HHS Public Access

Author manuscript

Pharmacol Ther. Author manuscript; available in PMC 2021 January 01.

Published in final edited form as:

Pharmacol Ther. 2020 January ; 205: 107424. doi:10.1016/j.pharmthera.2019.107424.

Role of Neutrophils in Ischemic Heart Failure

Vasundhara Kain, **Ganesh V. Halade***

Division of Cardiovascular Disease, Department of Medicine, the University of Alabama at Birmingham, Alabama, USA

Abstract

Amplified innate leukocytes (neutrophils and monocytes/macrophages) are associated with advanced ischemic and non-ischemic heart failure (HF). Intensified neutrophilic leukocytosis (neutrophilia) and sustained activation of neutrophils is the predominant factor that determines over activated inflammation in acute HF and the outcome of long-term chronic HF. After heart attack, the first wave of innate responsive and short-lived neutrophils is essential for the initiation of inflammation, resolution of inflammation, and cardiac repair, however uncontrolled and long-term activation of neutrophils leads to collateral damage of myocardium. In the presented review, we highlighted the interactive and integrative role of neutrophil phenotypes in cellular and molecular events of ischemic HF. In addition, we discussed the current, nonimmune, immune, and novel paradigms of neutrophils in HF associated with differential factors with a specific interest in non-resolving inflammation and resolution physiology.

Keywords

Cardiovascular disease; chronic inflammation; leukocytes; non-resolving inflammation; resolution of inflammation; obesity; aging

1. Introduction

The risk of atherosclerosis associated with the ischemic event of myocardial infarction (MI) is a key trigger to turn on acute inflammatory responses of cytokines, eicosanoids, and miRNA storms (Hansson, 2005). If remain unchecked, or activated for a prolonged time, it can lead to chronic inflammation with signs of advanced and chronic heart failure (HF). Despite the primordial requirement of inflammatory reaction, the essential, or detrimental, outcome depends on activated leukocyte entry (turn-on inflammation), repair time (resolution), and leukocyte exit (turn-off inflammation) in the cardiac healing phase. To comply with the healing process, differential leukocytes (neutrophils and monocytes/

***Corresponding author** Ganesh V. Halade, Ph.D., Department of Medicine, Division of Cardiovascular Disease, The University of Alabama at Birmingham, 703 19th Street South, MC 7755, Birmingham, AL 35233, USA, (Phone) 205-996-4139; (fax) 205-975-5150, ganeshhalade@uabmc.edu.

Conflicts of interest

The authors declare that there are no conflicts of interest.

macrophages) and their immune cell subsets participate in an inflammation-resolution program in a time-dependent manner. Among them, polymorphonuclear granulocytes (PMNs; neutrophils) serve as key effector cells and are the first responders to clear deceased, ischemic myocyte debris or invading pathogenic organism in myocarditis. In contrast, slow entry or delayed recruitment for debris clearance, or higher residual or transit time at the site of infarcted myocardium can be detrimental and facilitate collateral myocardium damage. Eventually, if neutrophils are not timely cleared from the site of infarct or the remote area, this can lead to non-resolving inflammation. Neutrophils (also known as neutrocytes) are the most abundant type of granulocytes (50% to 70%) type of white blood cells in most of the mammals while in mice their physiological levels are 20–30% in peripheral blood (Mestas & Hughes, 2004; O'Connell, et al., 2015). Neutrophils are an essential part of the innate immune system that coordinate inflammation-resolution and host defense mechanisms. The point of origin for neutrophils is bone marrow, where they are about 10 μm in diameter. Neutrophils are short-lived, non-professional, and highly mobile enabling entry in the tissue where other cells/molecules cannot invade (Nathan, 2006; Witko-Sarsat, Rieu, Descamps-Latscha, Lesavre, & Halbwachs-Mecarelli, 2000). Activated neutrophils play an important role in cardiac healing via polarizing monocytes/macrophages and clearing debris in HF (Horckmans, et al., 2017). In pathogen and risk free healthy mice (C57BL/6J) following myocardial infarction (MI), neutrophils endure infiltration peaks immediately and sustain this peak up until day 3, with a decrease beginning by day 5. In clinical setting, pathological increases in the neutrophil to lymphocyte ratio have been predicted to be major adverse cardiac events in MI patients (Akpek, et al.; Zahorec, 2001). The neutrophil to lymphocyte (N/Lmax) ratio has been associated with long-term mortality in patients with ST-segment elevation MI. The clinical study by Nunez et al. detailed a positive trend between mortality and N/L_{max} with the cohort of 470 patients excluding patients with cancer, inflammatory diseases, or premature death. The study showed hazardous risk ratio of N/L_{max} was 2.58 after adjusting standard risk factors (Nunez, et al., 2008). As such, in the clinical and pre-clinical setting, the expanded PMNs have been shown to mediate MI-induced ischemic/reperfusion injury (Nunez, et al.). Further, higher neutrophil levels during the first 12 hours after acute MI can predict the occurrence of chronic HF (Rashidi, et al.). Pre-clinical experimental studies and clinical evidence have shown an increased prevalence of MI in the morning and is associated with the over-activation of neutrophils during the active phase, indicative of oscillation of neutrophils due to circadian rhythm (Schloss, et al., 2016). Even though an increase in neutrophils worsens HF, the experimental depletion of neutrophils in mice subjected to MI worsens cardiac function and increases fibrosis, mortality, and progressively advanced HF (Horckmans, et al., 2017). Neutrophils serve as a time-dependent dual-edged sword during the post-MI cardiac healing and remodeling processes. This review aims to highlight the temporal and spatial role, timings, and risk factors (aging, physical activity/inactivity, circadian rhythm) that influence the clearance or on time exit of neutrophils to resolve post-MI inflammation during cardiac remodeling. Also, we discuss how neutrophils are responsive to the obesity-mediated lipidomic environment that dysregulates biosynthesis of pro-resolving lipid mediators in acute MI as potential sources of future therapeutic strategies.

