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Letter to the Editors-in-Chief

The role of Neutrophil Extracellular Traps in Covid-19: Only an hypothesis or a potential new field of research?

Neutrophils are the main cells of the innate immunity system. One of the mechanisms of neutrophils action is the formation of Neutrophil Extracellular Traps (NETs) [1].

Brinkman was the first to report the release of NETs in 2004 [2]. The discovery of NETs has spawned a new field of research in granulocytes investigation.

NETs are composed of nuclear chromatin, associated with nuclear histones and granular antimicrobial proteins. They are scaffolds, ideal for retaining microbes. The main function of NETs is trapping and killing pathogens, as such as bacteria, fungi, viruses and protozoa [1,2]. The trapping within DNA fibres prevents the spread of pathogens and facilitates the concentration of antimicrobial factors at the infection site [1].

The process of NETs generation, called NETosis, is a specific type of cell death, different from necrosis and apoptosis. It is a multi-step cell death program: enzymes from granules translocate to the nucleus and facilitate chromatin de-condensation. Then, internal membranes break down, and cytolysis releases NETs.

Both the nuclear and granular membranes disintegrate during NETosis, but plasmatic membrane integrity is maintained. This is in contrast to apoptosis or necrosis. NETosis is associated with disintegration of the nuclear envelope and mixing of nuclear and cytoplasmic material, loss of internal membranes and the disappearance of cytoplasmic organelles. More precisely, no peculiar signs of apoptosis are observed (membrane blebs, phosphatidylserine exposure, nuclear chromatin condensation and DNA fragmentation).

NETosis resembles necrosis in that both membranes are not intact, allowing intracellular proteins to leak outside the cells.

NETs release is a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent cellular death process. During activation, neutrophils produce reactive oxygen species (ROS), through the activation of NADPH oxidase [3]. ROS are involved in NETs release through a neutrophil elastase-mediated mechanism: it translocates from cytoplasmic granules to the nucleus and triggers chromatin degradation through histone cleavage [1–3]. Also myeloperoxidase contributes to the nuclear DNA de-condensation [1–3]. The role of oxidative stress in NETosis has been carefully reviewed [3,4].

An intriguing point about NETosis is that current evidence suggests that it is not only a death pathway: two different mechanisms have been described, and one of them could be considered the “vital” NETosis, as already carefully reviewed [5]. Also the “vital” NETosis allows NETs release. The principal differences between the two forms are the nature of the trigger stimulus, the timing and the mechanisms employed to make NETs release [5].

The involvement of NETosis in several diseases (other than infections) has been established, in particular autoimmune diseases, cancer, venous thromboembolism, atherosclerosis, diabetes, etc. [6–8].

1. NETs and Covid-19: What we need to know

Viruses are known for their ability to evade the body's immune response. Recently, it has been shown that they can act as triggers of NETosis processes [9–11].

In fact, many viruses can stimulate neutrophils to produce NETs. Different responses of neutrophils have been observed: classical NETosis, the production of antiviral agents or even the switch to apoptosis.

Virus-induced NETs (made up of complexes of double-stranded DNA, histones, granular proteins) can circulate in an uncontrolled way, leading to an extreme systemic response of the body with the production of immune complexes, cytokines, chemokines, finally favouring inflammation.

It is therefore clear that the virus-induced NETosis acts as a double-edged sword: there is the mechanical entrapment of the virus, but the inflammatory and immunological reaction triggered by the release of the NETs can be harmful by itself.

To date, there is no data in medical literature on the role of NETs in Covid-19 infection, a novel viral infection that leads to highly lethal interstitial pneumonia and for which there is currently no vaccine nor specific therapy [12].

In this scenario, the primary objective is understanding if NETs may be implicated in the response to Covid-19 and by which mechanisms. Concrete therapeutic proposals could derive from the knowledge of this form of innate immunity. To do this, it will be necessary to evaluate the activity of NETosis in patients with Covid-19 and evaluate whether the clinical course of the disease (clinical worsening or healing) may modulate NETosis.

Of note, it has been established that NETosis appears to be closely linked to the inflammatory response also in pulmonary diseases. In fact, NETs increase in patients with Acute Respiratory Distress Syndrome (ARDS) as shown in studies on bronchoalveolar lavage fluid [13,14], as well as in patients with acute respiratory failure during Chronic Obstructive Pulmonary Disease (COPD) exacerbation [15]. Similarly, advanced forms of Covid-19 are often characterized by hyper-inflammation (“cytokine storm”) with the development of an ARDS-like condition [12].

Up to now, many studies have confirmed the occurrence of several thrombotic complications in Covid-19 infection (both venous thromboembolism and arterial thrombotic complications) [16,17]. Furthermore, reports of micro and macro thrombotic phenomena such as microangiopathy, pulmonary embolism [18] have been frequently reported, which has led to a careful evaluation procedure for antithrombotic prophylaxis and/or coagulation in Covid-19 patients [16–18]. This issue is very important because it is related to the fact that NETosis seems to play an important role in all conditions characterized by venous and arterial thrombosis, as numerous evidences

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have confirmed [19–21]. NETosis has also been documented at the microvascular level, such as in many vasculitis, thrombotic microangiopathies such as Moschowitz syndrome [22].

Also the activity of DNAsi I (the enzyme implicated in the "digestion" of NETs) and the phagocytic activity of macrophages should be investigated in detail, as these are the two main mechanisms for regulating and self-limiting NETosis itself [1–2].

New frontiers in NETs evaluation in covid-19 may be represented by testing NETosis activity directly on bronchial alveolar fluid of patients after bronchoscopy or after sputum induction, using previously described approaches [12–15].

The final goal concerns the possibility of creating a NETs-oriented clinical trial. If it is true that the production of NETs occurs in conjunction with ROS increase, it is rational to study signal pathways involved in the response to oxidative stress, such as the pathway regulated by the Nuclear erytroid-related factor 2 (Nrf2) Nrf2/antioxidant related elements (ARE), the main transcription factor involved in antioxidant defence [23]. Possible therapeutic implications with Nrf2 activators (such as Resveratrol and Sulforaphane) [24,25] may then be considered.

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Chiara Mozzini*, Domenico Girelli

Department of Medicine, Section of Internal Medicine, University of Verona, Piazzale L.A. Scuro, 10, 37134 Verona, Italy

E-mail address: chiara.mozzini@univr.it (C. Mozzini).

* Corresponding author.