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Neutrophil Extracellular Trap (NET): The Interplay Between Infection, Inflammation and Thrombosis

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Abstract

While the observation that the interactions between infection and thrombosis is a well-known fact, the mechanism behind this connection was not clear. Also, it was known for a long period that thrombus contains neutrophils within its skeleton. Histon an intranuclear component, was known to have a very strong microbicidal property, the question was how could it reach its target? Could the discovery of this new mechanism in neutrophil biology; namely neutrophil extracellular trap (NET), answer these issues? Hopefully it could. This review will focus on the production of NET (NETosis), its implications in different diseases, its role in understanding the connection between infection, inflammation and thrombosis, finally we will look for the potentials of targeting it, for therapeutic benefits.

Keywords: Neutrophil Extracellular Trap; Inflammation; Thrombosis

1.Introduction

Neutrophils are the first line of defense against invading pathogens. The main mechanism of defending is through phagocytosis [1].

In 2004, a new mechanism of neutrophils activity was discovered, namely neutrophil extracellular traps (NETs). In which neutrophils degranulate releasing its cytoplasmic and nuclear contents. This content is called (NET) and the process of its formation is called NETosis. NETs are large polymer structures and are capable of sterilization of the surrounding space. NETs are backbones consisting of DNA/histones and are studded with anti-microbial peptides

that normally reside within the neutrophil granules [1].

2. Nets Function

The main role of NETs is to trap the invading pathogens preventing them from dispersion. Not only this, but at the same time the ensnared pathogens are to be exposed to a high concentration of anti-microbial effectors. These anti-microbial effectors include, the anti‑microbial proteins of the neutrophil granules and histone [2].

3. NET Morphology

Fully hydrated NETs have a cloud-like appearance and occupy a space that is 10-15-fold bigger than the volume of the neutrophil [2]. It consists of smooth filaments with a diameter of ~17 nm. These filaments are mainly composed of modified, nucleosomes that are stacked on each other. The surface of these modified nucleosomes are studded with granular proteins of globular shape with a diameter of ~50 nm [2] (Figure 1).

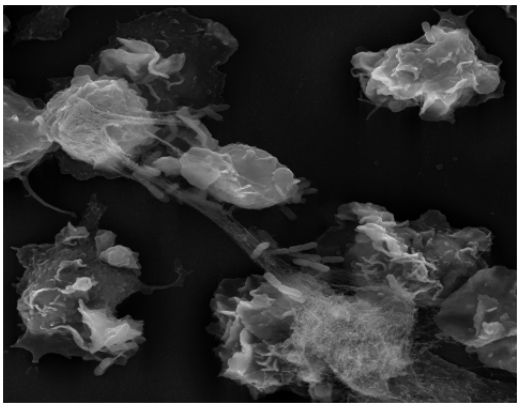

Figure 1: Scanning electron micrograph of NETs ensnaring Shigella flexneri Image courtesy of Volker Brinkmann.

4. Netosis is Not Apoptosis nor Necrosis

In apoptosis DNA is to be fragmented and the nucleus is to be shrinked, but no breakdown in the nuclear membrane. In sharp comparison, in NETosis there is a nuclear membrane breakdown, followed by decondensation of the chromatin, then the decondesed chromatin is to be mixed with the antimicrobial proteins from the neutrophil granules. Finally the cell wall ruptures and the contents are released outside the neutrophil [3-5]. Necrosis in the other hand, no changes in the nuclear membrane, however the typical lobulation of the neutrophil nucleus is lost [6].

5. NETosis

The hallmark of the process is the production of reactive oxygen species (ROS) by the neutrophil NADPH oxidase, without it NETosis is not possible. Failure of production of (ROS) means failure of production of NETs, with susceptibility to severe infections. Myeloperoxidase; another important enzyme in ROS formation, is needed for NETosis. Individuals missing this enzyme are also unable to make NETS [7]. Chromatin is to be decondesed by neutrophil elastase, the enzyme that can partially degrade histones .Only after this partial degradation of histone,

chromatin can now be decondesed [8]. Steps include the production of ROS, the migration of the protease neutrophil elastase (NE) and later myeloperoxidase (MPO) from granules to the nucleus, the decondensation of histones, and eventually the rupture of the cell [9].

