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Review of the NETs in infection and pathogenesis of ANCA associated Vasculitides

Ming Zhang[1], Chenglong Zhang[2], Guangqun Xing[1*]

[1]Department of Nephrology, Affiliated Hospital of Qingdao University, Qingdao, Shandong, 266003, China

[2]Department of Joint surgery, Affiliated Hospital of Qingdao University, Qingdao, Shandong, 266003, China

Abstract: **Currently, ANCA-associated vasculitides (AAV) has been testified to be associated with infectious diseases, in which Neutmphil Extracellular Traps (NETs) played a crucial role as a bridge. In this paper, the specific mechanism of NETs in infectious diseases and the pathogenesis of AAV is analyzed based on the characteristics of NETs.**

Keywords: NETs; AAV; Infection; Review

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*Corresponding Author: Guangqun Xing

1. Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitides (AAV) is a kind of progressive autoimmune disease that breaks out repeatedly and can hurt each system of the whole body[1]. Half of the patients have renal involvement[2-3] with ominous prognosis. According to the epidemiology statistics, the annual incidence of the AAV is about 46-184 per one million[4]. A number of studies have confirmed that ANCA has definite pathogenicity[5-8]. However, the pathogenesis of AAV has not been fully elucidated.

Recent studies have found that Neutrophil Extracellular Traps (NETs) played an important role in the occurrence and development of AAV. In 2009, Kessenbrocketc [9] first observed large amounts of NETs deposition in renal biopsy specimens of most renal involvement AAV patients. Abreu-velez[10] also found NETs deposition in skin biopsy of AAV patients with skin involvement. Subsequently, Söderberg[11] also found excessive NETs in the peripheral circulation of AAV patients, and the level of NETs in the peripheral circulation was significantly correlated with the activity of AAV disease. Meanwhile, as an important component of the innate immunity of the body, NETs may connect infectious events to the incidence of AAV. This paper mainly summarizes the characteristics of NETs and sorts out the role of NETs in infectious events and the pathogenesis of AAV.

2. Methods

The main databases of the literatures searched in this study are: PubMed, Wanfang database and CNKI. The search time is March 2020. The keywords “NETs”, “ANCA” and “infection” were used in the databases. After reading the title and abstract, the included studies are summarized.

3. Results

3.1 The characteristics of NETs

Neutrophils are the most abundant natural immune cells in the human immune system, and their cytoplasmic granules contain a wide spectrum of effective antibiotics. The release of these antibiotics is strictly controlled by phagocytosis, degranulation, and NETs[12], so as to prevent the damage of these cytoplasmic granules to their own tissues. Among them, the NETs are a large extracellular mesh structure with nucleus or loosened DNA from the depolymerization of mitochondria chromatin as its framework which winded by cytoplasmic protein and grain protein (including myeloperoxidase, protease-3, LL-37, elastase, lactoferrin, et.al) [13-15]. It can be used for trapping, neutralizing, and killing the invading pathogenic microorganisms[16], and prevent the spreading of pathogenic microorganisms in the body. The generation of NETs must involve peptidylarginine deiminase 4 (PAD4)[17-18], which can be inhibited by PAD4 inhibitors[19].

When neutrophils got external stimulus, PAD4, under the action of calcium ions, modified the arginine residues in the nucleus into citrulline residues through deimination, which caused the loosen and despiralization of chromatin structure in neutrophils' nuclei, and formed NETs by releasing a reticular structure outside the cell[16,19-21]. Currently, there are two ways that have been found for the generation of NETs. The first one is suicidal NETosis. To start the process, reactive oxygen ROS first triggered NE transferred from membrane related compound to cytoplasm, then combined and degraded actin[22]. After that, the nuclear membrane ruptured, so that loose nuclear chromatin can go into the cytoplasm of a complete cell, and fully mix with

particles within the cytoplasm. Then cell membrane began to permeate, and NETs were released extracellular[23]. The other one is active NETosis, in which neutrophils retained vital activity after release DNA from the nucleus[24], just as anucleate cells such as mature red blood cells. Although the two release modes were different, no necrosis was observed during the whole process when nuclear membrane cracked and DNA mixed with cytoplasmic particles.

The removal mechanism for NETs is not yet fully understood. However, current studies have confirmed that DNaseI can degrade NETs generated after infection[25]. Exogenous DNaseI injection can rapidly degrade NETs generated by staphylococcus aureus infection[26], but its endogenous removal mechanism is still not completely clear. In addition, the grain protein in NETs can still exist for a long time after the DNA's degradation[27]. It shows that the grain protein in NETs is removed by other mechanisms, which may involve the macrophage. Studies have also confirmed the removal of macrophage to NETs is a common regulation by both phenotypic dependence and time dependence [27]. But its specific removal mechanism is not fully clear.

3.2 Anomalous accumulation of NETs

Excessive generation and abnormal degradation of NETs caused by various reasons can lead to the accumulation of NETs in the body. When the auto-antigen contained in NETs was presented and immune tolerance was broken, auto-antibodies were generated and eventually lead to the occurrence of AAV. Drug-induced AAV also confirmed this hypothesis in terms of abnormal degradation. While most of Propylthiouracil (PTU) go through liver metabolism after entering the human body, a small number of PTU need the MPO modified metabolism of neutrophils. During the process when neutrophils metabolize PTU, the metabolites from the decomposition of PTU may cover the DNA in NETs, which hides the recognition sites of DNaseI and leads to long-time existence of NETs with an abnormal structure which cannot be degraded[28-29]. Animal experiments have confirmed that these structurally abnormal NETs can induce the production of MPO-ANCA[30]. In addition to drug-induced AAV, Fumihiko[31] found that the activity of DNaseI in AAV patients was significantly lower than normal people, and the activity of DNaseI was not directly related to the accumulation of NETs in the body. Genetic factors might determine the activity of DNaseI in the body. Nakazawa also found that AAV patients have a higher capacity to induce NETs' generation, but a lower capacity to degrade NETs[30]. This also provides a condition for the accumulation of NETs in AAV patients.

