Is apoptosis of vascular smooth muscle cells involved in the development of Takayasu arteritis? Suggestions from a case report

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Objectives. We report a female patient suffering from Takayasu arteritis (TA) who underwent surgical revascularization. Methods. By studying specimens obtained at surgery, we evaluated the cell composition of the arterial wall, along with the maturation pattern of vascular smooth muscle cells (VSMC) during the active phase of TA. Using TUNEL, we detected apoptotic cells within the tunica media.

Results. The highest percentage of apoptotic cells was found in areas where inflammatory infiltrate was present and the medial structure was more or less damaged. Apoptotic cells were also found in structurally preserved areas, where VSMC but not inflammatory cells were present.

Conclusions. Apoptosis involved not only inflammatory cells but also VSMC, particularly those of the immature type. We hypothesize a role for VSMC apoptosis in the development of TA.

KEY WORDS: Takayasu arteritis, Apoptosis, VSMC.

Takayasu arteritis (TA) is a chronic vasculitis affecting the aorta and its major branches, including renal and visceral arteries. Women aged 10–30 are usually affected [1]. Symptoms include intermittent claudication of the arms, legs and mesentery. When renal artery stenosis occurs, it may account for renovascular hypertension [1, 2]. TA consists of a continuous or patchy granulomatous inflammatory reaction due to macrophages, lymphocytes, histiocytes and multinucleated giant cells [1, 2]. The pathophysiological mechanisms underlying the disruption of the arterial wall are poorly understood, and this is particularly true for the potential role played by apoptosis.

Case report

A 35-yr-old woman was admitted to our department for severe hypertension refractory to therapy and bilateral claudication of lower limbs with reduced peripheral pulses. Blood pressure was 240/110 mmHg bilaterally.

Laboratory studies revealed the following: hypochromic anaemia with haemoglobin 8.8 g/dl, platelet count 481 000 mmc, ESR 52 mm/h, CRP 14.3 mg/l (normal range 0–5.0 mg/l), plasma creatinine 154/C22 mol/l (normal range 53–97/C22 mol/l) and creatinine clearance 26 ml/min (normal range 60–130 ml/min). Coagulation tests and levels of protein C and protein S were normal. Investigations for lupus anticoagulant, antinuclear antibodies and antiphospholipid antibodies were all negative. The patient was positive for HLA A23, A25, B18, B22 and BW6 antigens. Rheumatoid factor was negative, as were assays for the presence of immune complexes and cryoglobulins. Complement studies revealed only a slight decrease in the C3 component. Angiography of the lumbar aorta, renal and lower limb arteries showed bilateral subocclusion of the renal arteries, occlusion of the lumbar aorta and filling of the femoral arteries through the collateral circulation. This picture was suggestive of thrombosis from the lumbar aorta to the iliac communis of both sides. NMR study of the abdominal aorta disclosed a pattern suggestive of TA, namely a concentric and homogeneous thickening of the aortic wall. Neither angiography nor ultrasound examination showed any sign of involvement of the supraaortic trunks. Based on all these data, type II TA was diagnosed.

A fast impairment of renal function (plasma creatinine from 154 to 271/C22 μmol/l in 1 week) took place, which was due to ascending aortic thrombosis. Although vascular surgery is not recommended in TA, in this case it was considered mandatory in order to stop or delay the progression towards end-stage renal failure. Therefore, surgical revascularization was performed by aorto-bisiliac bypass grafting with a PTFE prosthesis, coupled with bilateral renal artery endarterectomy. Samples of aortic wall were collected during surgery. Histological examination was then carried out on samples of vascular tissue, which demonstrated a typical pattern of arteritis (see below). After revascularization, perfusion of the lower limbs improved, peripheral pulses became appreciable and a reduction in plasma creatinine level (125/C22 μmol/l) was observed. Satisfactory blood pressure control was achieved by nifedipine GITS (gastrointestinal therapeutic system) (30 mg once daily). With immunosuppressive therapy, including prednisone (100 mg daily) and methotrexate (10 mg weekly), the patient went into clinical and laboratory remission with normalization of ESR (11 mm/h) within 1 month.

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Informed written consent was obtained from the patient for this study.

Pathological data

This case reported represents a rare example of histological diagnosis of TA made ex vivo upon a specimen of abdominal aorta collected during surgery. The availability of vascular samples allowed us to evaluate the different cell types present in the aortic wall, the pattern of vascular smooth muscle cell (VSMC) differentiation and the occurrence of apoptosis. The presence of apoptotic cells was also tested in the aortic wall from three subjects with severe hypertension. Specimens were obtained from organ donors of the North Italy Transplantation (NIT) programme.

