Mast cells associate with T-cells and neointimal microvessels in giant cell arteritis

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ABSTRACT

Objective. *Mast cells (MCs) are known to be involved in the neovascularization and regulation of T cell responses. However, the presence of MCs in giant cell arteritis (GCA) is unknown. This prompted us to study the presence and phenotype of MCs in GCA.*

Methods. *Human GCA specimens collected for diagnostic purposes were examined with immunohistochemistry. Double immunostainings of MC tryptase with cathepsin G, vascular endothelial cell growth factor (VEGF), CD3, and CD31/D34 were performed.*

Results. *Double immunostainings showed that activated tryptase-, cathepsin G- and VEGF-expressing MCs associate with $CD3^+$ T cells and $CD31/CD34^+$ neointimal neovessels in the GCA lesions.*

Conclusions. *The results suggest that MCs may contribute to the pathogenesis of GCA putatively by regulating the functions of other inflammatory cells and resident vessel wall cells. Importantly, MCs promote neovascularization, which is considered as a prerequisite for the neointimal thickening in GCA.*

Introduction

Giant cell arteritis (GCA), also known as temporal arteritis, is the most common form of systemic vasculitis in adults. It is a vasculitis of medium- and large-sized arteries, and it commonly leads to symptoms, which include blindness and stroke. GCA is generally thought to be a disease in which the inflammatory response is dominated by macrophages and interferon-γ (IFN-γ) producing $CD3^+$ T cells (1). Dendritic cells are also thought to be important in the pathogenesis of GCA (2). One of the hallmarks of GCA is concentric intimal hyperplasia, which may lead to complete obstruction of the arterial lumen with ensuing end organ ischemia (3). A prerequisite for the growth of a thick neointima is the formation of intimal neovessels (4). Mast cells (MCs) are known to play an important role both in T cell regulation (5) and in neovascularization (6) in numerous tissues, but the presence of MCs in GCA has not been studied so far. The lack of data regarding MC presence in GCA is surprising considering the fact that bilateral interactions of MCs with T cells, dendritic cells, and, endothelial cells are known to fundamentally regulate immune responses (5, 7-10). Furthermore, endothelin-1, a potent vasoconstrictor and MC activator, was recently shown to be elevated in GCA (11). The apparent gap in knowledge combined with these pathogenic characteristics of GCA prompted us to search for the presence of MCs in the diseased temporal arteries. This is the first report on the presence of MCs in GCA.

Materials and methods

GCA samples

A total of 18 temporal artery biopsy specimens collected at Tampere University Hospital for suspected GCA were obtained for analysis. The study was approved by the Ethical Committee of University Hospital of Tampere. The samples were fixed with neutral buffered formalin, embedded in paraffin and sectioned for immunostainings. In histological analysis the biopsies of 13 patients (9 women, 4 men, mean age 76, range 65-99) fulfilled the criteria for GCA (3, 12) and a diagnosis of GCA was set. The samples from the remaining 5 patients showed no signs of inflammation or intimal thickening and the suspicion of GCA was cleared. The samples from these 5 patients were used as controls (4 women, 1 man, mean age 64, range 54-80).

Immunohistochemistry

Co-localization of MC-tryptase with neovascular endothelial cells (CD31/CD34) and cathepsin G was studied as

