Distinct macrophage phenotype and collagen organization within the intraluminal thrombus of abdominal aortic aneurysm

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Objective: **Little is known about the etiologic factors that lead to the occurrence of intraluminal thrombus (ILT) during abdominal aortic aneurysm (AAA) development. Recent work has suggested that macrophages may play an important role in progression of a number of other vascular diseases, including atherosclerosis; however, whether these cells are present within the ILT of a progressing AAA is unknown. The purpose of this work was to define the presence, phenotype, and spatial distribution of macrophages within the ILT excised from six patients. We hypothesized that the ILT contains a population of activated macrophages with a distinct, nonclassical phenotypic profile.**

Methods: **ILT samples were examined using histologic staining and immunofluorescent labeling for multiple markers of activated macrophages (cluster of differentiation [CD]45, CD68, human leukocyte antigen-DR, matrix metalloproteinase 9) and the additional markers α-smooth muscle actin, CD34, CD105, fetal liver kinase-1, and collagen I and III.**

Results: **Histologic staining revealed a distinct laminar organization of collagen within the shoulder region of the ILT lumen and a spatially heterogeneous cell composition within the ILT. Most of the cellular constituents of the ILT were in the luminal region and predominantly expressed markers of activated macrophages but also concurrently expressed α-smooth muscle actin, CD105, and synthesized collagen I and III.**

Conclusions: **This report presents evidence for the presence of a distinct macrophage population within the luminal region of AAA ILT. These cells express a set of markers indicative of a unique population of activated macrophages. The exact contributions of these previously unrecognized cells to ILT formation and AAA pathobiology remains unknown. (J Vasc Surg 2015;62:585-93.)**

Abdominal aortic aneurysm (AAA) is the 13th leading cause of death in the United States.[1] The pathogenesis of AAA has not been fully elucidated but is increasingly recognized to involve a chronic inflammatory process.[2-5] Multiple types of inflammatory cells (neutrophils, T cells, B cells, macrophages, mast cells, and natural killer cells) are present within the aortic wall during AAA progression, and these cells have been associated with increased levels of proinflammatory cytokines and diverse proteases. Taken together, this suggests an active inflammatory remodeling environment in the AAA wall that promotes progressive tissue degradation, focal dilatations, and potential rupture.[6-9] However, much less is known about the process that leads to the formation of an intraluminal thrombus (ILT), which occurs in >75% of AAA cases.[8]

Altered hemodynamics resulting from AAA are likely among the drivers of initial ILT formation and progression.[10,11] These hemodynamic disturbances are presumed to activate platelets, which leads to subsequent accumulation of fibrin and entrapment of erythrocytes within a layered structure.[12] A number of reports describe a three-layered structure consisting of luminal (in contact with flowing blood), medial, and abluminal (in contact with the AAA wall) layers, with most cells residing within the luminal layer.[13-16] Evidence also suggests that these layers possess a heterogeneous structure that matures with time and disease progression.

Although the cells within the ILT are predominantly platelets, lesser numbers of other cells, including macrophages, have also been observed.[9,13,17] However, due to their relatively small numbers compared with platelets, the exact phenotypes and roles of these cells in the formation or maturation of the ILT and overall pathology of AAA have gone largely unstudied. Given that recent evidence

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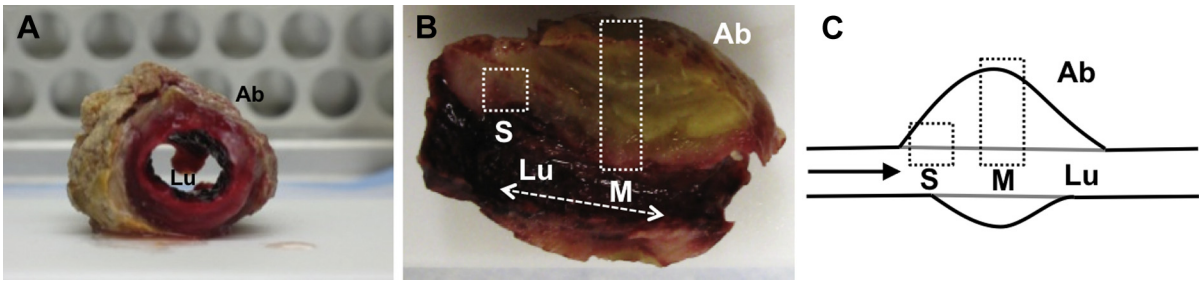

Fig 1. A and **B,** Cross sections show the gross morphologic appearance of the shoulder (*S*), luminal aspect (*Lu*), abluminal aspect (*Ab*) and midsection (*M*) of the intraluminal thrombus (ILT) at time of surgery. Note the layered structure. Samples were cut in two and then further dissected for microscopic investigation. **C,** A schematic view is shown of the ILT aspects depicted in panel **B**. The *boxes* represent the areas sampled.

