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Neutrophil Extracellular Traps in Respiratory Disease: guided anti-microbial traps or toxic webs?

B. Cortjens *, J.B.M. van Woensel, R.A. Bem

Paediatric Intensive Care Unit, Academic Medical Centre, Emma Children's Hospital AMC, Amsterdam

EDUCATIONAL AIMS:

The reader will be able:

- To define the process of NETosis and formation of NETs.
- To discuss the potential protective effects of NETs during respiratory diseases.
- To discuss the potential immunopathological effects of NETs during respiratory diseases.
- To discuss potential future treatment options targeting NETs in the airways and lungs.

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ABSTRACT

Neutrophil recruitment to the airways and lungs is a major hallmark of many respiratory diseases. One of the more recently discovered unique innate immune effector mechanisms of neutrophils is the formation of neutrophil extracellular traps (NETs), consisting of an extracellular network of DNA fibers studded with nuclear and granule proteins. Although in the respiratory system NETs contribute to capture and inactivation of bacteria, fungi and viruses, there is a delicate 'balance' between aid and damage to the host. Accumulating evidence now suggests that NETs can have direct cytotoxic effects to lung epithelial and endothelial cells and can contribute to airway obstruction. As such, NETs may play an important role in the pathogenesis of respiratory diseases. The purpose of this review is to give an up-to-date overview of the current status of NETs in respiratory diseases. We examine both experimental and clinical data concerning the role of NETs in host defence as well as immunopathology, with special attention paid to the literature relevant for the paediatric pulmonology community. Finally, we discuss future treatment strategies that may target the formation of NETs in the airways and lungs.

INTRODUCTION

The respiratory system, representing a primary entry site of many distinct microbes, is particularly dependent on strong innate immune surveillance. Neutrophil recruitment to the airways and lungs is a major hallmark of many respiratory diseases, ranging from pulmonary infections to asthma and acute respiratory distress syndrome (ARDS). In the classical paradigm, neutrophils utilize their effector functions in the host defence against bacteria and fungi [1,2]. However, strong recruitment and activation of neutrophils is also seen in viral respiratory infections [3–6]. Chronic neutrophil-dominant airway infiltrates are seen in paediatric cystic fibrosis (CF) [7–9] and in severe asthmatic patients [10], where neutrophils contribute to airway remodelling and mucus hypersecretion [11,12].

Neutrophils have three main effector mechanisms for direct anti-microbial activity (Fig. 1), although in the last decade new modulatory and effector functions (e.g. immune suppression, and tumor suppression [13,14]) have been discovered. First, neutrophils are known for their phagocytizing capacity, involving the engulfment and killing of opsonized extracellular pathogens with the aid of toxic granule proteins and production of reactive oxygen species (ROS). Second, neutrophils can act against extracellular microbes by secreting toxic proteins and enzymes, including myeloperoxidase (MPO), elastase and defensins, from their

* Corresponding author. Paediatric Intensive Care Unit, Emma Children's Hospital AMC, Meibergdreef 9, 1105 AZ, Amsterdam.
E-mail address: b.cortjens@amc.nl (B. Cortjens).