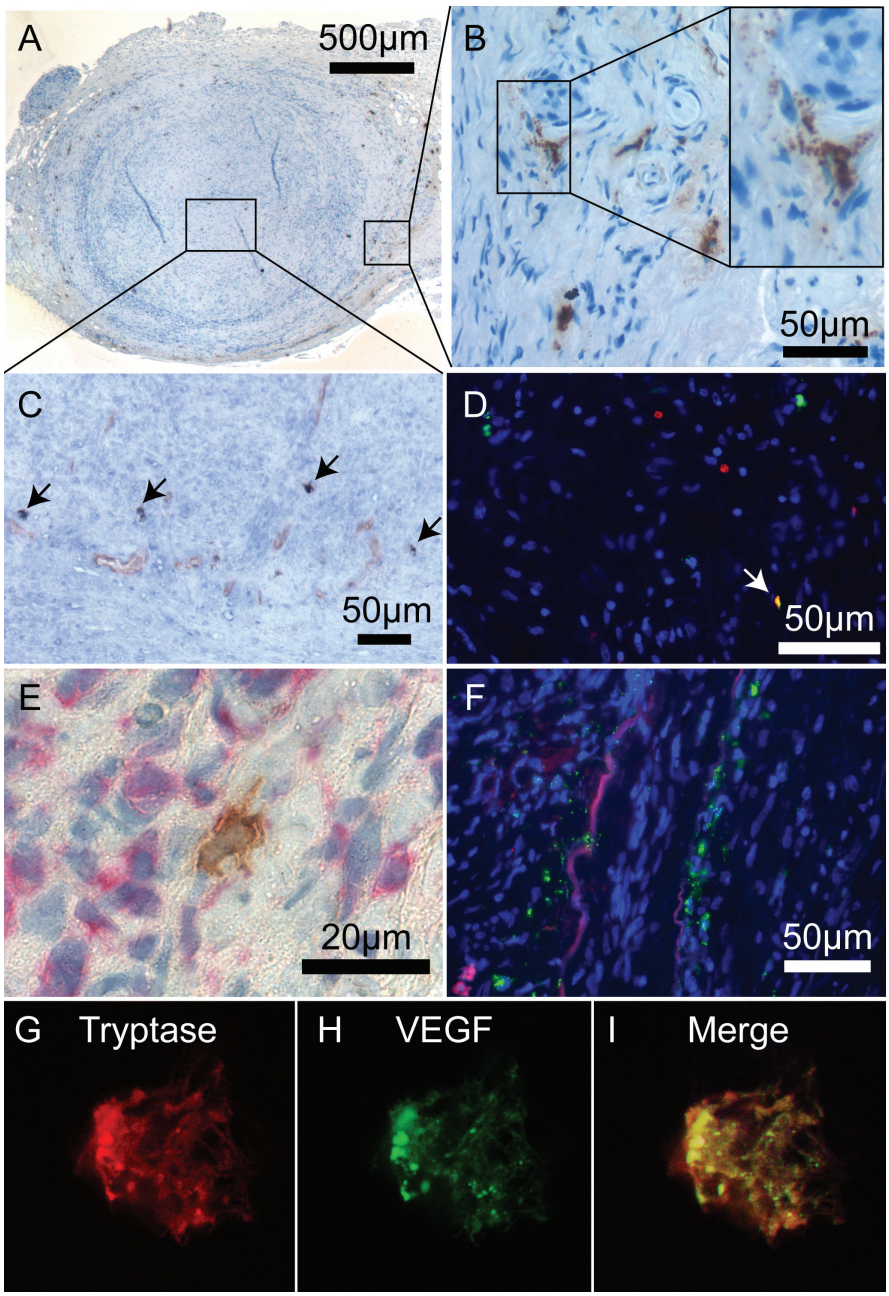

Fig. 1. (**A**) Low magnification of a cross section of a totally occluded temporal artery stained for MC-tryptase (brown, DAB). The rectangles in (**A**) indicate the areas shown in (**B**) and (**C**) at higher magnification. (**B**) Adventitial partly degranulating MCs at high magnification, stained for MC-tryptase (brown, DAB). The inset shows one degranulated mast cell at higher magnification. (**C**) Tryptase-positive MCs (arrows, nickel-DAB) and neovessels (reddish circular structures, AEC) in GCA neointima. (**D**) Only tryptase-positive (red) and tryptase- and cathepsin G- (green) double positive (white arrow, yellow) MC subpopulations were found in the GCA neointima. (**E**) Tryptase-positive MC (brown, DAB) in the GCA neointimal area occupied by CD3+ T cells (pink, fast-red). (**F**) Cells and extracellular granules negative for tryptase (red), but positive for cathepsin G (green) were commonly seen in the neointima and adjacent to elastic laminas. Here elastic laminas are visualized with faint red autofluorescence. The counterstain in (**A-C**), and (**E**) is Mayer's hematoxylin, and that in (**D**) and (**F**) 4',6-diamidino-2-phenylindole. (**G-I**) shows confocal microscope images of a neointimal tryptase- and VEGF-positive degranulated MC.

described previously (13). Only MCs within a distance of 50 µm from the nearest neovessel were considered as neovessel-associated. Co-localization of MC-tryptase with $CD3^+$ (polyclonal rabbit anti-human CD3, A0452, Dako, Glostrup, Denmark, working dilution 1:60) T cells was studied with the Zymed PicTure double staining kit (Zymed Laboratories Inc, San Francisco, CA), and co-localization of MC-tryptase with VEGF (polyclonal rabbit anti-human VEGF, clone A-20, Santa Cruz Biotechnology, Santa Cruz, CA, working dilution 1:100) was studied with an immunofluorescence double staining method using Alexa-conjugated secondary antibodies (Molecular Probes, Leiden, The Netherlands). Monoclonal mouse anti-human CD68 (clone PG-M1, working dilution 1:50) and smooth muscle cell (SMC) α-actin (clone 1A4, working dilution 1:500) antibodies were purchased from Dako. The specificity of each immunostaining was ensured as described previously (13).

