiltrated in

catalyst free durcupan mixture 1 as follows: propylene oxide, durcupan 1:1-30 minutes; propylene oxide, durcupan 1:3-30 minutes and absolute durcupan at 60<sup>o</sup>c in oven for one hour. The sections were then embedded in 100% durcupan with catalyst, and polymerized in an oven at 60<sup>o</sup>c, for 48 hours. Ultrathin sections made with Reichert ultramicrotome © were collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate (Glauert, 1965) and examined by EM 201 philips © electron microscope. Another set of sections were processed routinely for paraffin embedding and sectioning. Five micron sections were stained with Mason's Trichrome for light microscopic examination.

#### RESULTS

Tunica adventitia, the outermost coat of the aorta, is made of dense irregular fibroelastic connective tissue rich in cells, contains vasa vasora and merges with the subjacent connective tissue (Fig 1A). The connective tissue of the aortic tunica adventitia comprises elastic and collagen fibres, organised as bundles and fibres of variable sizes (Fig 1B, C). Elastic tissue of the tunica adventitia consists of elastic fibres interweaving with collagen (Fig 1D), and microfibrillar material which surrounds some of the cells, separating them from the adjacent collagen bundles (Fig 1E, F).

Cells of the tunica adventitia comprise a heterogeneous population. One category of the cells are elongated with long cytoplasmic extensions in all directions and a large euchromatic nucleus. Their long slender processes course between bundles of collagen,

and contain prominent rough endoplasmic reticulum (Fig 1D, E). A second category of cells has a large euchromatic eccentric nucleus, and several pseudopodia-like extensions (Fig 2A). Some of the extensions have vacuoles, while others seem to enclose parts of the extracellular matrix fibres. The nuclear free part of the cell cytoplasm is characterized by prominent rough endoplasmic reticulum, lysosome-like structures, and vacuoles, some of which contain dark particulate matter, resembling residual bodies. The third category comprises cells with long cytoplasmic extensions in all directions, and a relatively poor organelle disposition. Their long slender processes extend between bundles of collagen, and contain rough endoplasmic reticulum. Their nuclei are, however, large and euchromatic filling most of the cell (Fig 2B).

A fourth category of cells are observed in the neighbourhood of vasa vasora (Fig 2C-E). Some cells have flask shaped cell bodies, large euchromatic nuclei which fill most of the cell, leaving only a thin rim of cytoplasm. These cells have their own basal lamina, and several processes, some of which are situated between the endothelial cells of the vasa vasora, reaching the lumen, while others extend into the space between the vessels (Fig 2 D, E). The

processes which extend between endothelial cells share a basal lamina with the endothelial cells, like pericytes. Other perivascular cells embrace both the endothelial cell and the former perivascular cell, and do not bear remnants of basal lamina. This latter type of cells have a large euchromatic nucleus (Fig 2D). Between the vasa vasora there are other large perivascular cells with huge euchromatic nuclei and numerous cytoplasmic extensions (Fig 2E).