2. Neutrophils: physiology versus pathophysiology in cardiac healing

Immune response-activated neutrophils are complex cells capable of a vast array of specialized functions. Several lines of evidence indicate that over activated neutrophils contribute to chronic inflammatory conditions and drives expansion of innate and adaptive immune responses (Mantovani, Cassatella, Costantini, & Jaillon, 2011). Although neutrophils are undoubtedly major effectors of acute inflammation, depletion of neutrophils in the cardiac healing lead to exacerbated HF (Vulesevic, Sirois, Allen, de Denus, & White, 2018).

In humans, plaque formation (atherogenesis) is slow and progressive, accumulating from years to decades, and leads to MI as a major cardiovascular event (Hansson, 2005). Thus, the milieu is different with a number of variables, including aging, which is predominantly a co-variable along with other risk factors like co-medication, obesity, diabetes, and hypertension (Halade & Kain, 2017). When it comes to the pathologic context of rodents or experimental myocardial injury without risk factors, the neutrophils are recruited to the site of injury (‘get-in signal’) and the infiltrated neutrophils resolve via cell apoptosis, macrophage directed efferocytosis, or possible transmigration exit between 2 to 7 days after ischemic injury, depending on the surrounding milieu (Frangogiannis, 2012; Tourki & Halade, 2017). Apoptotic neutrophils release ‘find me’ signals that attract monocytes and neutrophil gelatinase-associated lipocalin (NGAL) is released by activated neutrophils from specific granules. NGAL in the neutrophil secretome is a key inducer of reparative M2c macrophages with a high efferocytosis capacity (Mishra, et al., 2005; Sørensen, et al., 2001; Yndestad, et al., 2009). After cardiac injury or infection, if short-lived neutrophils are residing or migrating at the site of infarction for longer than normal time, then dying neutrophils can liberate granule components to the extracellular environment and prolong the ongoing inflammatory response, facilitating advanced HF (Bratton & Henson, 2011). The dying neutrophils will release ‘find me signals’ which include lysophosphatidylcholine, fractalkine, nucleotide adenosine triphosphate (ATP) and uridine 5′ triphosphate (UTP), and sphingosine 1-phosphate (S1P) to attract monocytes (Elliott, et al., 2009; Truman, et al., 2008). The dead and apoptotic neutrophils express ‘eat me’ signals and many are bound ‘bridge’ molecules, which are soluble pattern-recognition proteins of the immune system indicating their job is completed at the site of injury. The ‘eat-me’ signal includes externalized phosphatidylserine (PS), altered lipids, changes in surface charges likely attributed to amino sugars, and exposure of intracellular molecules such as calreticulin, mitochondrial and nuclear constituents (K. S. Ravichandran, 2010). The clearance of apoptotic neutrophils appears to reprogram macrophages towards an anti-inflammatory phenotype, which is crucial for the resolution of inflammation after MI in mice. However, neutrophil expansion is a continued process in progressive HF in humans (Tracchi, et al., 2009). Thus, promoting the on-time neutrophils exit or removal from the tissue, contribute to the resolution of inflammation leading to cardiac healing and tissue repair (Canturk, et al., 2001). Neutrophils are essentially involved in cardiac repair to reach out and access deceased myocytes with expressions of many proteases that are important in order to facilitate tissue repair. It has been reported that neutrophils are the first source of MMP9 (matrix metallopeptidase 9), which is a major extracellular matrix (ECM)-digesting enzyme,

