NEUTROPHIL EXTRACELLULAR TRAPS (NETs): AN UPDATE

Rita Heuertz, PhD, MT(ASCP)

Department of Clinical Health Sciences
Doisy College of Health Sciences
Saint Louis University

Objectives

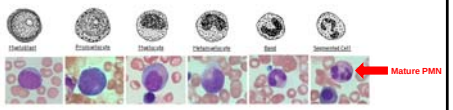
Learning Objective 1
Define a neutrophil extracellular trap.

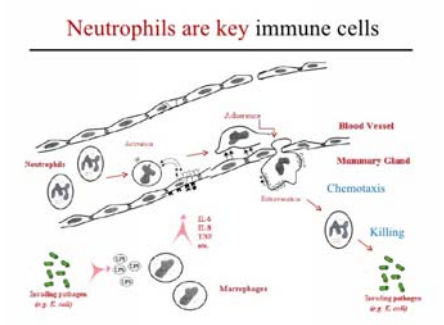
Learning Objective 2
Summarize functions of neutrophil extracellular traps.

Learning Objective 3
Relate neutrophil extracellular traps to microbial infections.

Neutrophils

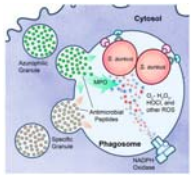
- Cellular components of the 1st line of immune defense/host response to infection
- Most abundant innate immune effector cells of the human immune system
- Potent phagocytes that contain a multitude of intracellular and extracellular mechanisms that lead to the demise of pathogenic bacteria
- Found in:
  - blood where they normally reside (with survival up to 8 hours) or
  - tissue where they have been actively recruited (with survival up to 7 days)





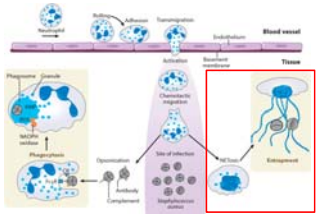
Neutrophils

- When PMN are actively recruited, they participate in microbe elimination
- Phagocytosis is a major function of PMN
  - process that causes production of potent antimicrobial agents that lead to demise of the microbe
    - Formation of phagosome within the PMN that contains the microbe
    - Release of azurophilic and specific granules (degranulation) into the phagosome
    - Formation and release of reactive oxygen species into the phagosome compartment
    - Microbe death



Neutrophils

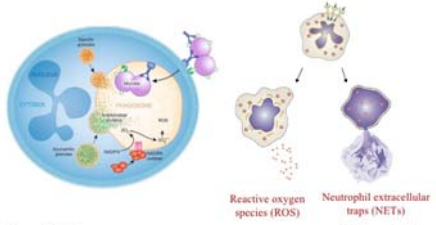
- An additional mechanism, and novel process, that leads to the demise of microbes has been identified. This process is known as NETosis and is the result of neutrophil extracellular trap (NET) formation.



Neutrophil killing mechanisms

INTRACELLULAR (phagocytosis, degranulation)

EXTRACELLULAR (ROS, NETs)



Reactive oxygen species (ROS)

Neutrophil extracellular traps (NETs)

Kennedy 2009

Brinkmann 2004

First Description of NETosis (2004)

- **Experiment**

  Assess PMN morphologically for activation and death after addition of activating agent (phorbol myristic acid: PMA)

- **Results Observed In Sequential Order (over total duration of 2-4 hours)**
  - Fusion of multi-lobed nucleus
  - Reduction in compactness (decondensation) of chromatin
  - Nuclear envelope breakdown yet maintenance of integrity of cytoplasmic organelles
  - Extracellular membrane disruption (due to ROS effect observed at 3 h)
  - Release of DNA into chromatin structures (now decondensed and spread out)
    - NOTE: decondensed chromatin occupies 3-5 times the volume of condensed
  - Formation of extracellular neutrophil traps (NETs)
  - Protein adherence to NETs
    - Proteins such as: histones, NE, MPO, lactoferrin, pentraxin 3, and more than 30 components of primary and secondary granules with bactericidal activity

- **Conclusion**

  Morphologic identification of a new PMN function was described. However, physiologic mechanisms, disease relevance and clinical translatability remained poorly understood at that time.