DNase I can degrade NETs in the blood stream, otherwise uncontrolled NETosis can be devastating. One of the devastating consequences of the failure of this degradation of NETS, is the generation of anti-self antibodies, with the development of autoimmunity, such as systemic lupus erythematosus (SLE) [10].

6. Stimuli for NETosis

Almost all kinds of infections can stimulate NETosis. Infections with bacteria, fungi, HIV and parasites induce NETs [9]. Reactive oxygen species (ROS), like hydrogen peroxide can also stimulate NETosis [4]. NET formation is also triggered, by antibodies [11], antibody–antigen complexes [12, 13], by microbial components such as lipopolysaccharide [14], M1 from B hemolytic Streptococcus [15], and phosphoglycans from Leishmania species [16].

Whatever the stimulus is, it should have the capacity to activate neutrophils through the interaction with the MAC-1 integrin receptors. This integrin receptor is not usually expressed in the circulating neutrophils, probably preventing excessive formation of NETs in circulation and avoiding thrombus formation [14].

7. NETS-Mechanisms of Microbicidal Activity

Trapping microorganisms and exposing it to a very high concentration of a very potent antimicrobials, is to be considered an ideal way of fighting microbes. This is what is really done through NETosis [2]. The antimicrobial effectors of NETs include histones, neutrophil elastase, cathepsins, proteinases, calgranulins, lysozymes, proteases, defensins and many others [1, 2, 21]. This antimicrobial effect is lost by digestion of NET by DNases. Accordingly, the expression of these DNases is essential for these bacteria to be pathogenic [10]. Microbes most likely stick to NETs through charge interactions [17, 18]. Pathogens can mask themselves with a capsule or by changing their surface charge, thus preventing binding to NETs [19].

Y. Weinrauch, et al found that Neutrophil elastase (NE) on the NETs can inactivate the virulence factors of Shigella flexneri, Salmonella typhimurium, and Yersinia enterocolitica [20]. Cathepsin G and Proteinase 3, are closely related to NE and are able to cleave many virulence factors of a different class of pathogens [21]. The ion chelator; calgranulin is responsible for the antifungal activity of NETs [22].

8. Histone, A Very Potent Antimicrobial Agent!

The antimicrobial activity of histone was discovered around the middle of the previous century [23]. This was further proved by finding that antibodies against histone, neutralize the antimicrobial activities of NETS [2]. Histones kill Gram-positive and -negative bacteria [24] and parasites [25]. One mole of histones kills ~100-fold more bacteria than other antimicrobials, such as defensins [25]. Histones also kill mammalian cells, so that histones

are implicated in the pathogenesis of multiorgane failure in sepsis [26]. The question was, how could this nuclear component be accessible to its target, without producing unwanted effects? Through NET formation, neutrophils provide histones with an opportunity to access its target, in close proximity [3].

9. Nets: The Interplay of Infection, Inflammation and Thrombosis

NETs provide a new link between innate immunity and thrombosis. NETs can stimulate, almost all steps of thrombosis. It can activate platelet adhesion, platelet aggregation, extrinsic pathway of coagulation and intrinsic pathway [27, 28]. Also, due to its large size, it may promote thrombus stability, in a similar way like Von Willbrand factor (VWF) and fibrinogen do [29].

Accordingly, NETs were found to be abundant, in experimental deep vein thrombosis in mice and baboons [30]. On the other hand, activated platelets can trigger neutrophils to release NETs [28]. S.R. Clark and colleagues found that activation of platelets through Toll-like receptor 4 (TLR-4), results in rapid NET formation [17]. The interactions between platelets and NETs is mediated through binding to an adhesion molecules such as fibrinogen, VWF and fibronectin [30, 31].