3.3 The vicious cycle of NETs and ANCA

The vicious cycle of NETs and ANCA may also play a crucial role in the pathogenesis of AAV. Kessenbrock[9] observed that PR3-ANCA in AAV patients could stimulate neutrophils to release NETs, and the ability to release NETs was positively correlated with the activity of AAV. Nakazawa[30] also found that MPO-ANCA could also induce neutrophils to generate NETs, and the stronger affinity of MPO-ANCA to MPO, the higher the activity of AAV, and the stronger the ability to generate NETs. In addition to classical ANCA, studies have reported that LAMP2-ANCA could also induce neutrophils' respiratory burst, promote ROS production, further promote neutrophils' NETosis, and induce the generation of NETs[17,32]. The abundant neutrophil granulocyte proteins contained in NETs have strong immunogenicity and can activate B lymphocytes through toll-like receptors to induce the production of ANCA[30]. Especially in the state of inflammation, the neutrophils would be activated by cytokines in vivo, and the activated neutrophils would express a small amount of ANCA target antigen on the surface. After binding with the target antigen, ANCA would further activate the neutrophils to promote the production of ROS and the degranulation reaction[18]. But a vicious cycle for ANCA and NETs theory is still controversial. In Kraaij et.al's study, although a significant increase of NETs was found in the patients with active AAV, there was no connection between the formation of excessive NETs and the levels of serum ANCA, and consumption of serum ANCA had no influence on the formation of excessive NETs. The excessive NETs in AAV patients didn't rely on the generation of ANCA[33]. At present, the role of the vicious cycle of ANCA and NETs in the pathogenesis of AAV still needs to be further confirmed by a large number of in vitro and in vivo experiments.

3.4 The vicious cycle of NETs and interferon-α (IFN-α)

The vicious circle of NETs and IFN-α also contributes to the pathogenesis of AAV. Some studies have found neutrophils aggregation, activation of plasmacytoid dendritic cell (pDCs), and co-localization of NETs, LL-37, and IFN-α in renal pathological specimens of AAV patients[9,34]. Meanwhile, the level of kidney IFN-α is positively correlated with LL-37[34]. Further studies have confirmed that NETs could activate pDCs through signaling pathways of Toll-like receptor 9(TLR-9) and Toll-like receptor 7 (TLR-7) and promote the expression of IFN-α[35-37]. The ability of NETs to activate pDCs depends on the association between DNA and endogenous Cathelicidin LL-37 (LL-37)[38], and the oxidation of DNA in NETs by reactive oxygen species (ROS) in mitochondria could

enhance the production of IFN-α by enhancing interferon gene stimulators[39-40]. At the same time, IFN-α could further stimulate neutrophils to form excessive NETs. Thus, the two can cause and effect each other in a continuous cycle[41]. The feedback of NETs and IFN-α provides a new model for explaining the incidence of AAV aggravated by NETs.

3.5 NETs act as a bridge between infection and AAV

Pereira[42] found that the MPO level was significantly increased in patients on the attack of chronic bronchial. NETs and bacteria were observed to intertwine in the sputum of these patients[43-44]. Meanwhile, Grabcanovic-Musija[45] found that NETs can also be observed in the sputum of patients in the remission phase of chronic bronchitis. Wright[46] confirmed that the mRNA expression of PAD4 in patients with chronic bronchitis was significantly up-regulated. Wang ZL[47] found that PAD4 in the peripheral circulation was significantly increased even in patients with remission of chronic bronchitis.

It can be seen that in pulmonary infectious diseases such as chronic bronchitis and bronchiectasis, there are repeated high levels of NETs generation in the lung infection. Meanwhile, during the remission period of the disease, the ability to generate NETs is significantly higher than that of normal people. In addition, the activity of DNaseI determined by genetic factors was significantly reduced, which provided a strong condition for the generation and accumulation of NETs. Besides, the number of alveolar macrophages in patients with Chronic bronchitis or bronchiectasis is significantly lower than that of in normal people.[46]. The phagocytosis of these small amounts of the alveolar macrophage has obvious damage which associated with the severity of disease[48]. As a result, excessive grain protein generated in NETs can not be cleared in time, which created further conditions for the exposure of autoantigen, the formation of ANCA and the onset of AAV.

The role of NETs as a bridge between infectious diseases and AAV has also been preliminarily confirmed in animal experiments. Li H[49] used subcutaneous stimulation of NETs in chronic bronchitis rat model, and found a significant increase of serum MPO-ANCA in model rats. Fan MH[50] found that serum NETs and MPO-ANCA levels were significantly increased in rats with chronic bronchitis during LPS stimulation of repeated pulmonary infection. The above studies suggest that long-term infection, NETs generated by repeated stimulation of neutrophils and repeated exposure of autoantigen may induce the occurrence of autoimmune diseases. As a bridge between infection and AAV, NETs may play a crucial role, but the specific mechanism still needs to be further confirmed by research.

4. Conclusion

In conclusion, the onset of AAV may be associated with long term infection. NETs as a bridge between infection and AAV participated in the pathogenesis of AAV. Still, a large number of experiments are needed to explore the crucial path between the participation of NETs in infection and generation of ANCA and onset of AAV , so as to reduce the occurrence of AAV through early identification of high-risk groups, early prevention, and close monitoring. At the same time, further exploration of the pathogenesis of AAV is also helpful to the development and application of specific innovative pharmaceuticals.

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