Brinkman et al. Neutrophil extracellular traps kill bacteria. Science 303:1532-1535, 2004. doi:10.1126/science.1092385

Neutrophil Extracellular Traps (NETs)

NET Definition

- large, filamentous web-like structures composed of cytosolic and granule proteins that are assembled on a scaffold of decondensed chromatin
- released by highly activated PMN in response to infectious agents, sterile inflammation, autoimmune stimuli, and cancer

NET Formation

- multifactorial process determined by the NET inducer
- nuclear envelope disintegrates and chromatin decondenses in a manner dependent on neutrophil elastase (NE) and peptidylarginine deiminase 4 (PAD4)
- proteins from the nucleus (histones) and PMN granules (e.g., elastase, lactoferrin, myeloperoxidase, antimicrobial peptides) bind to decondensed DNA (granule and nuclear contents fuse)
- total NET structure is ejected from the cell

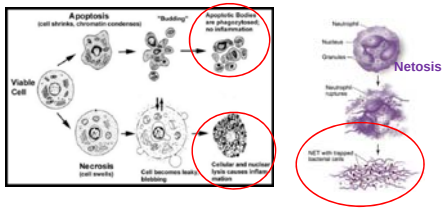
NET Action

- extruded DNA decorated with potent antimicrobials and proteases contain infection and its dissemination
- in sterile inflammatory conditions (those with no bacterial involvement such as atherosclerosis, systemic inflammatory response syndrome), NETs degrade cytokines and chemokines via serine proteases and may thereby decrease systemic inflammation

Ortmann & Kolaczkowska. Age is the work of art? Impact of neutrophil and organism age on NET formation. Cell Tissue Res 371(3):473-488, 2018. doi:10.1007/s00441-017-2751-4.

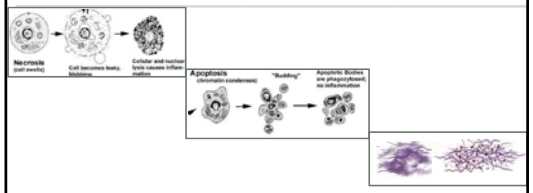
Definition of Neutrophil Extracellular Traps

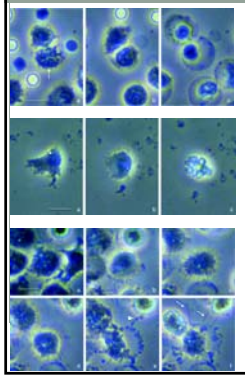
Neutrophil extracellular traps (NETs) consist of extruded nuclear DNA and histones decorated with PMN granule enzymes. NETs trap and kill bacteria by netosis, a process different from apoptosis and necrosis.



Description of NETosis

Necrosis	Apoptosis	Netosis
Membrane and organelle disintegration	Membrane blebbing	Vacuolization
Phosphatidylserine exposure during early steps of necrosis	Phosphatidylserine exposure	No exposure to Phosphatidylserine
Cellular swelling and bursting	Nuclear chromatin condensation without disintegration of the nuclear membrane	Nuclear chromatin decondensation with disintegration of the nuclear membrane
Cell damage releasing the intracellular contents	Programmed cell death	Programmed cell death





Morphological changes in **necrotic** PMN (LPS induction)
a. Lobes of nucleus fuse (arrow)
b. Cell becomes round
c. Necrotic cells have terminally compromised membranes that allows cells to swell

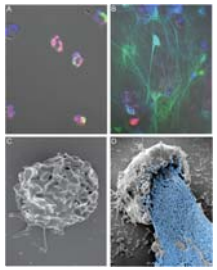
Morphological changes in **apoptotic** PMN (LPS induction)
a. Early morphology: condensed nucleus (arrow)
b. Cell rounds with perinuclear aggregation of chromatin but maintains cytoplasmic integrity
c. Late stage: nucleus collapses; cytoplasmic granules are tightly packed; blebs are formed by plasma membrane

Morphological changes in **NETosis** (induced by 10 nM PMA)
a. nucleus loses its lobes
b. chromatin decondenses
c. nuclear envelope disaggregates
d. nucleoplasm becomes homogenous
e. cell membrane ruptures; cell interior is ejected (arrowhead)
f. chromatin is expelled (arrows)

All inductions are done *in vitro*. All photos are time lapse images. Each scale bar represents 10-µm length.

Iba et al. Neutrophil cell death in response to infection and its relation to coagulation. J Intensive Care 1(13), 2013. doi:10.1186/2052-0492-1-13.

