
Neutrophils and neutrophil extracellular traps orchestrate initiation and resolution of inflammation

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ABSTRACT

Neutrophils, the most abundant leukocytes in the human body, are considered to be the first line of defense in the fight against microorganisms. In this fight neutrophils employ weaponry such as reactive oxygen species produced via the NADPH oxidase complex 2 together with the release of intracellular granules containing antimicrobial agents. The discovery that activated neutrophils release decondensed chromatin as DNase-sensitive neutrophil extracellular traps (NETs) lead to a renewed interest in these leukocytes and the function of NETs *in vivo*. In this review, we will focus on desirable as well as detrimental features of NETs by the example of gout and pancreatitis. In our models we observed that neutrophils drive the initiation of inflammation and are required for the resolution of inflammation.

Introduction

The immune system is a complex network of cells, molecules and organs with the main function to protect the body from invading and endogenous pathogens. It further participates in the prevention of damage caused by attacking micro- and macroorganisms. Their contribution in the resolution of inflammation has only recently been described in several models of health and disease. However, so far few biological mechanisms are known that lead to the removal of a specific target without causing collateral damage of surrounding cells and tissues (1, 2). The neutrophil, the most abundant leukocyte in the human body, is considered to be the first line of defense in the fight against microorganisms. Since neutrophils deploy a rather unspecific weaponry, controlling neutrophilic inflammation is strictly necessary to avoid collateral damage (3, 4). Neutrophils were long considered as simple downstream executors of immune responses and their mode of ac-

tion was thought to be limited: Phagocytosis of pathogens with their subsequent killing and degradation, and release of antimicrobial agents by degranulation. Both intra- and extracellular killing of microorganism is closely related to production of reactive oxygen species (ROS) via the NADPH oxidase complex 2 (5, 6). However, recent research revealed neutrophils as more sophisticated immune cells that are able to precisely regulate their granular enzymes by ion fluxes, release immunomodulatory cytokines and chemokines (*e.g.*, IL-8, CXC chemokine receptor-2 ligands), interact with various components of the immune system, and therefore can play a key role in (auto)immunity (7-11). Furthermore, neutrophils are involved in processes they were considered to be excluded from, like the killing of intracellular pathogens and their participation in the regulation of the adaptive immune system. They are also important players in several diseases including allergy, atherosclerosis, thrombus formation and metabolic disorders (12-14). Several of these new attributes have been associated with the ability of activated neutrophils to release decondensed chromatin decorated with granular content as DNase-sensitive neutrophil extracellular traps (NETs) (9). NETs act as a scaffold for the aggregation of viable, necrotic and apoptotic cells as well as particulate matter such as crystals and microbes (15). The process of NETosis preferentially takes place in preformed cavities and to a lesser extent inside the connective tissues. This additional attribute of neutrophils has started an intense discussion over the role and function of NETs *in vivo*. Specific conditions influence whether a NET will act inflammatory or anti-inflammatory and the question for researchers and clinicians remains, which conditions direct the mode of action of NETs in specific models of disease.

'Good' NETs in gout

Gouty arthritis is an acute inflammatory reaction which is initiated by precipitation of oversaturated solutions of uric acid as monosodium urate crystals (MSU). Untreated, gout can cause significant structural damage, mostly of bone, cartilage and tendons (16-18). Formation of MSU crystal aggregates, denoted as tophi, are associated with inflammation due to their ability to continuously remodel and recruit monocytes (15, 19, 20). Recently, the involvement of NETosis in gout has become of particular interest. Schauer *et al.* observed that NETs release IL-8, a key chemokine involved in the recruitment of neutrophils. At high neutrophil concentration, solitary NETs form larger conglomerates, so called aggregated NETs (aggNETs). Moreover, Schauer *et al.* observed that pro-inflammatory mediators such as IL-1 β and IL-6 are degraded by aggNETs *in vitro* via serine proteases attached to their meshwork (15). IL-1 β is a potent pro-inflammatory cytokine and a strong stimulator of bone resorption via the upregulation of RANKL (21). Similarly, IL-6 promotes synovitis and joint destruction by stimulating neutrophil migration and osteoclast maturation (22). Therefore, therapeutic targeting of either IL-1 β or IL-6 might be a promising way to ameliorate acute inflammation and bone remodelling in this model. The authors further examined the *in vivo* relevance of cytokine degradation via aggNETs using the *Ncf1*^{**} mouse strain that displays a drastically reduced capacity to execute ROS dependent NETosis upon stimulation with MSU. They found significantly higher concentrations of pro-inflammatory mediators in MSU-injected air pouches of *Ncf1*^{**} mice compared to wild type, suggesting an impaired degradation of cytokines/chemokines. Strikingly, transfer of aggNETs from WT mice into air pouches of *Ncf1*^{**} mice restored their ability to reduce inflammatory mediators. Furthermore, *Ncf1*^{**} mice showed a chronification of disease in a murine model of MSU induced-arthritis (15). This study highlights the importance of the oxidative burst and the formation of aggNETs in the reso-