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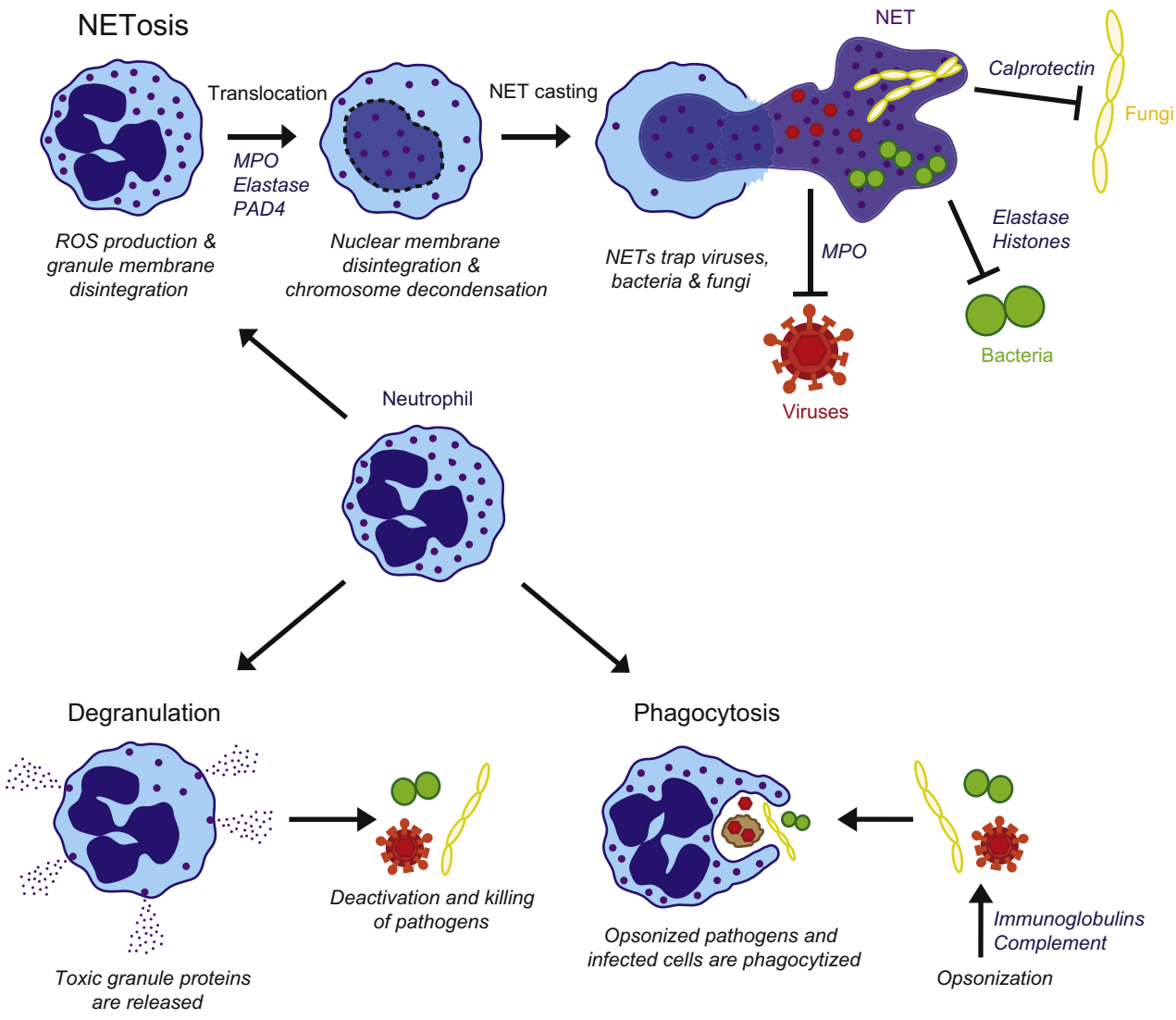

Fig. 1. Neutrophil defences against pathogens.
Neutrophils undergo the process of NETosis, which leads to the formation of NETs under influence of various triggers (e.g. cytokines, fungi, viruses, bacteria). These NETs, consisting of extracellular DNA packed with nuclear (e.g. histones) and granule (e.g. MPO, elastase) proteins, can trap and/or neutralize pathogens. Other main effector functions of neutrophils include the classical mechanisms of degranulation with secretion of anti-microbial proteins and phagocytosis of pathogens. This latter process is enhanced through opsonization by the complement system or immunoglobulins. *NETs;* neutrophil extracellular traps, *MPO;* myeloperoxidase, *PAD4;* peptidylarginine deiminase 4

granules by the process of degranulation. In addition, ROS and a number of pro-inflammatory cytokines are released to the extracellular microenvironment aiding in the innate immune response.

A third, unique killing mechanism, discovered more recently in addition to phagosome- and granule-mediated killing, involves the formation of neutrophil extracellular traps (NETs) (Fig. 1). These traps are produced by neutrophils as a last resort suicide mechanism, ensuring effective pathogen killing even after their death. As will be described in more detail below, NETs are large extracellular network-like structures consisting of DNA studded with several granule proteins (e.g. MPO, elastase) and nuclear proteins (e.g. histones). The genomic DNA-strands form the backbone of the NET, organized by the release of modified histones. NETs can expand up to 15 times the size of the originating cell [15,16], which tremendously increases the range for effective capture of various large, but also small sized pathogens with subsequent killing or neutralization by the toxic proteins coated on the NETs. NETs were discovered in 2004 by Brinkmann and colleagues who stimulated isolated neutrophils *in vitro* with potent neutrophil activators (Phorbol 12-myristate 13-acetate (PMA), LPS and CXCL-8/IL-8) to produce NETs and observed bacterial killing by these structures [16]. Currently, the cellular events leading to the formation of NETs are being defined, as well as their precise functions and clinical implications [17]. Given the unique neutrophil biology and their high numbers under both physiologic and pathophysiological conditions in the lungs, research on NETs will have an impact on our knowledge regarding the pathophysiology and treatment of many respiratory diseases. In this review, we therefore aim to provide an overview of the current literature regarding NETs and their role in respiratory diseases, with special attention to studies relevant for respiratory diseases in children.

NETosis

Before discussing NETs in the context of respiratory disease, we here present a brief overview of NETs biology. The cascade of events leading to NET formation is termed NETosis. NETosis can be initiated by various triggers, including direct stimulation by pathogens (bacteria, viruses or fungi) and pro-inflammatory cytokines (e.g. CXCL8/IL-8), and appears dependent on activation of Toll-like receptor (TLR) pathways [18,19]. NADPH oxidase