Visualization of NETs



different magnifications of isolated human PMN as viewed by immunofluorescence & SEM

Histone 2A (red) MPO (green) DNA (blue)

Fluorescent Microscopy
(A) Unstimulated PMN

(B) NETs formed after electropermeabilization

Scanning Electron Microscopy (SEM)
(C) PMN not electropermeabilized

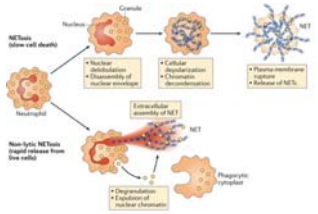
(D) After electropermeabilization

Malachowa et al. NET confusion.
Front Immunol 2016. doi:10.3389/fimmun.2016.00259

NETs Basics

- types of NET release
  - NETosis (suicidal: PMN death)
  - non-lytic NETosis (vital: PMN cytoplast survives)
- triggers of NET release
  - microbes (whole or microbial fragments)
  - inflammation-associated agents
- components released during netosis
  - components of primary (azurophilic) and specific granules of PMN
  - others, such as pentraxin-3
- disease relevance of NET release
  - antimicrobic action
  - other consequences of NET action

Types of NET Release (NETs Pathways)



FIRST PATHWAY is a cell death pathway (**suicidal NETosis**) associated with nuclear delobulation, disassembly of nuclear envelope, loss of cellular polarization, chromatin decondensation, plasma membrane rupture

SECOND PATHWAY is a non-lytic form of NETosis (**vital NETosis**) that occurs independent of PMN death: involves secreted expulsion of nuclear chromatin, release of granule proteins (degranulation), chromatin/protein assembly extracellularly, residual active anucleated cytoplast that continues to microbe ingestion

Papayannopoulos V. Neutrophil extracellular traps in immunity and disease.
Nature Rev Immunol 18:134-147, 2018. doi:10.1038/nri.2017.105

Triggers of NET Formation and Release

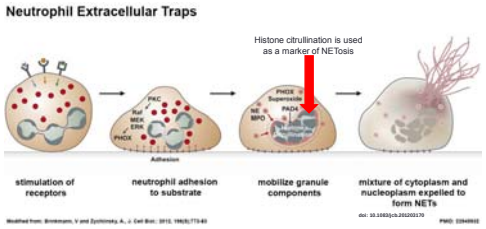
- Autoantibodies
- Bacteria
- Cigarette smoke
- Complement-derived peptides
- Endotoxin (bacterial component, LPS)
- Fungi
- Hydrogen peroxide (substrate of MPO)
- Interleukin 8 (IL-8)
- Ionophores such as A23187
- Lipid mediators (2-chlorofatty acid: aka 2-CIFA) *
- Platelets that are activated
- Protozoa
- Urate crystals
- Viruses

REVIEWED IN: Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nature Rev Immunol 18:134-147, 2018. doi:10.1038/nri.2017.105

* Palladino et al. 2-chlorofatty acids: lipid mediators of neutrophil extracellular trap formation. J Lipid Res 2018, doi:10.1194/jlr.M084731

FUN FACT
citrullination is frequently used as a NET biomarker

Indicator of PMN Activation as a NET



EXAMPLE of Citrullination as NET Marker. Meher et al. Novel role of IL (Interleukin)-1β in neutrophil extracellular trap formation and abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol 38(4):843-853, 2018. doi: 10.1161/ATVBAHA.117.309897

Components Released During Netosis

- Calprotectin
- Cathelicidins
- Cathepsin G
- Defensins
- Elastase (NE)
- Gelatinase
- Histones
- Lactoferrin
- Leukocyte proteinase 3 (PR3)
- Lysozyme C
- Myeloperoxidase (MPO)

REVIEWED IN: Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nature Rev Immunol 18:134-147, 2018. doi:10.1038/nri.2017.105

Disease Relevance of NET Release

The Good of NETs

- Granular contents and nuclear histones are extruded from the PMN in a manner such that the DNA is decorated with potent antimicrobials and proteases. These agents act to:
  - contain infection and its dissemination
  - degrade cytokines and chemokines via serine proteases (in sterile inflammation)

The Bad of NETs

- Overproduction of NETs, their inadequate removal or their prolonged presence in tissue or vasculature can lead to "bystander" or "collateral" damage or initiation of diseases
  - injury occurs to unintended targets, such as normal healthy cells or tissue

The Good of NETs

- NETs are active against Gram positive and Gram negative bacteria with bactericidal effects
- NETs are active against fungi
  - Kill yeast and hyphal forms of *Candida albicans*
- NETs are active against parasites
  - Kill *Plasmodium falciparum*

REVIEWED IN: Brinkmann. Neutrophil extracellular traps in the second decade. J Innate Immun. 2018. doi:10.1159/000489829

