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Research Progress on the Effect and Detection Methods of Neutrophil Extracellular Trap

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Abstract

The neutrophil extracellular trap net is an important component naturally existing in the body's immune system, which is formed and released by neutrophils. It contains a variety of protein components and enzymes with bactericidal activity, which can inhibit or kill pathogens invading the body and participate in a variety of immune reactions. However, under certain conditions, it will have harmful effects on the body such as tissue cell damage, inflammatory reaction, thrombosis, etc., which can promote the development of some diseases and aggravate the illness. In this study, the immune function, damage and advantages and disadvantages of NETs detection methods were reviewed.

Keywords

NETs; Immunity; Injury effect; Detection method; Research progress.

1. Introduction

Neutrophils play an important role in innate immunity. They originate from bone marrow and are the most abundant in peripheral blood. They have a wide range of nonspecific immunity. Neutrophil Extracellular Traps (NETs) are a major research direction in the study of the mechanism of action of neutrophils in recent years. The formation mechanism of NETs can be divided into early insolubility and late solubility according to Boeltz S in his paper [1]. Neutrophil activation leads to the formation of both soluble and insoluble NETs structures, which are activated by multiple different receptors in recognition of internal environmental infection and sterile environmental stimuli [2]. Many scholars study shows that the NETs in the host has a "double-edged sword" role in the steady state, can not only kill pathogens that invade the host and at the same time also can cause tissue damage, acute, chronic inflammation, thrombosis blocking blood vessels, causing the body's physiological pathology and organization structure change, leading to autoimmune, inflammatory bowel disease and metabolic disease, and affect the body microcirculation. [3-6].

2. The Immune Effect of 1.NETs

NETs have a variety of protein components attached to them, Including histone H2A/H2B, cathepsin G, lactoferrin, penetrating protein, gelatinase, peptidoglycan binding protein, calprotectin, poreforming protein, dermal D(GSDMD) and other bactericidal active proteins, chromatin, leukocyte proteinase 3, antimicrobial peptides, neutrophil elastase and myeloperoxidase. It can not only directly kill pathogens, but also inhibit the spread of pathogens [7].

2.1. Anti-pathogen Mechanism

Xu Lifeng showed in his study that the main components with anti-pathogen effect are histone, calvatin, neutrophil elastase (NE) and myeloperoxidase (MPO) [8]. Histones have a highly effective antibacterial effect, but their functions include mediating cytotoxicity, which can cause

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damage to tissues and cells of the body. Calvatin inhibits pathogens by combining with metal ions to form chelates, thus inhibiting the reproduction of pathogenic microorganisms [9]. Neutrophil elastase can directly kill bacteria and degrade their virulence; In contrast, NETs cannot be formed in the body due to complete myeloperoxidase deficiency [8]. In our study, We also identified pathogens that can stimulate the formation and release of NETs by neutrophils, including 16 types of bacteria including Salmonella typhimurium, Vibrio cholerae, Staphylococcus aureus, and Pseudomonas aeruginosa, 3 types of mycoplasma including Mycoplasma pneumoniae, and 4 types of fungi including Candida albicans and Aspergillus fumigatus. Nine groups of viruses, including HIV, feline leukemia virus and hepatitis B virus, and 10 groups of parasites, including Leishmania protozoa, Plasmodium falciparum and Trypanosoma cruzi. Stimulation of neutrophils to produce NETs by the listed pathogens not only inhibits the growth, reproduction and metastasis of the pathogens, but also may cause tissue cell damage.

2.2. Antiviral Effect

Studies have shown that neutrophils play a critical role in the antiviral immune mechanism produced by the body [10, 11] and that viral stimulation of NETs has a strong antiviral effect and can also cause severe cell damage to tissues. The antiviral process of NETs mainly involves the stimulation of neutrophil formation and release of NETs by the virus, and then the main components of NETs inhibit proliferation and kill the virus, while the viral escape mechanism is to weaken the antiviral effect and immunopathological response of NETs during the formation of NETs [12]. At the same time, during the antiviral process, tissue cells are damaged, and even case effects occur, such as dyspnea, autoimmune diseases and thrombosis [13].

2.3. Antiparasitic Effect

Lismania protozoa, Plasmodium falciparum, Trypanosoma cruzi, histolytica amoeba, Eimeria, Toxoplasma, neospora, Schistosoma japonicum, hemorrhoedia, and nematode spirochete are the parasites that can currently induce the formation and release of NETs [8]. Studies have shown that NETs in vitro can kill some protozoans, such as Leishmania protozoa or T. gondii tachyzoites. But there are also parasites that cannot be killed, such as the tissue lytic amoeba trophoblast. NETs had only limited effect on larvae of worms and almost no effect on adults. At the same time, NETs may aggravate the local inflammatory response, resulting in tissue damage [14].

3. NETs Damage

NETs role is widely, such as respiratory damage, acute myocardial infarction (mi), pancreatic disease, ischemic stroke, acute lung injury (ali) and acute respiratory distress syndrome damage effects of airway inflammation and other diseases, this article on respiratory damage, acute myocardial infarction (mi), pancreatic disease, ischemic brain died of acute lung injury (ali) as an example.