and are stimulated by the phorbol ester N-formylmethionyl-leucyl-phenylalanine (formyl-Met-Leu-Phe), tumor necrosis factor (*TNF*)-*α*, and interleukin (*IL*)-*8* (Cauwe, Martens, Proost, & Opdenakker, 2009). Neutrophils-derived MMP9 is known to drive localized proximal and chronic distal inflammation in cardiovascular disease (Halade, Jin, & Lindsey, 2013). A robust MMP9 release is reported from neutrophils isolated from human blood and stimulated with *IL-8* (Chakrabarti & Patel, 2005). Also, neutrophils are the main source of annexin 1 and other pro-resolving peptides that are released via a micro-particle/micro-vesicle pathway, facilitating annexin 1 interactions with the endothelium. Annexin 1 signals via ALX/FPRs (formyl peptide receptors) to terminate neutrophil migration in a MAPK-dependent manner (X. Liu, et al., 2012).

Traditionally, neutrophils perceived inflammatory cells, however after the onset of cardinal signs of inflammation (rubor, calor, tumor, and dolor), neutrophils, start to produce pro-resolving lipid mediators, including lipoxin A_4 (LXA_4) (Serhan, 2010),(Serhan, Chiang, & Van Dyke, 2008). LXA_4 not only inhibits ALX/FPR1-dependent neutrophil migration but also initiates non-phlogistic (phagocytosis of apoptotic leukocytes which does not provoke the release of pro-inflammatory mediators) recruitment of monocytes and phagocytosis of apoptotic neutrophils by macrophages. A unique mode of LXA_4 action is that it induces changes in the phosphorylation of proteins of the cytoskeleton, leading to inhibition of neutrophil migration (Kolaczkowska & Kubes, 2013). Closely related to LXA_4 and AT-LXA_4 are specialized pro-resolving mediators, including resolvins and protectins produced by the interaction of neutrophils and endothelial cell while maresins are produced by macrophages (Serhan, 2010). Specialized pro-resolving mediators (Lipoxin A_4, D-and E-series resolvins) also limit neutrophil migration, and protectin D1 additionally increases expression of C-C chemokine receptor type (CCR)5, which on late apoptotic neutrophils acts as a scavenger for C-C motif chemokine ligand (CCL)3 and CCL5 (Spite, Clària, & Serhan, 2014). Therefore, a switch in neutrophil phenotype (pro-versus anti-inflammatory) manifested as a change in lipid mediators profile seems to occur which decides the fate (physiological healing vs pathological healing) in HF (Figure 1).

Post-ischemic injury, the neutrophils polarization is a continuous process, thus anti-inflammatory N2 neutrophils exist in the infarcted LV, with N1 pro-inflammatory being the predominant phenotype along the post-MI (Ma, et al., 2016). N1 negatively correlates with infarct wall thinning. This indicates that N2 neutrophils might be protective in early healing post-MI and should maintain that may partially explain why neutrophil depletion is deleterious for myocardium healing post-MI (Cuartero, et al., 2013). The study by Daseke et al have shown neutrophil mapping and phenotypic changes in response to MI. They concluded post-MI day (d)1 neutrophils had a high degranulation profile with increased matrix metalloproteinase (MMP) activity. At post-MI d3 neutrophil profiles showed upregulation of apoptosis and induction of extracellular matrix (ECM) organization which is further amplified to ECM reorganization profile at d5 post-MI. The reparative signatures marked with expression of fibronectin, galectin 3, and fibrinogen were noted at d7 to contribute to scar formation by stimulating ECM reorganization (Daseke, et al., 2019). We speculate that the timely entry, calibrated quantity of neutrophils, and secretion products are required in the local cardiac microenvironment to promote reparative macrophage

polarization. This is a prerequisite for regulated fibrosis, mature scar formation, and physiological cardiac remodeling (Wan, et al., 2013).