Microbes Known to Induce NETosis

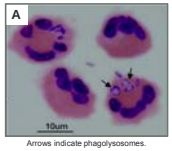
Microbes	Pathologies	NETosis
S. aureus	Osteomyelitis, endocarditis, bacteremia, gastroenteritis	Vital
S. pneumoniae	COPD, pneumonia, emphysema	Suicidal
E. coli	Enteritis, UTI, meningitis, sepsis	Suicidal
C. difficile	Diarrhea, pseudomembranous colitis	ND
S. flexneri	Dysentery	Suicidal
S. typhimurium	Infectious gatroenteritis	Suicidal
Yersinia	Yersiniosis, enterocolitis	Suicidal
M. tuberculosis	Tuberculosis	Suicidal
V. cholerae	Cholera	Suicidal
Influenza virus	Influenza A (H1N1)	Suicidal
Dengue virus	Dengue	Suicidal
HIV	Acquired immune deficiency	Suicidal
RSV	Acute bronchitis	Suicidal
C. albicans	Candidiasis	Vital
A. fumigatus	Invasive aspergillosis	Suicidal
C. neoformans	Cryptococcal meningitis	Suicidal
P. falciparum	Malaria	Suicidal
T. gondii	Toxoplasmosis	Suicidal

Delgado-Rizo et al. Neutrophil extracellular traps and its implications in inflammation: an overview. Front Immunol 2018. doi:10.3389/fimmu.2017.00081.

NET Interactions with Microbes

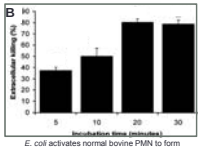
- GNR: *Escherichia coli*

INTRACELLULAR KILLING (phagocytosis, degranulation)



Arrows indicate phagolysosomes.

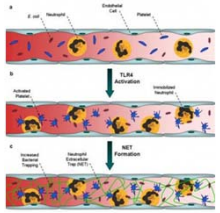
EXTRACELLULAR KILLING (NETs)



E. coli activates normal bovine PMN to form NETs which are highly bactericidal.

Grinberg et al. Beta-hydroxybutyrate abrogates formation of bovine NETs and bactericidal activity against mammary pathogenic *E. coli*. Infect Immun 76(6):2802-2807. 2008. doi:10.1128/IAI.00051-08.

Consequences of NET Interactions: Killing of *E. coli* (Sepsis Example)



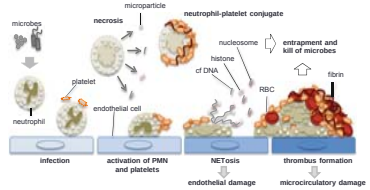
(a) Prior to detection of bacteria, inactivated PMN and platelets circulate through the microvasculature. The presence of *E. coli* leads to TLR4 activation.

(b) PMN detect LPS and are recruited to EC lining the microvasculature. TLR4-activated platelets are recruited to the adherent PMN where they bind to immobilized PMN.

(c) This leads to robust PMN activation and NET formation (suicide NETosis). A greater number of *E. coli* are now trapped within the microvasculature by the NETs, where they can be killed and cleared.

Ma & Kubes. Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis. J Thromb Haemost 6(3):415-420. 2008. doi: 10.1111/j.1538-7836.2007.02865.x

Sepsis: NETosis & Microthrobmus Formation

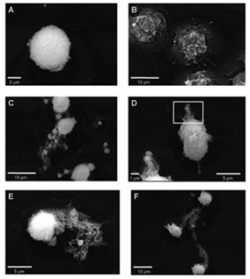


- Microbes activate PMN which then activate platelets by expelling microparticles, nuclear components, granular proteins.
- PMN accumulate and adhere to endothelium in collaboration with platelets where the PMN expel NETs that kill bacteria and activate coagulation.
- NETs serve as scaffold for thrombus formation.
- Thrombi substantially lead to microcirculatory damage and organ dysfunction in sepsis.

Iba et al. Neutrophil cell death in response to infection and its relation to coagulation. J Intensive Care 1(13). 2013. doi:10.1186/2052-0492-1-13.

NET Interactions with Microbes

- GPC: *Staphylococcus aureus*



SEM of NETs after incubation with S. aureus

Method
PMN and S. aureus were incubated (1 h) on autologous plasma-coated slides, fixed with glutaraldehyde, stained with gold film and studied by SEM. Images are representative of 3 experiments with PMN from different blood donors.