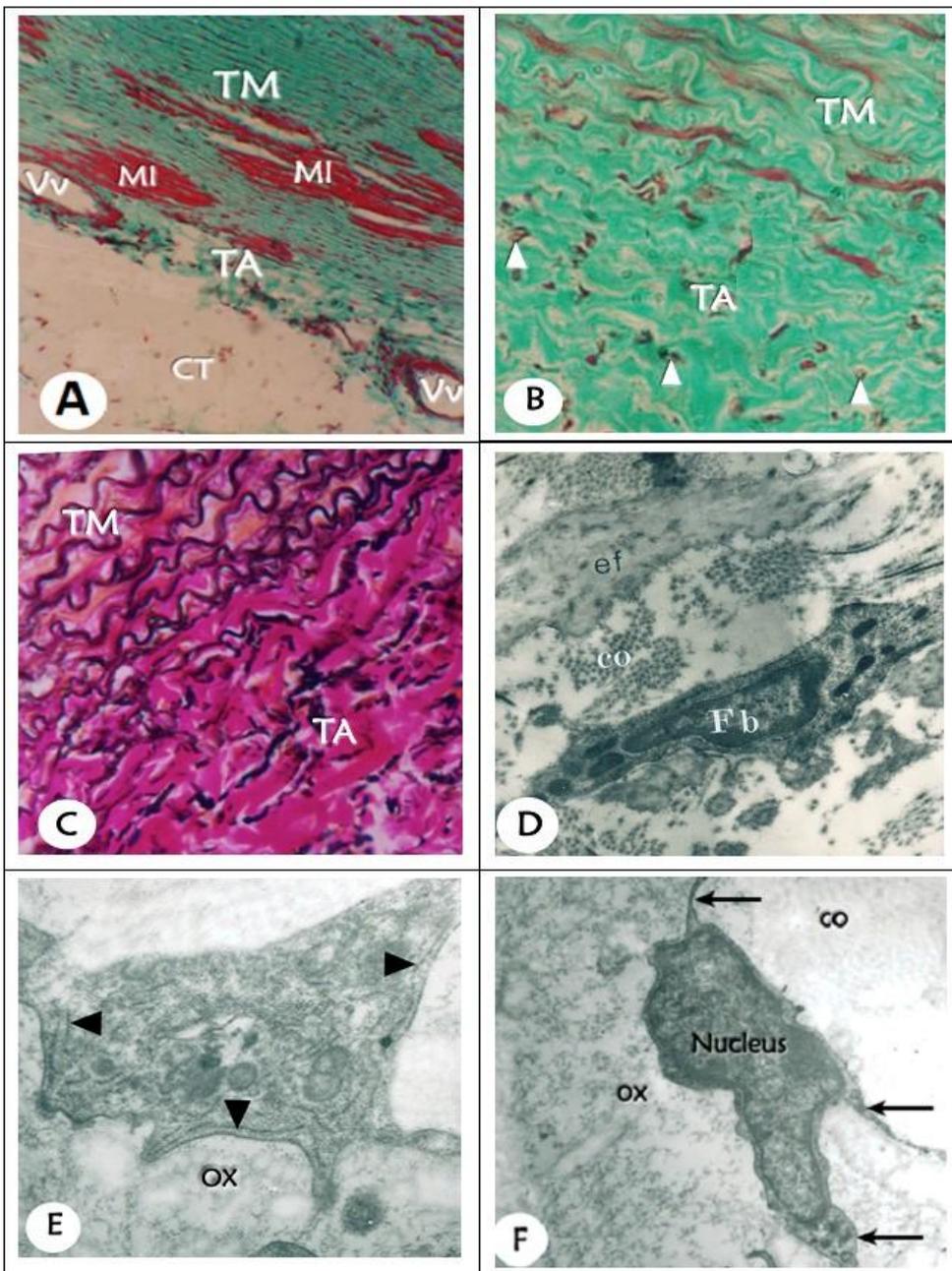


Figure 1A-F: In micrographs the tunica adventitia (TA) of abdominal aorta of goat showing fibres and cell types. TM = Tunica Media. A: Vasa vasora (vv). The tunica adventitia merges with subjacent connective tissue (CT). Mason's Trichrome stain. x100. B: Haphazardly arranged collagen fibres (green), and various types of cells (arrowheads). Mason's trichrome stain. x 400. C: Irregularly arranged collagen fibres (stained pink) mixed with elastic fibres (stained black). Weigert's elastic stain. x400. D: A fibroblast (Fb) surrounded by collagen (co) and elastic fibres (ef) running in various directions. x8,760. E: A cytoplasmic extension of fibroblast containing abundant rough endoplasmic reticulum (arrowheads). Note also the microfibrils (ox) adjacent to the cell. x63,400. F: A cell with large euchromatic nucleus, and cytoplasmic extensions (arrows). Note that the cell is surrounded by collagen (co), on one side and microfibrils (ox) on the other. x27,800.