3. Neutrophils: heterogeneity modifiers after myocardial infarction

Neutrophils play multiple and diversified roles in the immune-mediated opsonize system, such as intracellular receptor-mediated phagocytosis, degranulation with antimicrobial activity, and formation of neutrophil extracellular traps (NETs) (Mayadas, Cullere, & Lowell, 2014). Neutrophils initiate innate responses and coordinate infectious and non-infectious inflammation (sterile injury) repair and tissue remodeling. Neutrophils express various pattern recognition receptors, that include Toll-like receptors (TLRs), C-type lectin receptors, NOD-like receptors (NLRs), and cytoplastic sensors for RNA, including retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation associated protein 5 (MDA5). Immune responsive chemokines initiate the ‘get-in’ signal to neutrophils at the site of ischemic injury and assist monocyte/macrophage signaling by amplifying inflammatory protein-2α (MIP-2α, CXCL2, GRO-β), leukotriene B_4 (LTB_4), cytokine-induced neutrophil chemoattractant 1 (CINC-1, IL-8,CXCL8, and complement 5a) (Ma, Yabluchanskiy, & Lindsey, 2013). Damage associated molecular patterns (DAMPs) are produced from host tissue or immune cells in response to injury or stress. MI-associated DAMPs include heat shock proteins, high-mobility group box (HMGB)-1, low molecular hyaluronic acid, and fibronectin fragments, which aids in the inactivation of neutrophils (Ma, et al., 2013). Neutrophil apoptosis/departure or exit, followed by a clearance from the site of injury, is a symbol of inflammation-resolution or wound healing that requires the activation of many stop signals (lipoxins) or inhibitory pathways. For example, injury sites, pathogens, or apoptotic cells generate physiological ‘get in’ signals to neutrophil infiltration and activate ‘find-me’, ‘eat-me’ signals at the site of injury (infarcted zone) that facilitate monocyte/macrophage scavengers during the activation of resolution of inflammation (Halade & Ma, 2016). ‘Find me’ signals usually include lipids such as lyso-phosphatidyl-choline (LPC) and sphingosine-1-phosphate (S1P) and are known to stimulate neutrophil chemotaxis as well (Kodi S. Ravichandran, 2011).

In response to sterile injury/ bacterial or fungal infection, the pro-inflammatory/resolving milieu (e.g., with the release of cytokines, bioactive lipid mediators, peptides, and growth factors) and neutrophils undergo respiratory outbursts and release large amounts of reactive oxygen species (ROS) mediated by a multi-component enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. To date, the physiological and pathological role of ROS is under investigation due to the limitation of quantitative methods and sources of organ specific role of ROS (Brandes Ralf, Rezende, & Schröder, 2018). Upon activation of neutrophils, the subunits of NADPH oxidase assemble to form an active enzyme complex that catalyzes the production of ROS, which is responsible for neutrophil antimicrobial activity (Winterbourn, Kettle, & Hampton, 2016). Excessive production of ROS can damage host tissue by modifying amino acids, proteins,carbohydrates, and lipids that impair cellular or organ active biological functions (Yu, Robotham, & Yoon, 2006). Figueiredo et al., have shown that the ROS is generated by multiple NOXs (NADPH oxidase-2 and −4) and contribute to insulin resistance in mice fed a high fat diet, triggering obesity (Souto Padron de Figueiredo, et al., 2015).

An important role of neutrophils is the protection against both pathogenic organisms and non-infectious injuries or wounds by exocytosis and the release of neutrophil granule components. There are four main types of neutrophil granules: azurophilic granules, specific granules, gelatinase granules, and secretory granule. Azurophilic granules are the largest and primary granules that were formed during maturation of PMNs and contain a heme enzyme; myeloperoxidase (MPO), serine proteases, azurocidin, α-defensins, lysozyme, and bactericidal proteins (Ma, et al., 2013). Primary granules contain inflammatory and resolving defensins, cathepsin G and lactoferrin, suggestive of a neutrophil counterbalance physiological role in cardinal inflammation (Ganz, 2002). Specific granules are secondary and are smaller than azurophilic granules in diameter, they contain lactoferrin, NGAL, cathelicidin, and lysozyme. Gelatinase granules are tertiary, smaller than specific granules, and contain multiple matrix metalloproteinases (MMP-8 and −9) and a few microbicidal proteins. Secretory granules consist primarily of complement receptor 1, plasma protein albumin, clusters of differentiation (CD)13 (aminopeptidase N), CD14, and CD16 (Fc gamma receptor III) (Borregaard, Kjeldsen, Lollike, & Sengelov, 1995; Faurschou & Borregaard, 2003; Ma, et al., 2013). In addition to antimicrobial activity, these granule components are also involved in several inflammation-associated diseases. The temporal chemical dynamics in the biosynthesis of lipid mediators and peptides with possible receptors in relevance to the importance of these granules in the MI setting, however, needs to be investigated in acute and chronic HF.