Results
A. PMN alone (unstimulated) no NET production
B. PMN stimulated with PMA (diacylglycerol mimic): cell spreading but no extracellular structures formed
C. PMN + S. aureus: early NET release from PMN remaining intact
D. PMN + S. aureus: bacteria covered by NETs
E. PMN + S. aureus: NETs formed lattice-like structures and entangled bacteria
F. PMN + S. aureus: NETs emanate from small area on PMN surface and fuse with other NETs

Pliszczek et al. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. J Immunol 185(12):7413-25, 2010. doi.org/10.4049/jimmunol.1000675.

Consequences of NET Interactions: *S. aureus* Killing

- Very rapid killing (5-60 min) that requires no PMN lysis
- Identification of **vital** NETosis

Pliszczek et al. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. J Immunol 185(12):7413-25, 2010. doi.org/10.4049/jimmunol.1000675.

NET Interactions with Microbes

- GNR: *Pseudomonas aeruginosa*

SEM of purified human PMN that have produced NETs after cocultivation with *P. aeruginosa* for 3 h.

REVIEWED IN: Brinkmann. Neutrophil extracellular traps in the second decade. J Innate Immun 2018. doi 10.1159/00489829

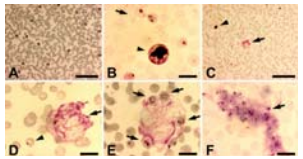
NET Interactions with Microbes

- Yeast: *Candida albicans*
  - NETs kill both yeast and hyphae forms of *C. albicans*
  - However, it was observed that more NETs were produced to hyphae than to yeast
  - Which led to the conclusion that PMN sense size of pathogen and produce more NETs when pathogen is large
  - Good for Innate Immunity: since large structures are not easily phagocytosed by PMN
- **Hyphae selectively induce NETosis**

Branzk et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nat Immunol 15(11):1017–1025, 2014. doi: 10.1038/ni.2987

NET Interactions with Microbes

- Parasite: *Plasmodium falciparum*



Giemsa-stained peripheral fingerprick blood smears from children 5 years old and younger presenting with uncomplicated malaria.
(A) low magnification field of a control smear from child presenting with fever but no detectable *P. falciparum*
(B) field showing PMN exhibiting toxic granulation and lack of distinct nuclear membrane (arrowhead) and fibrous material (arrow)
(C) field showing NET (arrow) and *P. falciparum*-infected erythrocyte with a young trophozoite (arrowhead)
(D) higher magnification image of NET field from (C)
(E) Fibrous aggregate interpreted to be NET containing parasitized erythrocytes (arrows)
(F) Fibrous aggregate interpreted to be NET sequestering parasitized erythrocytes

Baker et al. Cytokine-associated neutrophil extracellular traps and antinuclear antibodies in *P. falciparum* infected children under 6 years of age. Malar J 7:41, 2008. doi:10.1186/1475-2875-7-41

NET Interactions with Microbes

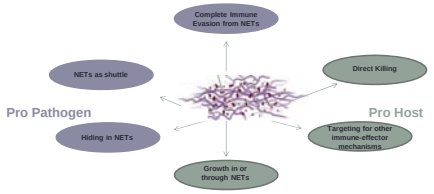
- Viruses
  - Virus-induced NETs have limited capacity to clear bacterial infections suggesting that NETosis performed in bacteria is fundamentally different from NETosis done by viruses
  - Viruses known to induce NET formation include:
    - Influenza A
    - HIV-1
    - Herpes
    - Hantavirus
    - Respiratory syncytial virus
- Jenne and Kubes state

"Protection: But at What Price?"

  - NETs are extremely cytotoxic
  - sticky NET structures are covered with molecules designed to kill pathogens and can fail to distinguish between "friend and foe"
  - individual NET components (such as elastase, histones) are cytotoxic and can cause serious endothelial damage, exposure of subendothelium, coagulation, exacerbated inflammation
  - NETs prevent diffusion of PMN granular proteins and cause concentration of antimicrobic (cytotoxic) molecules that then can act on host cells.
    - Because of DNA "stickiness", cytotoxic molecules adhere to surface of host cells with end result being significant potential for **collateral damage**

Jenne & Kubes. Virus-induced NETs – critical component of host defense or pathogenic mediator? Plos Pathogens 11(1): e1004546. 199-210, 2015. doi:10.1371/ journal.ppat.1004546

Hypothetical Fates of NET-Trapped Pathogens



While it is clear that NETs trap pathogens, outcomes of the encounter between the two remain unclear. Several fates of NET-trapped pathogens are possible.