3.1. Respiratory Tract Injury

NETs have anti-bacterial, anti-fungal, and anti-viral effects in the respiratory tract, but under certain conditions they can also damage the respiratory tract and cause severe respiratory illness. Such as acute lung injury, acute respiratory distress syndrome, airway obstruction, tuberculosis, etc. [15]. Acute lung injury and acute respiratory distress syndrome during the acute phase, m1-type alveolar macrophages destroy tissues and simultaneously produce tumor necrosis factor -α and γ infectious agents to stimulate neutrophils to produce and release NETs, which can aggravate lung injury [16]. High levels of DNA in NETs can increase the viscosity of mucus, which can cause airway blockage when the viscosity reaches certain levels [17, 18]. In

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the early stage of tuberculosis infection, neutrophils are the first to gather in the infected lungs and become a new host of mycobacterium tuberculosis and stimulate the production of NETs, but NETs can only capture mycobacterium tuberculosis, but cannot kill or inhibit mycobacterium tuberculosis, thus aggravating the damage of the infected lungs [19, 20].

3.2. Acute Myocardial Infarction

The main pathogenesis of myocardial infarction is rupture of atherosclerotic plaque, thrombosis, and obstructing of blood circulation, resulting in myocardial infarction. NETs may be involved in the rupture of atherosclerotic plaque and play an important role in unstable plaque [21]. In the early stage of thrombolysis, neutrophils form and release NETs, and some studies have shown a positive correlation between NETs and mi size. NETs may play a damaging role in the vascular site of the offender in MI by stimulating thrombosis and promoting inflammatory response [22]. The mechanism of ACTION of NETs in ami remains unclear.

3.3. Pancreatic Diseases

Acute pancreatitis and pancreatic cancer are the most common diseases of the pancreas. Some scholars indicate that in acute pancreatitis, can inhibit the release of the NETs, can reduce the inflammatory response and reduce the damage to the cells of tissues, especially of pancreatic tissue damage, and the NETs is to promote the mechanism of acute pancreatitis by promoting bile pancreatic if formation and blocked bile pancreatic duct, bile and pancreatic juice outflow obstruction, form circumfluence, It eventually develops into acute pancreatitis [23]. NETs promotes proliferation, metastasis, and thrombosis in pancreatic cancer.

4. NET S Detection Method

NETs play a double-edged role in protecting the body from pathogens such as bacteria, fungi, mycoplasma, viruses and parasites. On the other hand, it can cause injury, inflammatory reaction and thrombosis to body tissues and cells, and promote the formation of diseases such as respiratory tract injury, acute myocardial infarction, pancreatic disease, ischemic stroke, acute lung injury and airway inflammation of acute respiratory distress syndrome. The following table lists the latest detection methods at home and abroad and their advantages and disadvantages [24].

5. Summary and Outlook

Neutrophil extracellular traps (NETs) are generated by the formation of neutrophils. Neutrophils are one of the most important immune cells to protect against external infection in the body. Their bactericidal modes of phagocytosis and degranulation play an important role in the innate immune system. However, when neutrophils reach the site of infection, the pathogen can stimulate the formation and release of NETs. With the accumulation of a large number of NETs, the body can be damaged, such as tissue damage, inflammatory response, autoimmune diseases, thrombosis, etc., and can also participate in the occurrence and development of various diseases. The MICROSCOPY technique used in NETs is direct, but is cumbersome, time-consuming, and subject to subjective influences. Gradually with the development of technology, to achieve high throughput, automation testing, and by the in situ and in vitro were tested in vivo test development, implementation of various diseases of NETs in the process of development levels and trends, the future can be used as the auxiliary diagnosis methods of some diseases, but also need to automation, simple, cheap, improvement and innovation of higher accuracy.

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Table 1. The latest detection methods at home and abroad and their advantages and disadvantages

NETs test method		The advantages and disadvantages
In vitro detection method	Enzyme linked immunosorbent	The method is sensitive, rapid, low cost and can be automated, but has poor repeatability. It has certain value for the early diagnosis of autoimmune diseases.
	Immunofluorescence staining of cells	Simple operation, can be qualitative, location and quantitative detection; The interpretation of the results differs greatly between different observers and is easily disturbed by subjective assumptions.
	Fluorescence spectroscopy	High-throughput, rapid assay was achieved with a high degree of objectivity and quantification, but with errors. Not all CF-DNA was derived from NETs, and dead cells needed to be excluded to increase assay specificity.
	Flow cytometry	Efficient, rapid, precise, multi-parameter, and high-throughput, neutrophils do not need to be purified, but cells that have dissolved or are in advanced NETosis may be ignored.
	Electron microscopy	High resolution, high magnification was used for qualitative testing, but the morphological difference between NETs and blood fibrin was not significant and the results needed to be validated again by immunofluorescence microscopy
In situ and in vivo detection methods	Western blotting	The advantage is that NETs associated proteins can be qualitatively and semi-quantitatively analyzed in terms of protein expression, but the disadvantage is that the preparation process of protein samples is time consuming, variable, and susceptible to various factors
	Tissue immunofluorescence staining	Its application in vivo detection of NETs is limited due to complicated operation procedures, easy removal of NETs during washing, and observer influence during interpretation of results.
	In vivo imaging technique	A special case of NETs in situ testing is where live cell imaging is performed immediately after a laboratory animal is killed, without the need to immobilize a specific organ.
	Vivisection microscopy	Qualitative and quantitative detection can be carried out. The advantages of this method are high throughput, objective and simple, without special instrument detection, but a large amount of data is needed to train the model to satisfy the image processing process

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