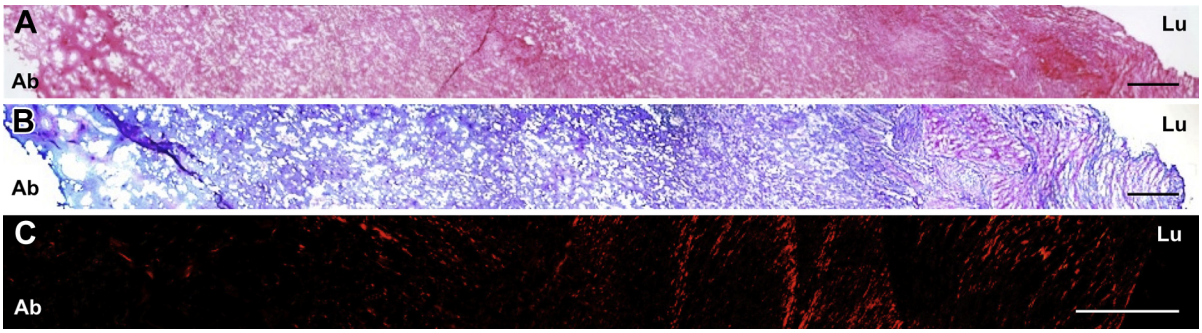

Fig 2. Asymmetric composition of the intraluminal thrombus (ILT) is seen in representative histologic appearance of samples. Samples were stained with **(A)** hematoxylin and eosin, **(B)** Herovici's polychrome, and **(C)** picrosirius red (PSR). Note the distinct appearances of the abluminal (*Ab*), medial, and luminal (*Lu*) regions of the full-thickness ILT sample. **B** and **C,** Herovici's polychrome and PSR staining suggest the presence of collagen within the luminal region of the ILT. *Scale bar* = 250 μm.

suggests that activated macrophages play an important and determinant role in the pathology of a number vascular diseases,[17-20] the purpose of this work was to define the presence, phenotype, and distribution of macrophages within the ILT of patients with AAA. We hypothesized that the ILT contains a population of activated macrophages with a distinct, nonclassical phenotypic profile.

METHODS

This study met the necessary requirements of the U.S. Department of Health and Human Services Code of Federal Regulation Title 45, Part 46.101(b)(4) to be designated as "research with no human subjects involved"; that is, research involving the collection or study of pathologic specimens collected in such a manner that patients cannot be identified, directly or through identifiers linked to the patients. Therefore, this study was exempt for the need for patient consent and was approved by the University of Pittsburgh Institutional Review Board.

Patients. For this study, AAA wall and ILT samples were obtained from nine men (eight were smokers), aged between 66 and 75 years, who were undergoing elective AAA repair surgery. All patients were taking statins and antiplatelet drugs. The aortic dilatation for these patients was between 43 and 76 mm. All of the AAA studied were degenerative, fusiform atherosclerotic aneurysms and were not mycotic or associated with genetic abnormalities.

Sample procurement. Tissue was obtained from the operating room immediately after excision, deidentified, provided to the research team, and transported to the laboratory in ice-cold phosphate buffered saline (PBS, pH 7.4). ILT samples were processed for histologic examination (six patients) or isolation of cells for culture experiments (three patients) as described below.

Sample preparation. Samples were prepared for histologic examination and immunofluorescent labeling. Briefly, the ILT was washed thoroughly in PBS, placed immediately in 4% paraformaldehyde, and kept at 4°C for 24 hours. Samples were transferred to a 30% sucrose solution for at least 24 hours before being cut luminally to abluminally through the thickness of the thrombus in two locations, the shoulder region and the midsection (Fig 1). The resulting pieces were loaded into optimal cutting temperature mounting media (Tissue-Tek; 4583 Sakura Finetek USA Inc, Torrance, Calif) in cryomolds and frozen at −80°C for 24 hours before being sectioned at 6 to 7 μm thickness. Samples for cell isolation were left unfixed and processed as described below.