3.1 Lifespan of activated and non-activated neutrophils

Neutrophils are highly responsive and non-professional chemoattractant cells that sense the invasion or injury within seconds to minutes and initiate cardinal signs of acute responses for vascular adhesion, trans-endothelial migration, and arriving at the site of infection/injury (Faurschou & Borregaard, 2003). Bone marrow is a reserve pool of mature neutrophils, containing 20 times more neutrophils than the overall quantity within the circulation of humans. Physiologically, neutrophils are released in blood 2 days after (apparent) maturation in the bone marrow. In response to injury or infection, approximately 10^{12} neutrophils are released immediately, compared to the usual 10^{11} per day (Roos, 1998; Tak, Tesselaar, Pillay, Borghans, & Koenderman, 2013). Average circulation time of neutrophils varies from 6–12 hours after moving to the tissues, where they remain active for 2–6 days (Basu, Hodgson, Katz, & Dunn, 2002; Dancey, Deubelbeiss, Harker, & Finch, 1976). One of the study has shown that the neutrophil lifespan is 5.4 days in the circulation of humans and 0.75 days in mice using 2H-adenosine in neutrophils (Simon & Kim, 2010). In early cardiac healing of ischemic injury, neutrophils drive safe clearance in myocardium wound healing (MI), which is the first critical step of resolution of inflammation thereby delayed advanced HF, if uncontrolled or dysregulated lead to non-resolving inflammation (Soehnlein, Steffens, Hidalgo, & Weber, 2017). Neutrophils' lifespan and time course is prescisely defined in MI, but the differential roles of neutrophils within non-resolving and resolving inflammation in advanced HF remain to be under-investigated.

3.2 Neutrophils set the resolution platform in ischemic injury

The timely entry of neutrophils or the on-time get-in signal balances both the host-defense and clearance mechanisms after injury or infection and signals for the resolution of

inflammation. If this process is dysregulated because of delayed 'get-in' signal or impaired 'get-out' signal due to dysregulated neutrophil kinetics, it can contribute to non-resolving inflammation (Figure 1). Historically, the resolution of inflammation was acknowledged in 1907 by Opie et al. which showed that disappearance of fluid enriched in fibrin and leukocytes after 5 days along with poly-nuclear leukocytes clearance and the presence of mononuclear cells in the pleural cavity in dogs (Opie, 1907). According to the traditional and conceptual viewpoints, the resolution of inflammation was considered a passive phenomenon that removes inflammatory stimuli and dilutes chemokine gradients to prevent leukocyte recruitment over time (Table 1). However, within the last decade, the resolution has been well established as an active phenomenon needed to resume normal physiological function (Kain, Prabhu, & Halade, 2014; Serhan, et al., 2008). With the cardinal signs of inflammation, neutrophils generate several local endogenous peptide/bioactive lipids that promote the active resolution of inflammation and termination of neutrophil recruitment, which is marked by the resolution phase and, ultimately, homoeostasis.

Neutrophils are one of the key regulators of resolution of inflammation in MI. Popular anti-inflammatory therapies, like non-steroidal anti-inflammatory drugs (NSAIDs), has developed to reduce the neutrophil-driven response in acute post-MI injury (Halade, Kain, Wright, & Jadapalli, 2018). Post-MI, neutrophils increase within 24 hours, marking a mandatory active inflammatory response, which is essential for cardiac healing (Kain, et al., 2014; Ma, et al., 2016) (Halade, Norris, Kain, Serhan, & Ingle, 2018). Horckmans et al. demonstrated that neutrophils orchestrate the phenotype and number of monocytes/macrophages post-MI (Horckmans, et al., 2017). Depleting neutrophils with a monoclonal Ly-6G antibody in C57BL/6 mice with coronary ligation (MI) results in a decrease in the $Ly6C^{hi}$ monocyte release from the splenic reservoir; which alters the macrophage proliferation phenotype, leading to excessive fibrosis, increased stiffness, and reduced cardiac function ultimately resulting in HF and kidney injury (Horckmans, et al., 2017) (Ma, et al., 2016). Activated neutrophils highly express GPCR (G-protein-coupled receptors), ALX/FPR2, which is essential for resolution process and regulating leukocytes via generating pro-inflammatory and pro-resolving mediators (Figure 2) (M. Liu, et al., 2012) (Kain, Jadapalli, Tourki, & Halade, 2019). Recent study unfolded the simultaneous activation of neutrophils correlation between SPMs and proinflammatory mediators response in temporal manner. The study showed SPMs regulates resolution mechanisms, which are activated early during STEMI. After MI, increase in neutrophil counts strongly correlate with protectins that biosynthesized as part of innate response independent of CRP (C-reactive protein) activation (Fosshaug, et al., 2019). From a clinical perspective, the role of neutrophils after cardiac injury is under studied in inflammation-resolution signaling and the process of how neutrophils regulate myocyte health at early or late time points after MI still needs to be explored.