Immunocytochemical analysis with monoclonal antibodies specific to different cell types (VSMC, macrophages and lymphocytes) was carried out according to the procedures in use in our laboratory. Furthermore, we used terminal deoxynucleotide transferase-mediated dUTP nick end labelling (TUNEL) to detect apoptotic cells. The histological examination displayed a severe granulomatous inflammation of the aorta and renal artery characterized by lymphoplasmacytic infiltrate, histiocytes, rare giant cells and intimal proliferation associated with patchy destruction of the medial musculo-elastic lamellae and necrosis. A diagnosis of active-phase Takayasu’s arteritis with thrombosis was made (Fig. 1). Even in areas free from inflammatory cells (A-type areas; Fig. 2), the tunica media showed a reduced number of VSMC and of elastin layers. The large majority of VSMC were double-labelled with smooth muscle (SM)-E7 and non-muscle (NM)-F6 monoclonal antibodies (Fig. 2A and B), indicating co-expression of smooth muscle and non-muscle myosin heavy chains Aplα1; that is, the fetal type in our nomenclature (for review see [3]). In these areas, a number of cells were TUNEL-positive (Fig. 2D). In medial areas of aorta characterized by discrete accumulation of inflammatory cells (B-type areas; Fig. 3A), the number of VSMC and of elastin layers was dramatically reduced (Fig. 3B) and TUNEL-positive cells were more frequently found (Fig. 3C). Inflammatory cells were mainly stained by the macrophage-specific monoclonal antibody HAM 56 (Fig. 3A). Moreover, some cells were recognized by the anti-lymphocyte CD45, and a few cells were positive to the antibody specific to CD4 lymphocytes (not shown). The intima and the media also showed very few VSMC stained by SM-E7 only, a pattern typical of VSMC expressing smooth muscle myosin heavy chains exclusively, i.e. well differentiated VSMC [3].

In order to better define the rate of apoptosis in the aortic media, the number of TUNEL-positive cells was assessed by computed image analysis (AT-IBAS; Kontron, Eching, Germany) in five fields (26 x 10⁵ μm² each) from medial areas of type A, B and C (the latter, full infiltration of inflammatory cells with complete loss of structure). The highest rate of apoptosis was present in areas where inflammatory cells were present and medial structure was more or less lost (C-type areas, 25.3 ± 12.7% vs B-type areas, 23.7 ± 7.3%; F = 4.02, P = 0.046; ANOVA and Student–Neuman–Keuls multiple comparison test).

![Fig. 1. Histological sections of the aortic wall. (A) Granulomatous inflammatory cell infiltration of the media characterized by lymphoplasmacytoid cells and histiocytes. Haematoxylin–eosin stain, ×200. (B) Note the patchy destruction of the medial musculo-elastic lamellae. Elastic Van Gieson stain, ×200. (C) Diffuse inflammatory cell infiltration of the media with giant cells. Haematoxylin–eosin stain, ×150. (D) Close-up view of C. Haematoxylin–eosin stain, ×400.](https://academic.oup.com/rheumatology/article-abstract/44/4/484/1774659)
Nevertheless, a remarkable percentage of apoptotic cells was also found in relatively preserved areas (A-type areas, 10.6 ± 5.3%, \( P = 0.05 \) vs B-type areas, \( P = 0.032 \) vs C-type areas) where VSMC but not inflammatory cells were found. Indeed, apoptosis involved a number of VSMC of the aortic media and this may be part of the process leading to loss of vascular structure in TA.

Apoptotic cells were not found in the media layer of the three hypertensive subjects, ruling out the possibility that VSMC apoptosis in non-inflamed areas could be a consequence of severe hypertension.

Discussion

It is known that in the MRL strain of mice, which develop spontaneous granulomatous vasculitis similar to human TA, some macrophages avoid apoptosis and play a role in disrupting arterial wall structure [4]. However, the contribution of VSMC apoptosis to the progression of arteritis is still undefined. Our data based on TUNEL analysis suggest that in TA, medial VSMC, mainly of the fetal type, undergo diffuse apoptosis. This cell type plays a key role in the development of vascular disease as fetal type VSMC increase in the plaque and the underlying media of the atherosclerotic vessels and represent the bulk of medial hypertrophy [3].

On the other hand, apoptosis of VSMC is potentially involved in the physiological regulation of vascular structure, as it may regulate cell mass in the normal arterial wall. The higher rates of apoptosis seen in VSMC of atherosclerotic plaque may contribute to its rupture [5]. Although apoptosis may represent a homeostatic mechanism, a high rate of programmed cell death could be viewed as a major pathophysiological aspect of autoimmune diseases such as TA. The finding of a remarkable degree of apoptosis even in areas that are not occupied by inflammatory cells is intriguing. It suggests that the start of the apoptosis process could be due to interaction of some soluble factors released by these cells into the vascular milieu (such as TNF-\( \alpha \) or Fas ligand) with their specific receptors.
Although we could not carry out further evaluation of the apoptosis process due to the very small amount of aortic tissue available to us, these data support the involvement of VSMC in apoptosis and hence in the development of the wall remodelling observed in TA.

The authors have declared no conflicts of interest.

References


