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EDITORIAL VIEWPOINT

The Differences Between Neovascularization of Chronic Total Occlusion and Intraplaque Angiogenesis*

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A number of patients with coronary artery disease present with chronic total occlusion (CTO), in which the extent of distal reperfusion and myocardial salvage is dependent on collateral circulation. These patients represent a real challenge to the interventionalist, because there is no proven therapy. Interventional or surgical candidates generally succumb to higher rates of complications. At this point, there is little understanding of the pathophysiology of CTO, and a need for new treatment strategies to promote recanalization to help avoid the morbidity associated with interventional or surgical procedures.

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The incidence of CTO is reported as high as 22% in patients presenting with sudden coronary death as their first manifestation of atherosclerotic disease, with even greater frequency (32%) among diabetic patients (1–3). CTO develops from total luminal obstruction of an artery by a thrombus, with subsequent organization and varying degrees of recanalization; often times, these events are clinically silent. The process of thrombus organization coincides with the development of intraluminal microvessels accompanied by inflammatory cells, followed by infiltrating smooth muscle cells and deposition of proteoglycan matrix. Eventually, there is replacement of proteoglycans by type I collagen, where the cross-linking of collagen results in negative remodeling (4). Clinically, optimum treatment results are achieved if catheter-based revascularization can be successfully performed within 1 year of the occlusion, specifically, during earlier phases of thrombus healing.

In this issue of *iJACC*, Munce et al. (5) expand our knowledge of CTO and revascularization using a rabbit femoral artery model in which occlusive thrombi were produced by thrombin injection, with recovery up to 24 weeks. Elegant imaging studies using micro-computed tomography (μCT) revealed 2 distinct types of vascular structures, referred to as circumferential "extravascular" and longitudinally oriented "intravascular" microvessels with unique temporal distributions. The extravascular microvessels are maximal at 2 weeks and few of these vessels remain at 12 weeks, whereas the longitudinally oriented intravascular microvessels are maximal at 6 weeks and progressively decline thereafter. What is more intriguing is the direct communication of the 2 vascular structures throughout the length of the occlusion, which is tapered at both proximal and distal ends.

From the pathologist's perspective, there are 3 main differences separating the rabbit model from naturally occurring CTO in patients. First, the time period of healing in rabbits is accelerated relative to humans, as shown with vascular stents (6). Second, a lack of underlying disease in the rabbit CTO model presents another variable, because expansion of vasa vasorum (VV) and revascularization of the thrombus are codependent on an atherosclerotic process. In humans, plaque progression is invariably associated with an increased adventitial VV. Third, and perhaps most important, the thrombin-induced thrombus substrate in the rabbit is likely fibrin-rich whereas in humans, the thrombus is always platelet-rich at the culprit site, although the propagated throm-

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bus may show greater fibrin at the proximal and distal end, in particular with longer occlusion lengths. Therefore, these relative differences raise the question as to how relevant the rabbit thrombin-induced model of CTO is to human disease.

In both humans and animals, the normal arterial wall is supplied by a network of VV whose function is to deliver nutrients and oxygen and remove "waste products" from the artery (7). The development and progression of atherosclerosis occurs in coordination with the expansion of VV from the adventitia into the plaque (8). Microvessels within the plaque have been found to increase with lesion advancement, so that early lesions showed greater adventitial VV while densities of intraplaque microvessels in late lesions were similar to the adventitia (9). More interesting, intraplaque microvascular endothelial cells frequently showed abnormalities of membrane blebbing and cytoplasmic vacuoles, in addition to disrupted cell-to cell contacts, and basement membrane defects (9). Further, the stabilization of microvessels by mural smooth muscle cells was an infrequent finding, suggesting an immature vascular phenotype. Although intraplaque VV are often tortuous, they travel at right angles from main branches within the adventitia, generally through a disrupted medial wall where they further branch. On the contrary, microvessels associated with CTO, as shown by Munce et al. (5), develop at the proximal end of the thrombus, with established communication with the distal open artery. Therefore, vascular structures that develop in the thrombin model of CTO traverse the lumen primarily in a longitudinal orientation, although they are similarly tortuous in nature as in human CTO. Whether the vasculature structures associated with CTO in the animal model differ from intraplaque microvessels is unknown, as no details were provided regarding the morphologic characteristics of the neoangiogenic vessels within CTO in reference to mural pericytes, endothelial competence, or basement membrane integrity.

The report by Munce et al. (5) also suggests that neoangiogenesis associated with CTO may be a physiologic response to low oxygen tension, where hypoxic areas were identified within the occluded lumen and deeper medial layers at proximal and distal sites at 2 weeks, but not at 12 weeks. Although the origin of neoangiogenesis in CTO is likely from the proximal and distal nonoccluded ends, it also might be driven by circulating endothelial progenitor cells, as reported in venous thrombi (10). Microvascular development within plaques has similarly been shown to be hypoxia dependent as angiogenesis is only observed once the diffusion limits of oxygen exceed ~350 μm (11).