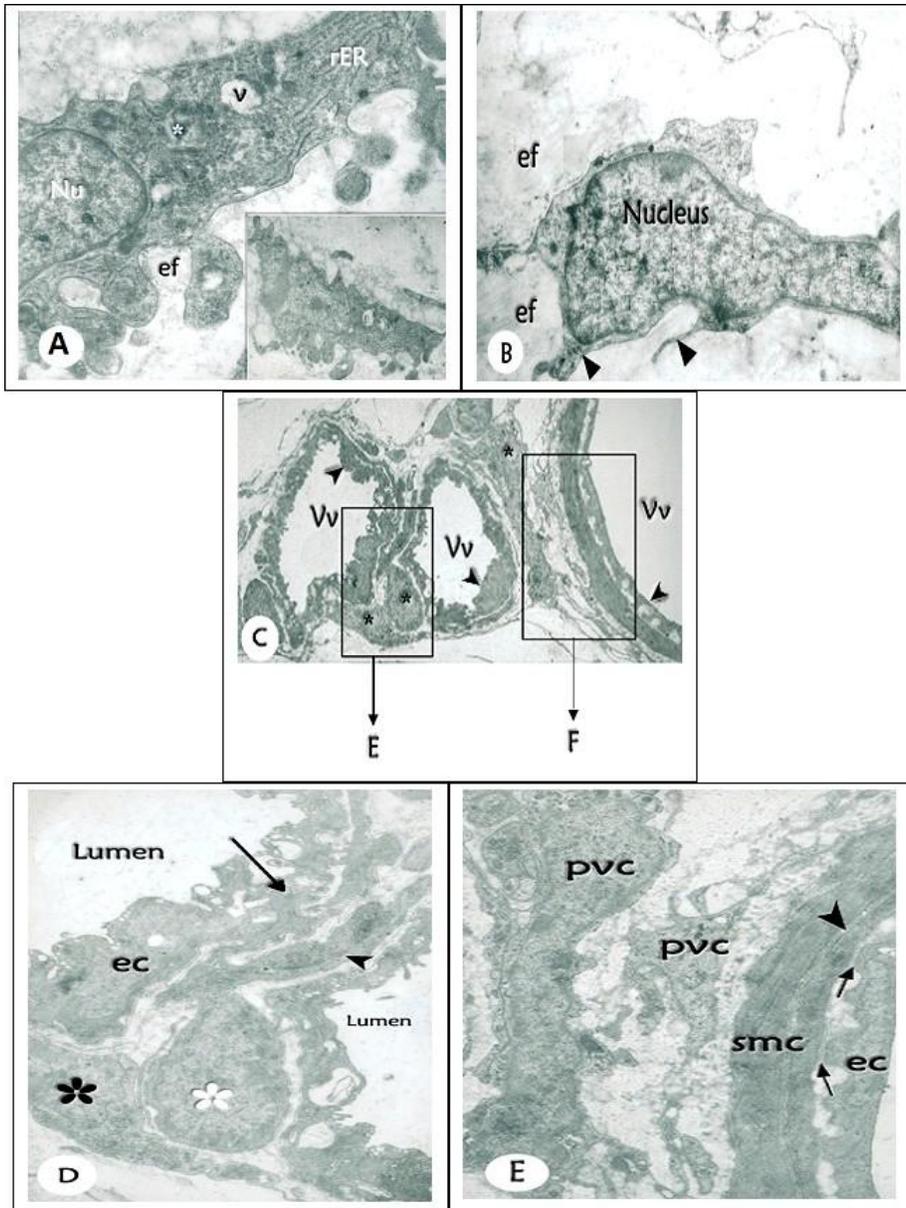


Figure 2A -E: Electron micrographs of tunica adventitia of goat aorta showing various types of capillaries and cell types. A: A cell with a euchromatic nucleus (Nu), rough endoplasmic reticulum (rER) and vacuoles (v) some of which contain dark material (star). Note some of the cell processes engulfing elastic fibres (ef). x27,800. B: A cell with large euchromatic nucleus, cytoplasmic extensions (arrowheads), and relatively poor organelle content. Note the elastic fibres (ef) around the cell. x8,760. C: Three adjacent vasa vasora (vv). Note endothelial (arrowheads), and perivascular cells (stars). x4000. D: Flask shaped perivascular cell (white star) with one process (arrow) extending through a gap in the endothelium (ec), and the other process extending between the two vessels (arrowhead). The other cell (black star) embraces the first one, Magnification x27,800. E: The wall of one of the vasa vasora. Note the two layers of smooth muscle cell (smc), sharing a basal lamina (arrowhead). Note also the endothelial cell (ec), with basal membrane irregularities. Endothelial cells share basal lamina with smooth muscle cell (arrows). The perivascular cells (pvc) have large nuclei and cytoplasmic extensions. x27,800.