The stained samples were photographed with a digital camera (Spot RT colour operated with Spot advanced software, version 4.1, Diagnostic Instruments, Sterling Heights, MI) attached to a Nikon Eclipse E600 microscope or with a TCS SP1 laser scanning confocal microscope (Leica, Wetzlar, Germany). All MCs and neovessel-associated MCs in each entire section were counted from the photomicrographs and the results are expressed as average number per mm^2. For counting the MC distribution density (MCs/mm^2) the areas of sections, and of intima, media, and adventitia, were measured with computer-aided planimetry (Image-Pro Plus, version 4.5, Media Cybernetics Inc. Silver Spring, MD). As indirect indication of MC activation, the presence of extracellular MC granules was histologically evaluated as previously described (14).

Results

The histological structure of the GCA samples was distorted compared to the healthy control samples. In the GCA specimens the lamina elastica interna and externa were mostly degraded, and the adventitial-medial border, medial layer and deep parts of neointima were filled with dense inflammatory cell infiltrates composed mainly of $CD3^+$ T cells and $CD68^+$ monocyte-macrophages. Medial layer and neointima were typically mainly composed of SMC α-actin positive cells. Multi-

nucleated giant cells were seen in all GCA specimens. Furthermore, 6 out of the 13 GCA specimens were totally occluded, while the remaining ones were subtotally occluded. The totally and subtotally occluded GCA specimens were similar in regards of populations of all studied cell types. These pathogenic characteristics are in line with previous reports (1, 15). The healthy control arteries had a normal-sized lumen and showed no signs of neointimal growth or inflammation.

Tryptase stainings (Figs 1A and B) and tryptase-CD31/CD34 double stainings (Fig. 1C), revealed MCs in all layers of the GCA specimens, *i.e.*, in the adventitia, the media, and the intima. Degranulated MCs as a sign of MC activation were also found in the adventitial and intimal layers of the GCA specimens. Overall, MCs were most abundant in the adventitial layer of the GCA samples (mean 30 MC/mm^2). In the GCA samples, MCs were numerous also in the neointima occupying the area of the original lumen (mean 12 MC/mm^2). Indeed, in the GCA samples, 58% of the MCs were present in the adventitia, 5% in the media, 6% in the intima and 31% in the neointima. Of the neointimal MCs, 67% were associated with neovessels (Fig. 1C). These findings of the GCA specimens sharply contrasted with those of the healthy control samples, in which MCs were observed in the adventitia only (mean 13 MC/mm^2).

The tryptase-CD3 double stainings confirmed the presence of MCs in the areas also occupied by CD3$^+$ T cells (Fig. 1E). These areas were generally populated also by CD68+ monocyte-macrophages (not shown).

Tryptase-cathepsin G double stainings (Fig. 1D) showed that a subpopulation of MCs in the GCA samples was also positive for cathepsin G. In addition, cells and granules negative for tryptase, but positive for cathepsin G (likely neutrophils and neutrophil-derived granules) were commonly seen in the neointima and in the vicinity of elastic laminae (Figs. 1D and F, respectively). Tryptase-VEGF double stainings revealed that a subpopulation of neointimal MCs also expressed VEGF (Figs. 1G-I).

Discussion

Here we demonstrate, for the first time, the presence of MCs in neointima of temporal arteries affected by GCA. By showing close spatial association of MCs with neovessels and T cells, we provide suggestive evidence for a proangiogenic and immunoregulatory role of MCs in this disease. Importantly, a subpopulation of the neointimal neovessel-associated MCs showed signs of activation, *i.e.*, they were degranulated. Accordingly, these neovessel-associated MCs are capable of releasing a variety of granule-associated mediators such as histamine, heparin, TNF-α, VEGF, bFGF, and adrenomedullin (6). Activated MCs may also participate in the remodelling of the affected arteries by regulating SMC growth and death (16). Thus, activated MCs may accelerate the formation of a neointima by triggering the release of matrix-bound growth factors such as TGF-β, which stimulate SMCs to proliferate (17). On the other hand, the activated MCs may also attenuate the formation of a neointima by releasing heparin, which prevents SMC proliferation, or by proteolytically degrading pericellular matrix components, which induces SMC apoptosis (18). In addition, the secretion of proangiogenic and proinflammatory mediators as well as direct cell-cell interactions may regulate the responses of other inflammatory cells, including T cells, involved in the pathogenesis of GCA (5, 7-10). MCs may also recruit other inflammatory cells into the GCA lesion, by promoting expression of endothelial cell adhesion molecules and chemokines (19, 20). Furthermore, it is possible that MCs promote endothelial endothelin-1 production (21). However, they may also participate in the degradation of this vasoconstrictive mediator by releasing carboxypeptidase A (22). MC cathepsin G and chymase may also participate in the increased angiotensin II formation observed in GCA (23). Taken together, the MCs present in GCA lesions may participate in the pathogenesis of GCA in multiple ways.

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