Inflammation also plays an important role in creating a hypoxic environment in the arterial wall, mainly resulting from the high-metabolic demand

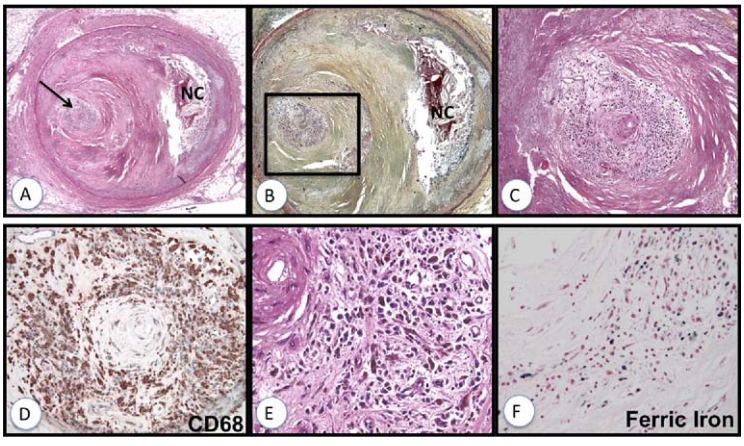

Figure 1. Role of Inflammation in Formation of Neoangiogenesis in CTO

(A) The coronary section of a fibroatheromatous plaque with a necrotic core (NC) shows chronic total occlusion (CTO) where the lumen is obstructed by a thrombus **(arrow)**, hematoxylin and eosin stain. **(B)** Similar sections as in **(A)** are shown, stained by Movat pentachrome. **(C)** A higher power view of the area within the black box in **(B)** shows a thrombus with extensive inflammation and neoangiogenesis. **(D)** Numerous CD68-positive macrophages are seen in the central lumen. **(E)** The majority of macrophages within the thrombus contain hemosiderin **(brown pigment)**, hematoxylin and eosin stain. **(F)** Pearl's iron stain reveals iron-positive macrophages **(blue)**.

of macrophage-derived foam cells (12). Angiogenesis in plaques is often accompanied by macrophages expressing hypoxia-inducible factor-1, which remains biologically active in the absence of oxygen (13). Under hypoxic conditions, a stabilized hypoxia-inducible factor-1α initiates the expression of many target genes involved in regulation and coordination of angiogenic responses. Similarly, inflammation is likely an important contributor towards pathologic angiogenesis in CTO, where densities of macrophages are generally higher in occlusions of <1-year duration as compared with >1-year duration (Fig. 1).

There are insufficient data for human CTO to confirm whether there is communication between luminal microvessels of the thrombus and arterial VV, although the report by Munce et al. (5) in normal rabbit arteries implies such connections exist. Unlike the rabbit CTO model, to the best of our knowledge, there are no published reports of human CTO imaged by micro-CT. The few published reports of human CTO mostly rely on techniques of postmortem angiography by barium gelatin infused at physiologic pressure to visualize vascular channels within the thrombus. In a study of 18 cases of human coronary CTO with postmortem angiography from our laboratory, no definite connections between microvessels associated with the thrombus and arterial VV were noted in either serial cross sections or longitudinally cut segments (Fig. 2) where proximal and distal ends of the total occlusion were visible (R. Virmani, 2009).

Another autopsy study of 10 subjects with coronary CTO documented by angiography within 3 months of death showed that the proximal ends of the occlusion were tapered in 5 lesions, with abrupt occlusion in the remaining 5; 7 occlusions were short, <15 mm, whereas 3 involved longer segment lengths. The abrupt type of occlusion was commonly associated with side branches in the absence of recanalization, although there were small vascular channels in the proximal segment without apparent communication with arterial VV. Reconstruction of short segments with tapering showed microvessels with varying diameters of 160 to 230 μm, suggest-