lution of gouty inflammation and might be applied to other disease models like systemic lupus erythematosus (SLE) (15). Mice with impaired ROS production via NOX2 show increased basal levels of lupus auto-antibodies, and an augmented deposition of complement C3 and IgG in glomeruli (23). Moreover, introduction of NOX2-deficiency in the lupus-prone MRL.*Fas*^{lpr} mouse strain precipitates the disease course (24).

'Bad' NETs in pancreatitis

Common risk factors of acute pancreatitis, a leading cause for admissions to hospitals for gastrointestinal disorders, are formation of gallstones and alcohol abuse (25). Obstruction of the pancreatic duct leads to upstream blockage of pancreatic secretion, which is accompanied by the premature activation of zymogens, mediating self-digestion of the pancreas and thus causing severe inflammation (26). Neutrophils infiltrate the pancreatic parenchyma during this inflammatory response (26). The destroyed secretory parenchyma will be replaced by fatty tissue, typical of chronic pancreatitis, if the underlying cause of the acute pancreatitis is not resolved (27). Moreover, new forms of pancreatitis, such as autoimmune pancreatitis type 2, which displays granulocytic epithelial lesions containing intra- and periductal granulocyte aggregates, further support the involvement of neutrophils in pancreatitis (28). Therefore, Leppkes and colleagues hypothesised that the formation of intraductal aggNETs and subsequent occlusion might be involved in the clinical symptoms of chronic pancreatitis. Indeed, they observed that neutrophils enter the ducts under inflammatory conditions and form aggNETs, which in turn might hamper secretory flow and thereby drive focal pancreatitis and parenchymal remodelling (29). The authors further detected that these intraductal aggregates contained interleukin-17A (IL-17A), a pro-inflammatory cytokine which triggers the recruitment of innate immune cells. These observations lead to two different approaches, transgenic and vector-based, for the systemic delivery of IL-17A in mice.

The overexpression of IL-17A induced inflammatory pancreatitis which was accompanied by neutrophilia, increased mobilisation of neutrophils, elevated tryptic activity of the pancreas homogenate and a striking myeloid inflammatory infiltration (29). Closer observation of the intraductal aggregates revealed a strong co-localisation of citrullinated histone H3 (citH3) with extranuclear DNA, a hallmark characteristic of NETs. Moreover, citH3, a surrogate of PAD4 activity was predominantly observed inside pancreatic ducts and in the lumen of acini undergoing ductal metaplasia (29). PAD4 activity is crucial for neutrophil chromatin decondensation during NETosis (30). Importantly, *PADI4* deficiency strongly protected mice from the development of IL-17A-induced pancreatitis leading to the conclusion that PAD4-mediated arginine citrullination vitally contributes to aggNET formation in the context of ductal occlusion (29). The ability of NETs to occlude ducts and potentially cause pancreatitis allows for the possibility of therapeutic intervention to prevent excessive neutrophil activation as a treatment option.

Conclusion

In recent years, the traditional view of neutrophils as simple eliminators of pathogens changed dramatically after they were found to be engaged in many other physiological and pathological processes. Most interestingly, many of these newly discovered functions of neutrophils seem to be related to their ability to release nuclear content decorated with granular proteins via the formation of NETs. However, the release of NETs is a double-edged sword. On the one hand, 'bad' NETs are involved in the obstruction of vessels and ducts as well as thrombus formation and acute inflammation; on the other hand 'good' NETs are able to contribute to the resolution of inflammation as demonstrated for gouty tophi. These recent findings have led to the conclusion that NETs are neither the villains nor the hero in autoimmunity, but can act as a key component of initiation as well as resolution of inflammation. It also became apparent that a certain shift in the

balance is needed for a neutrophil to act pro- or anti-inflammatory. How this balance is maintained on a physiological level and what interferes with it is the focus of further investigations. This is of particular importance as mechanisms that disturb the balance and lead to the formation of 'bad' NETs are potential therapeutic targets.

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