3.3 Responsive neutrophils to the metabolic and lipidomic milieu in obesity

Cardiometabolic disease and metabolic inflammation (meta-inflammation) are influenced by dietary micro/macro-nutrient intake, sedentary lifestyles, dysregulated sleep/wake cycle, and are major contributor to the failure of preventing metabolic disease. Undernutrition, referred to as malnutrition, is a result of inadequate diet and/or malabsorption of nutrients that leads

to increased susceptibility to infection by weakening the immune system (Bourke, Berkley, & Prendergast, 2016). The study by Takele and colleagues has shown that the ability of neutrophils from malnourished individuals to produce ROS was significantly impaired, suggesting that these neutrophils are less capable of killing pathogens and unable to control the growth of pathogenic microorganisms (Takele, et al., 2016). Iron deficiency, a type of malnutrition, is the most common trace element deficiency worldwide, affecting 20%–50% of the world's population and is associated with impairments in cell-mediated immunity and reductions in neutrophil action, with decreased bacterial and myeloperoxidase activity (Garner & Brabin, 1994). In contrast, over-nutrition can cause the neutrophils to be pre-activated or over-active and present in abundance.

A clinical study involving obese individuals has shown that the neutrophil/lymphocyte ratio (NLR) is significantly higher compared to age-matched healthy controls suggest a robust indicator of inflammation (Aydin, et al., 2015). Another study has shown that the neutrophils population and production of superoxide in an obese individual is higher compared with those of a leaner individual (Brotfain, et al., 2015). Comprehensive nutrition based study by Hu et al. reported that dietary fats, not protein or carbohydrates, are responsible for obesity in laboratory rodents (Hu, et al., 2018). Our study identified that when a calorie-enriched diet, polyunsaturated fatty acid (PUFA-10% w/w safflower oil), was supplemented to aging mice for period of two months that increases 12(S)-HETE, a pro-inflammatory mediator which is responsible for vascular permeability to extravasate neutrophil endothelial transmigration in accelerating inflammation (Halade, Kain, Black, Prabhu, & Ingle, 2016; Lopez, et al., 2015). Aging mice study demonstrated that the leukocyte response in both young and aging mice fed PUFA showed higher levels of $Ly6G^+$ cells (neutrophils swarming) that amplifies inflammation markers post-MI, indicating neutrophil activity/quality and timing are essential to define the resolution of inflammation (Halade, et al., 2016; Lopez, et al., 2015). Balanced energy intake and diet are essential for calibrated neutrophil function and population and an optimal immune response, thus future investigation is warranted to define neutrophils role in inflammation-resolution pathway in HF.

3.4 Neutrophil dynamics in exercise versus sedentary pathophysiology

Physical activity/exercise is essential to adapt a healthy lifestyle if performed precisely in the right amount and time. Both physical activity and a sedentary lifestyle influence immune response and activation. Exercise induces an inflammatory response and neutrophil activation in skeletal muscle likely starts immediately after exercise (Frenette, Cai, & Tidball, 2000). Animal studies have also demonstrated a clear influence of neutrophils on muscle regeneration after exercise. In mice, the depletion of neutrophils before muscle injury impairs skeletal muscle regeneration, likely as a result of a reduced capacity to remove muscle tissue debris by neutrophil-dependent phagocytosis thereby slowing the regenerative process (Toumi, F'Guyer, & Best, 2006). A study by Kawamura et al. indicated that, regardless of the types of muscle-damaging exercise, peripheral neutrophils infiltrate the damaged tissue within several hours and cause inflammatory reactions by producing ROS (Kawamura, et al., 2018). Gene networks displayed an association between the transcriptome profiles of skeletal muscle and blood leukocytes in response to exercise or