DEFENSE OF HOST
- ❖ NETs provide direct antimicrobial activity
- ❖ NETs immobilize pathogens for attack by other immune effector mechanisms
- ❖ NETs may not harm pathogens but may allow them to grow in/through NETs thereby preventing their attachment to host cells

DEFENSE OF MICROBE
- ❖ pathogens may completely evade NETs being formed
- ❖ microbes exploit NETs to hide from host immune system or subvert NET-mediated host defense mechanisms
- ❖ pathogens hijack NETs as shuttles for dissemination in the host

Stephan & Fabri. The NET, the trap, and the pathogen: neutrophil extracellular traps in cutaneous immunity. Exp Dermatol 2014. doi:10.1111/exd.12599000489829

The Bad of NETs

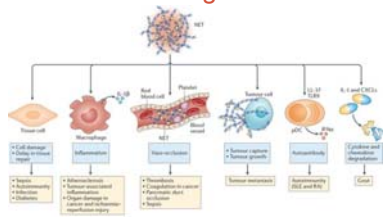
If numbers of NETs and their removal as well as site (location) of NET formation are not tightly regulated processes, their presence can have serious consequences. For example, they have been implicated in endothelial cell injury and microcirculatory plugging.

Pathologies for which links are known/postulated include:

- Adverse cardiovascular events in patients with atrial fibrillation, such as stroke, acute coronary syndrome, acute heart failure, vascular death (Arroyo et al. Arterioscler Thromb Vasc Biol 38(4):892-902. 2018. doi: 10.1161/ATVBAHA.117.310597)
- Autoimmune/auto-inflammatory diseases such as rheumatoid arthritis, systemic inflammatory response syndrome, sepsis, endothelial injury, venous thrombosis, small vessel vasculitis, psoriasis, gout, systemic lupus erythematosus (SLE)
- Cancer: Cancel cells induce metastasis-supporting NETs (Park et al. Sci Transl Med 2016 8:361ra138)
- Cardiovascular disease: when human PMN come in contact with cholesterol crystals, they spontaneously undergo NETosis, which plays a significant role in the progression of plaque formation. (reviewed in Brinkmann. J Innate Immun 2018 doi:10.1159/000489829).
- Chronic obstructive pulmonary disease (Pedersen et al. Eur Respir J 51(4). 2018. doi 10.1183/13993003.00970.2017)
- Lung diseases: cystic fibrosis lung mucus contains much DNA, most of which is complexed with NETs: amount of extracellular DNA directly correlates with poor pulmonary function (reviewed in Brinkman. J Innate Immun 2018. doi:10.1159/000489829).
- Rheumatoid arthritis: NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis (Khandpur et al. Sci Transl Med 2013. 5:178ra140)
- Thrombosis: increased NET formation promotes thrombosis (Wolach et al. Sci Transl Med 10(436), 2018. doi:10.1126/scitranslmed.aan8292)

REVIEWED IN: Brinkmann. Neutrophil extracellular traps in the second decade. J Innate Immun. 2018. doi:10.1159/000489829

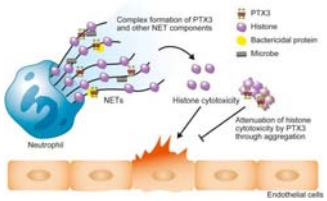
Disease Relevance of NET Release: NET-Mediated Pathologies



DIRECT cell damage is implicated in infection, sepsis, autoimmunity and diabetes.
MACROPHAGE ACTIVATION turns on macrophages for inflammation; NETs drive atherosclerosis.
PROMOTION OF INFLAMMATION leads to organ damage in cancer and ischemia–reperfusion injury.
NETs IN CIRCULATION promote coagulation, vascular occlusion, thrombosis. NETs IN CAPILLARIES promote tumor metastasis.
NET ACCUMULATION promotes resolution of inflammation through degradation of cytokines and chemokines.

Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nature Rev Immunol 18:134-147. 2018. doi:10.1038/nri.2017.105

Are There Any Known EC Protectants against NET Injury? Pentraxin 3 (PTX3)



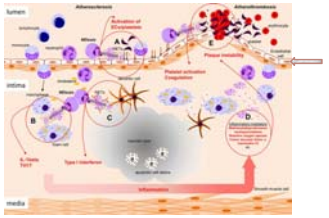
Long pentraxin 3 (PTX3) protects endothelial cells from extracellular histone cytotoxicity.
Extracellular histones are considered to be the main cause of septic death due to their cytotoxic effect on endothelial cells. PTX3 present in NETs forms a complex with histones and other NET component proteins, including bactericidal proteins. This may enhance microbial clearance via synergistic effects. NET histones exert cytotoxicity toward endothelial cells, but PTX3 attenuates this cytotoxicity through aggregation. Thus, PTX3 functions to maintain balance between the beneficial and detrimental effects of neutrophils. Intraperitoneal administration of PTX3 reduces mortality in mouse models of sepsis.

Daigo et al. The protective effect against extracellular histones afforded by long pentraxin PTX3 as a regulator of NETs. Front Immunol 2016. doi:10.3389/fimmu.2016.00344

Examples of Pathologies

that Involve NETs

Emerging Role of NETs in Atherosclerosis and Atherothrombosis



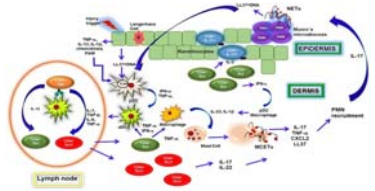
(a) NET formation by PMN in capillary lumen activates WBC, platelets, EC to create proinflammatory milieu resulting in endothelial dysfunction, an initial trigger of lesion formation.

(b/c) NET presence in lesions initiate IL-1β/TH17, type I interferon response leading to WBC activation and release of proinflammatory mediators at lesion site.

(d/e) NET-driven proinflammatory responses cause inflammatory environments that favor plaque destabilization/rupture. During atherothrombosis, NETs trigger activation of coagulation cascade and increase thrombus stability thus orchestrating arterial occlusion.

Doring et al. Neutrophil extracellular traps in atherosclerosis and atherothrombosis. Circ Res 120(4):736-743. 2017. doi: 10.1161/CIRCRESAHA.116.309692

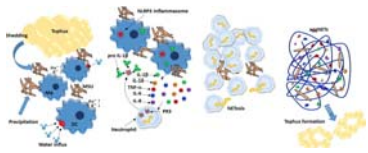
NET Involvement in Psoriasis



NET formation in psoriatic plaques. Intracellular content of mast cells is released along with IL-17 and other cytokines that induce PMN infiltration into epidermis and formation of microabscesses. PMN encounter high concentrations of IL-23 and IL-1β in this microenvironment, which makes them susceptible to NET formation/release. Through NET formation, they secrete cellular contents including IL-17, thus amplifying the inflammatory process and increasing cells recruitment and keratinocytes activation. Finally, NETs are important sources of LL37–DNA complexes and IL-17, both of which activate dendritic cells and keratinocytes that, in turn, produce IFN-α and LL37, respectively. Th1 cells induce activation of T CD8 IL-17-producing cells in epidermis and dendritic cells within dermis, perpetuating the inflammatory environment in psoriatic lesions.

Delgado-Rizo et al. Neutrophil extracellular traps and its implications in inflammation: an overview. Front Immunol 2018. doi:10.3389/fimmu.2017.00081.

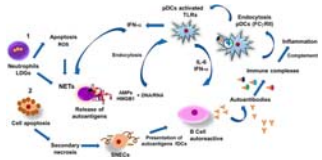
NET Function in Gout (Anti-Inflammatory)



NETs alleviate inflammation caused by monosodium urate (MSU) crystals. MSU crystals are deposited in joints by precipitation and by shedding from a large aggregate (called tophus) and further ingested by phagocytes (e.g., dendritic cells, macrophages). As a consequence, the increased intracellular [Na] induce cells to enhance H_2O uptake, thus diluting K levels below inflammasome-activation concentration thresholds. IL-18 and IL-1β are secreted and mediate recruitment of PMN to inflamed joints; PMN further increase IL-1b levels by cleaving pro-IL-1b in a process mediated by proteinase 3 (PR3). Upon ingesting MSU crystals, PMN undergo NETosis that not only degrades MSU but also encapsulates it, effectively reducing its inflammatory potential. Finally, aggregates of NETs (aggNETs) further stop inflammation by degrading *in situ* cytokines.

Delgado-Rizo et al. Neutrophil extracellular traps and its implications in inflammation: an overview. Front Immunol 2018. doi:10.3389/fimmu.2017.00081.