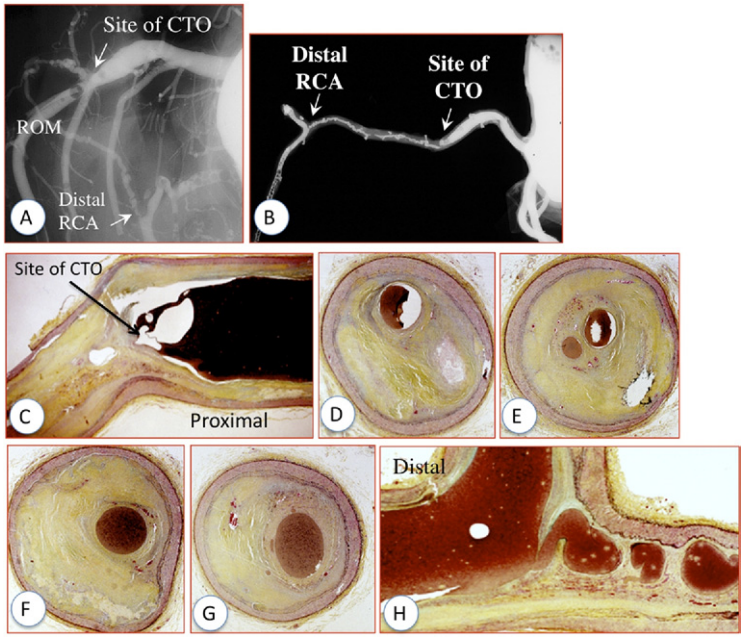

Figure 2. Radiographic and Histologic Images

This series of radiographic and histologic images correspond to a 46-year-old white man with a history of smoking, hypertension, and back pain who had a cardiac arrest while exercising. A postmortem angiogram with barium gelatin perfusion shows the chronic total occlusion (CTO) of the mid-right coronary artery (RCA) with 95% stenosis of the left circumflex and healed subendocardial posterolateral myocardial infarction (**A** and **B**). The recanalized channels of the right coronary are torturous. The proximal (**C**) and distal (**H**) right coronary artery was cut longitudinally to demonstrate the total occlusion with the distal segment demonstrating tortuous recanalized vessels. Serial cross sections (**D** to **G**) demonstrate the recanalized segment. The **red/brown** indicates recanalized channels containing barium. ROM = right obtuse marginal artery.

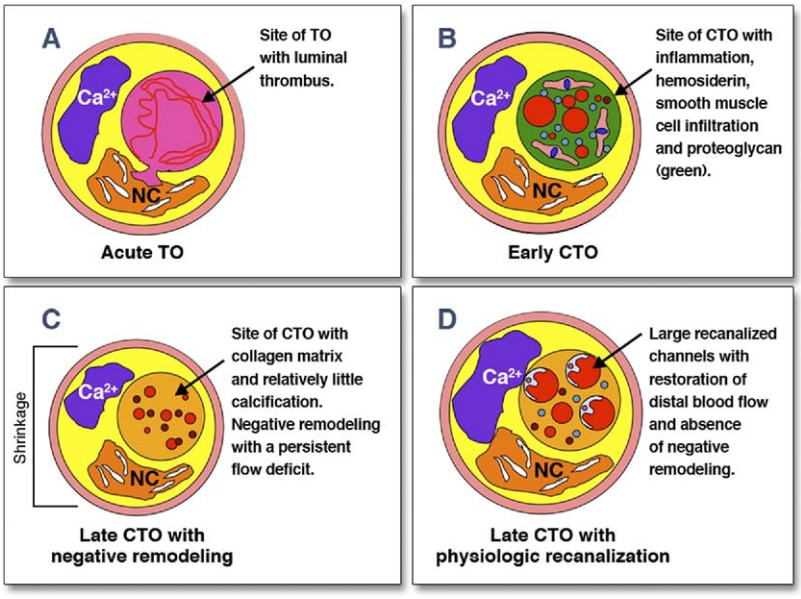

Figure 3. Morphologic Characteristics of CTO

(A) Acute plaque rupture is shown, with luminal thrombus resulting in chronic total occlusion (CTO). **(B)** Inflammation with early thrombus organization is shown. Note early recanalization accompanied by proteoglycan matrix **(green)** in the area of total occlusion (TO). **(C)** The late chronic phase of healed TO is shown, with deposition of collagen type I where the cross-linking of collagen promotes negative remodeling of the vessel. **(D)** Physiologic recanalization is accompanied by restoration of normal flow distally, thus preventing negative remodeling.

ing that such cases likely favor successful wire passage and balloon angioplasty (14). Similarly, in the study by Munce et al. (5), CTO with tapering at the proximal ("entry") end together with >85% of the length of the occlusion traversed by microchannels would lead to a greater success of wire crossing of CTO. The authors suggest that the reason for the wire entry, as well as stent placement in a false lumen, may be attributed to the location of the communicating channels between arterial wall VV and neoangiogenic vessels of the total occlusion.

From our experience, cross-talk between arterial VV and the microvasculature associated with CTO in humans is uncommon, and dissections likely result from difficulties in passing a wire at the proximal entry of the thrombus. Moreover, unlike the rabbit model, the occurrence of side branches in human CTO favors the abrupt rather than tapering occlusion, whereas the former rarely exhibits revascularization. Further, the success or failure of wire passage is highly dependent on the organization of the developing matrix within the lumen and negative remodeling, which increases with duration of the total occlusion (Fig. 3). In particular, age-related changes in intimal plaque composition from cholesterol-rich to fibrocalcific plaques may explain the adverse revascularization profile of older CTO (15).

In conclusion, the work of Munce et al. (5) expands our understanding of the sequence of healing involved in CTO. The growth of angiogenic vessels in response to total occlusion is greatest at 2 weeks in the experimental model, where, unlike the rabbit, the temporal sequence of microvascular development in human CTO remains unknown but is likely more delayed. These observations suggest that interventions aimed at promoting recanalization of the thrombus should be instituted early, before the occurrence of negative remodeling. A better understanding of the regulation of neovascularization in occlusive thrombi at the molecular level might allow for the development of new medical therapies targeted at enhancing physiologic recanalization in an attempt to restore blood flow.

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