other physiological stressors in eight trained men before and 3, 48, and 96 hours after 2 hours of strenous exercise (cycling and/or running) (Broadbent, et al., 2017). The participants displayed two gene network pathways related to mitochondria and confirmed that post-translational processes are preserved (Broadbent, et al., 2017). It has been reported that the impaired neutrophil migration in older adults (i.e. reduced velocity and accuracy) could be partially caused by physical inactivity (Bartlett, et al., 2016) (Sapey, et al., 2014). In humans, it has been observed that acute severe exercise (ASE) and chronic moderate exercise (CME) affect neutrophil functions differently. ASE was found to be ineffective and CME improved neutrophil functions, explaining the low risk of infection in physically active individuals (Syu, Chen, & Jen, 2012). Another study showed that ASE in sedentary, but not active subjects, facilitated neutrophil extracellular trap (NET) formation via elevating the NADPH oxidase-generated ROS and suppressing the $\Delta\Psi m$ (Syu, Chen, & Jen, 2013).

3.5 Neutrophils kinetics in the circadian cycle

A circadian rhythm is a 24-hour oscillative and physiological process of all living beings, including plants, animals, fungi, and cyanobacteria. Based on this dynamic homeostatic process, circulating leukocytes oscillate between blood and peripheral tissue of mice, peaking in the blood at Zeitgeber time ZT5 (where ZT0 refers to lights on and ZT12 to lights off) and in the muscle and bone marrow at ZT13 (Scheiermann, Kunisaki, & Frenette, 2013). MI performed at ZT13 had higher neutrophil counts in the heart compared to surgeries performed at ZT5. After MI, neutrophils were mobilized from the bone marrow, resulting in a significant decrease in femur neutrophil counts 12–24 h post-MI, especially in the ZT13 group (Schloss, et al., 2016). A baseline granulocyte–monocyte progenitor (GMP) was used for neutrophil counts in the bone marrow and showed the ZT13 group was higher 24h after MI compared to the ZT5 group. The levels of retention signal *CXCL12* in the bone marrow decreased from ZT5 to ZT13. The increased neutrophil mobilization after MI at ZT13 might be triggered by enhanced circulating levels of neutrophil chemoattractants. The levels of *TNF-α*, *CXCL1*, *CXCL2*, *CCL3*, *CCL5*, and *G-CSF* were upregulated in the plasma of ZT13 versus ZT5-operated mice 24 hours post-MI (Schloss, et al., 2016). These proinflammatory cytokines and chemokines and their receptors closely associate with the temporal and spatial recruitment of neutrophils to injury during the inflammatory phase and promote the clearance of necrotic neutrophils during the transition to the resolving phase. This inflated neutrophil migration into the heart, if kept unchecked, shifts the inflammation-resolution axis to more inflammatory conditions and leads to cardiac cell death and a decrease in tissue reparative capacity. Studies have shown that the neutrophils oscillate in steady-state with distinct rates and rhythmicity confirmed by using fate mapping parabiosis studies. Nonimmune neutrophils are densely populated in bone marrow, spleen, lung and liver to operate basal physiology and homeostasis, but neutrophils presence is limited in ovaries, testis, and brain (Casanova-Acebes, et al., 2018; Gibbs, et al., 2014). Diurnal compartmentalization of neutrophils, driven by an internal timer, coordinates immune defense and vascular protection (Adrover, et al., 2019). A recent comprehensive study by Swirski lab shows that mice subjected to sleep fragmentation produce more $Ly6C^{high}$ monocytes and amplified neutrophils with larger atherosclerotic lesions development and produce less hypocretin—a stimulatory and wake-promoting neuropeptide–in the lateral hypothalamus (McAlpine, et al., 2019). In contrast to sleep, daily sunlight manipulates

hypoxic pathways and provides essential cardioprotection to ischemic heart, however the role of neutrophils of sunlight directed rhythmic neutrophils activation is unclear (Oyama, et al., 2019). The rhythm based oscillation of neutrophils on the time of day (sleep and sunlight exposure) has emerged as an important and under-investigator gatekeeper of immune systems biology.