## DISCUSSION

The fibroelastic nature of the aorta, observed in the current study, is consistent with that reported in dogs (Orsi et al., 2004; Nowrozani, 2011); rabbits (Viegas et al., 2001) guinea pigs and albino rats (Mello et al., 2004). The collagen confers high tensile strength to the adventitia to enable it bear high pressures, which the tunica media is unable to cope with while the elastic fibres allow reversible stretchability (Kielty et al., 2002). Accordingly, it is plausible, as has been suggested by Orsi et al., (2004), that the fibroelastic composition of the tunica adventitia enables it to maintain the structural and functional integrity of the aorta.

A striking feature of the tunica adventitia, observed in the present study, is the presence of several capillaries and small arterioles. Capillaries are the vascular-tissue interphase where exchange of oxygen, substrates and metabolites occurs, and their increased density signifies high metabolic activity (Kacklick et al., 2007; Poole et al., 2013). This supports the reports from experimental/pathology studies that tunica adventitia harbours a dynamic microvasculature (Majesky et al., 2011; Simionescu and Sima, 2012). Their presence in otherwise healthy aortic wall suggests that they are inherently present to provide continuous blood supply to sustain high metabolic activity. Pertinent to this suggestion are assertions by previous workers that the TA is metabolically active (Stenmark et al., 2012, 2013)

Anatomical studies report the tunica adventitia to comprise only fibroblasts and macrophages (Tonar et al., 2010; Nowrozani et al., 2011). Observations of the current study reveal, at variance with this, that TA has a wider variety of cells. This finding of multiple populations of cells in the tunica adventitia is consistent with the observations that the TA houses a wide variety of cells including fibroblasts,

immunoregulating, progenitor, endothelial cells and pericytes (Simionescu and Sima, 2012; Stenmark et al., 2012, 2013). Some of these cells may represent an important source of pericytes for angiogenesis during remodeling of the artery in physiological and pathological processes (Torsney et al., 2005; Corselli et al., 2012), and also provide a convenient supply of mural cells for vascular bioengineering applications (Howson et al., 2005).

Many of the cells observed in the present study resemble fibroblasts. These findings support the conventional view that, fibroblasts are the major cell type in the tunica adventitia (Tonar et al., 2010; Nowrozani, 2011). It is possible that the other relatively poorly differentiated cells constitute fibroblast precursors, namely mesenchymal stem cells. Some of them, on the other hand, may be pericytes or myofibroblasts, both of which on their own, or by transformation into smooth muscle cells are involved in matrix synthesis (Torsney et al., 2005; Howson et al., 2005; Stenmark et al., 2012; 2013).

Two types of cells were observed around capillaries. This is concordant with recent reports of two types of perivascular cells, namely pericytes and adventitial cells. These cells have been implicated in constituting multipotent progenitor cells similar to mesenchymal stem cells (Corselli et al., 2012; Stenmark et al., 2013) involved in routine remodeling and response to injury. Some of them may also be involved in immune surveillance. Pertinent to this suggestion is the observation that some of their processes were seen penetrating into the lumen through endothelial pores.

The other cells resemble those of the mononuclear phagocytic system, thus

supporting reports that the tunica adventitia of the aorta contains macrophages and vascular dendritic cells (Majesky et al., 2011, 2012; Stenmark et al., 2012; 2013). These workers suggested that these cells are responsible for maintaining immune surveillance and inflammatory cell trafficking, therefore protecting the vascular wall. Accordingly, it has been suggested that resident macrophages and dendritic cells comprise a large population of immune cells which may be involved in homeostatic regulation of immune responses within the aortic wall (Majesky et al., 2011; Stenmark et al., 2013).

In conclusion, the tunica adventitia of the aorta is an active vascular compartment with

microvasculature and multiple populations of cells usually involved in synthesis of ECM, immunosurveillance and remodeling of the aorta. These are the features that probably enable it to maintain structural and functional integrity and respond to vascular injury.

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