After thrombolysis takes place, NETs need to be degraded, like fibrin and VWF. NETs are to be degraded by DNAase, while fibrin is to be degraded by plasminogen system and VWF by ADAMTS13 [32]. The role of DNAase in thrombolysis was demonstrated by the elegant work of Tobias and colleagues. In his work, Tobias et al. observed that the clotted specimen missing DNAase was not lysed even in the presence of plasmin, while only the specimen containing both plasmin and DNAase was lysed [32, 33].

Not only this, but even before the discovery of NETs, Nucleic acids was shown to be able to activate coagulation, with RNA binding both factor XII and XI in the intrinsic pathway [29]. Also, histones was demonstrated to be a very powerful stimulus for thrombin generation, platelet activation and platelet aggregation in a platelet-dependent manner [27].

10. Nets is Also Implicated in Diseases

From previous discussion, it is now obvious that NETs could be incriminated in thrombotic disorders by acting as a scaffold for thrombus formation [4]. NETs was detected in venous thrombosis model in mice [33]. Any failure of proper degradation of NETs will expose the hidden antigens to the immune system, with consequences of autoimmunity. In this regard it was shown that the neutrophils isolated from SLE patients form excessive amounts of NETS in comparison to normal people, particularly in response to antibody complexes [34].

The reason for this high propensity for NET formation in SLE patients is hypothesized to be due to decreased degradation. This decrease in NET degradation was demonstrated and was found to be due to either the presence of DNase1 inhibitors or a high titer of anti-NET antibodies [36, 37]. Mutation in DNase1 or to DNase1-like 3, is

associated with high propensity for development of SLE [35]. Immune complexes isolated from other autoimmune diseases, such as small vessel vasculitis or Wegener's disease, also was found to induce NET formation [11].

Patients with Felty's syndrome, a form of rheumatoid arthritis, produce autoantibodies against citrullinated histones [3].

In ulcerative colitis, DNA-bound lactoferrin was found to be the major target for antineutrophil perinuclear cytoplasmic antibodies. DNA-bound lactoferrin is a neoantigen that is present in NETs [38]. Excessive NET formation, as it occurs in sepsis was associated with tumor metastasis [39, 40]. This is being hypothesized due to the promotion of early adhesive events between NETs bound to tumor cells and endothelial cells of blood vessels [41].

11. Manipulating Nets for Therapeutic Benefits

DNAse administration was effective in preventing thrombotic complications arising as a result of NET formation in murine models of malignancy [42]. The binding of NETs to circulating tumor cells was abrogated by NET inhibition with DNAse and/or a neutrophil elastase inhibitor [41]. One interesting approach comes from studying of thrombomodulin. Thrombomodulin was found to be protective against endothelial dysfunctions in sepsis, with favorable therapeutic profile against sepsis induced coagulopathy [43].

Accordingly, recombinant human-soluble TM (rTM) is now in use for the treatment of disseminated intravascular coagulation in sepsis, in Japan [44]. What is interested is the Shimomura et al observation of the ability of rTM to fully inhibit NETosis in neutrophils cultured with platelets and in the presence of LPS [45]. From electrical charge point of view, NETs are polyanionic polymers.Using the Polyamidoamine (PAMAM) dendrimers, such as spermine, as poly-cationic inhibitor,Jain S and colleagues demonstrated a promising effects in inhibiting nucleic acid and NET mediated coagulation both *in vivo* and *in vitro* [46].

12. Future Directions

The role of NETs in thrombosis should lead a search for any abnormalities in what could be labelled (NET system), in thrombophilia. Likewise, atherosclerotic diseases which represent a major health burden, is it the time to look at it from another view? Could this view be (NET system)?

The role of this system in the pathogenesis of autoimmunity, should lead for a novel therapeutics dealing with this system. Also, its role in cancers and cancer metastasis is expected to deserve more search in the near future.

As NET is a product of neutrophils, which in turn are the cells responsible for fighting invading microbes, so that it is possible that the abnormalities in NETS are a consequence of any abnormalities in microbes. This point is expected to be studied extensively in the near future, creating a new insight for pathogenesis of different diseases.

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