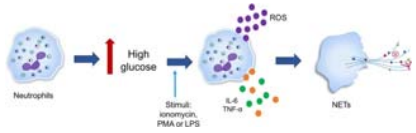
NET Involvement in SLE



Pathogenesis of systemic erythematosus lupus (SLE).
(1) Low-density granulocytes (LDGs) undergo apoptosis and release ROS and autoantigens, thus stimulating formation of NETs as well as release of both antimicrobial peptides (AMPs) and nucleic acids (DNA, RNA). Nucleic acids and AMPs form complexes capable of binding high-mobility group box 1 (HMGB1) protein, which can be recognized by toll-like receptors in plasmacytoid dendritic cells (pDCs), which respond by synthesizing interferon alpha (IFN-α), thus promoting formation of NETs and IL-6. Both cytokines induce differentiation of autoantibody-secreting autoreactive B cells, leading to formation of immune complexes that activate the complement system and also are susceptible to be internalized by pDCs through type II Fcγ receptors (FcγRII)-mediated endocytosis. Endosomes associate to toll-like receptors (TLRs)-containing vesicles, which results in activation of pDCs and synthesis of IFN-α, further inducing NETs and tissular inflammation. In addition, necrotic cells-derived DNA–HMGB1 complexes activate B cells resulting in production of autoantibodies and formation of immune complexes that activate pDCs, leading to IFN-α synthesis and thus establishing a positive feedback.
(2) Another pathway capable of inducing autoantibodies production is mediated by release of autoantigens from apoptotic cells that undergo secondary necrosis and generate secondary necrotic cells (SNECs); accumulation of such cells in germinal centers of secondary lymphoid organs facilitates presentation of autoantigens by follicular dendritic cells (FDCs) to autoreactive B cells and subsequent formation of immune complexes that lead to persistent inflammatory process that causes tissular injury in SEL patients.

Delgado-Rizo et al. Neutrophil extracellular traps and its implications in inflammation: an overview. Front Immunol 2018. doi:10.3389/fimmu.2017.00081.

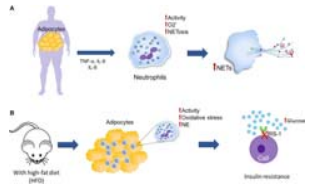
NET Formation in Diabetes



High glucose primes PMN to undergo NETosis. Neutrophils, in response to inflammatory stimuli (ionomycin, PMA, LPS), generate oxidative stress. In addition cytokine production, such as IL-6 and TNF-α, is triggered by high glucose in type 2 diabetes.

Delgado-Rizo et al. Neutrophil extracellular traps and its implications in inflammation: an overview. Front Immunol 2018. doi:10.3389/fimmu.2017.00081.

NETs and Obesity



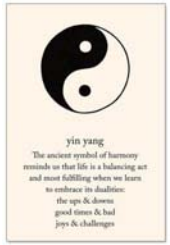
NETs and adipose tissue.
(A) Obesity is characterized by increased adipose tissue and chronic low-grade inflammation. Adipocytes secrete adipokines (such as TNF-α, IL-6, IL-8), which have been associated with increased activity of peripheral PMN (such as generation of superoxide anion, induction of NETosis).
(B) In mice on a high-fat diet, increased PMN recruitment into adipose tissue is observed with concurrent increased PMN elastase (NE) activity. It is possible that PMN may be promoting insulin resistance through degradation of the insulin receptor substrate-1 (IRS-1).

Delgado-Rizo et al. Neutrophil extracellular traps and its implications in inflammation: an overview. Front Immunol 2018. doi:10.3389/fimmu.2017.00081.

Yin Yang: Balance Between Good and Bad

* * * * * * * * * *

as relevant to NETs as it is to life in general



More References

- Kenny et al. Diverse stimuli engage different neutrophil extracellular trap pathways. eLife. 2017. doi:10.7554/eLife24437.001
- Ortmann & Kolaczkowska. Age is the work of art? Impact of neutrophil and organism age on NET formation. Cell Tissue Res 371(3):473-488, 2018. doi:10.1007/s00441-017-2751-4.
- Brinkmann et al. Immunodetection of NETs in paraffin-embedded tissue. Front Immunol 7, 2016. doi: 10.3389/fimmu2016.00513
- Berger-Achituv et al. A proposed role for neutrophil extracellular traps in cancer immunoediting. Front Immunol 4:48, 2013. doi 10.3389/fimmun.2013.00048
- Brinkmann and Zuchlinsky. Neutrophil extracellular traps: is immunity the second function of chromatin? J Cell Biol 198(5):773-783, 2012. doi: 10.1083/jcb.201203170.

Questions?