NETosis in Disease Condition

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Ischemic thrombotic disease, characterized by the formation of obstructive blood clots within arteries or veins, is a condition associated with life-threatening events, such as stroke, myocardial infarction, deep vein thrombosis, and pulmonary embolism. The conventional therapeutic strategy relies on treatments with anticoagulants that unfortunately pose an inherent risk of bleeding complications. These anticoagulants primarily target clotting factors, often overlooking upstream events, including the release of neutrophil extracellular traps (NETs). Neutrophils are integral components of the innate immune system, traditionally known for their role in combating pathogens through NET formation. Emerging evidence has now revealed that NETs contribute to a prothrombotic milieu by promoting platelet activation, increasing thrombin generation, and providing a scaffold for clot formation.

Keywords: thrombosis ; NLRP3 ; inflammasome ; NETs

1. Introduction

In recent years, NETosis has emerged as a critical player in the context of aging and age-associated diseases [1]. Neutrophils from aged individuals or aged mice exhibit altered NETosis kinetics, with some reports suggesting an enhanced NET formation with age [2][3], or in aged individuals with severe vasculitis [4][5], while others indicate minimal or no change in NETosis in otherwise healthy aged mice [6]. The age-related changes in NETosis may contribute to chronic inflammation, a hallmark of aging, and may also impact the immune response to infections in the elderly. Therefore, understanding the mechanisms underlying the age-related alterations in NETosis is crucial for unraveling the complexities of aging.

2. NETosis in Acute Ischemic Conditions

Elevated plasma levels of extracellular histones have been reported in ischemic or thrombotic conditions, such as myocardial infarction [7], stroke [8][9], ischemic outcomes after angioplasty [10], and DVT [11][12]. Stakos et al. observed that neutrophils isolated from IRA aspiration in STEMI patients were more prone to the release of NETs in comparison to non-infarct-related coronary arteries and control individuals [13]. NETs were found to be constitutively present in the thrombi retrieved during endovascular therapy in patients with acute ischemic stroke [14]. However, the authors could not find any significant association between the circulating markers of NETs and the final thrombolysis in cerebral infarction (TICI) score [14]. Borissoff et al. reported that the circulating markers of NETosis were independently associated with the severity of coronary atherosclerosis, the occurrence of major adverse cardiac events, and the presence of a prothrombotic state [15]. Surprisingly, a deficiency of PAD-4 in hematopoietic cells did not display a significant impact on the progression of atheromatous plaques in hypercholesterolemic mice [16], despite detecting the presence of NETs in atherosclerotic lesions from human carotid endarterectomy tissue [16]. This may indicate that the mechanisms may differ between mice and humans. An alternative explanation could be that the process of inducing disease conditions in mice may not necessarily phenocopy human disease pathology. Retinal vein occlusion (RVO), which refers to the obstruction of the retinal venous system due to thrombus formation, has emerged as the second most common retinal vascular disorders [17][18]. Recently, Wan et al. reported that the elevated plasma levels of NETs are associated with RVO and can be exploited as a potential biomarker [19]. In summary, NETs have been identified in several ischemic conditions but their mechanistic contributions to pathological ischemia is not fully understood in most of the disease states.

3. NETosis in Cancer-Associated Thrombosis

Several tumors and cancer cells secrete a cytokine called the granulocyte colony-stimulating factor (G-CSF) [20][21][22][23][24], which exhibits the potential to induce NETosis and promote thrombosis [25]. Higher levels of circulating NET markers predicted the occurrence of DVT in cancer patients and was associated with a poor prognosis in a large cohort in the Vienna Cancer and Thrombosis Study (CATS) [26]. This was an important study since it included patients with malignancy

at varied sites, such as brain, breast, bronchus, stomach, prostate etc. [26]. The elevated circulating levels of MPO-DNA was also associated with thrombosis in patients with myeloproliferative neoplasms (MPN) [27]. Similarly, histone–DNA complexes have been demonstrated in the thrombi in patients with different cancer types [28][29] and circulating H3Cit has been associated with the markers of thrombosis [28].

There was also in vivo evidence for NETs promoting venous thrombosis in mouse cancer models. Demers et al. [25], using murine models of lung and breast carcinoma and chronic myelogenous leukemia, demonstrated an increased sensitivity of neutrophils undergoing NETosis. Hisada et al. observed elevations of H3Cit and cfDNA in the plasma and thrombi from mice bearing human pancreatic tumors, and the administration of DNase 1 or the depletion of neutrophils reduced the thrombus size in mice bearing human tumors [30]. Similar observations have been made by others in mammary cancer [31][32][33]. Wolach et al. further extended the findings on the role of NETs in animal models of cancer-associated thrombosis to MPN represented by the $Jak2^{V617F}$ mutation [34]. A $Jak2^{WT}$ mouse transplanted with $Jak2^{V617F}$ bone marrow had elevated NETs compared to the $Jak2^{WT}$ mice and developed spontaneous pulmonary thrombosis, which was absent when the mice were engrafted with PAD-4-deficient $Jak2^{V617F}$ bone marrow. The $Jak2^{V617F}$ mice treated with DNase 1 or the JAK inhibitor Ruxolitinib had a reduced thrombus size when subjected to the IVC stenosis model for DVT. Taken together, these findings implicated a mechanistic role of NETs in cancer-associated ischemic thrombosis.

4. NETosis and Age in the Context of COVID-19

NETosis is by far the major underlying pathway observed in COVID-19-associated thrombosis [35][36][37][38][39][40]. Age may exert a profound influence on the intricate dynamics of the immune response during COVID-19 [41][42]. Understanding this relationship is vital because it offers valuable insights into why older individuals often experience more severe forms of COVID-19. As individuals age, their immune systems undergo several changes, including alterations in neutrophil function and a predisposition to chronic inflammation, creating an environment that may prime the immune response for heightened NETosis [43]. These age-related changes can result in a more robust NETosis response when confronted with viral infections like SARS-CoV-2. Consequently, the amplification of NETosis in older individuals can contribute to increased inflammation, tissue damage, and a more severe clinical course of COVID-19 [44].

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