4. Microbiome

The mammalian immune system is a complex network of innate and adaptive components endowed with an extraordinary capacity to defense, adapt, and respond to highly diverse challenges. Complex microbiota supports large fraction of immune machinery to maintain a symbiotic relationship. The gut microbiome dysbiosis dysregulates the immune system defense plan. The various studies have show neutrophils are critically regulated by gut microbiota milieu. The studies have shown that neutrophil is important for human health via regulating microbiota. High number of neutrophils are recruited to the oral cavity in dysbiotic state. The neutrophils are responsible for clearance of infection with anaerobic bacteria, several periodontal pathogens such as *Porphyromonas gingivalis* are resistant to oxidative killing there decreasing oral inflammation (Berezow & Darveau, 2011; Mydel, et al., 2006). Neutrophils clear inflammation in lung infection by shifting profile from by $CD18^{hi}CD62L^{-/lo}$ inflammatory neutrophils to $CD18^{-/lo}CD62L^{hi}$ pro-resolution neutrophils (Felix, et al., 2018). The wild type C57BL/6 mice (which are innately resistant to *E. histiolytica* infection), when treated with antibiotics prior to cecal challenge with E. histolytica, desplayed more severe colitis and delayed clearance of *E. histolytica* which is due to decreased neutrophil recruitment to the gut (Watanabe, et al., 2017). Our recent study suggests that aging superimposed on obesity leads to dysbiosis leading to nonresolving inflammation. Intake of calorie-enriched diet in aging lead to expansion of genus *Allobaculum* that expanded neutrophils post-MI, dysregulated splenic leukocyte profiling with decrease in CD169+ macrophages (V. Kain, et al., 2019). Clinical studies have shown that in healthy controls, the blood and neutrophil-associated microbiomes in the patients with severe acute pancreatitis (SAP) were significantly altered, with an expansion in Bacteroidetes and Firmicutes as well as a decrease in Actinobacteria. Variations in the microbiome composition in SAP patients were observed to be correlated with immunological disorders, including altered lymphocyte subgroups, elevated levels of serum cytokines and altered proteomic profiles of neutrophils (Li, et al., 2018). The microbiome plays important role in regulating and maintaing neutrophil function which opens new avenues for devloping novel targets for therapeutics.

5. Summary and conclusion

Activated neutrophils are the first responder to facilitate the clearance of inflammation due to pathogens, injury, and stress-induced cellular dysfunction as part of physiological host defense. On-time entry of the activated neutrophils' to the site of injury/infection ('get-in signal'), transit time at site of injury/infection ('residual time'), and on-time exit or apoptosis ('get-out or safe clerance signal') are critical to define whether inflammation will lead to non-resolving inflammation or outline the resolving response. Life-style-related imbalanced intake of macro/micro-nutrients, sedentary work, and circadian rhythm that integrate with

microbiome are newly emerging regulators of neutrophil activity. The understanding of immune responsive lipid mediators microenvironment with circadian cycle and microbiome distribution that controls the entry, residual time, and exit of neutrophil trafficking is essential for developing new therapeutics to target the resolution of inflammation in different disease pathologies, including cardiovascular disease, cancer, and autoimmunity.

Acknowledgements

Authors acknowledge the support from National Institutes of Health AT006704, HL132989, HL132989, and UAB Pittman Scholar Award to GVH. The authors would like to thank Servier Medical Art imagebank that is used to create the schematics in figure 1–2.

List of non-standard abbreviations

CD	Cluster of differentiation
CCL	C-C Motif chemokine ligand
HF	Heart failure
LV	Left ventricle
MI	Myocardial infarction
MMP	Matrix metallopeptidase
NADPH	Nicotinamide adenine dinucleotide phosphate
NETs	Neutrophil extracellular traps
NGAL	Neutrophil gelatinase-associated lipocalin
NSAIDs	Nonsteroidal anti-inflammatory drugs
PUFA	Polyunsaturated fatty acids
PMN	Polymorphonuclear neutrophils
ROS	Reactive oxygen species
ZT	Zeitgeber

Glossary

Circadian clock
(or circadian oscillator) is a biochemical oscillator that cycles with a stable phase and is synchronized with solar time.

‘Don’t-eat me’ signal
are markers exposed on the cell surface for inhibiting phagocytosis. ‘Don’t-eat-me’ signals include CD47, which when expressed on the surface of a cell, inhibit phagocytosis of that cell, by activating signal regulatory protein alpha (SIRP)-alpha receptors on the phagocyte.

‘Eat-me’ signal
are makers a cell exposes on its surface being opsonized that binds soluble proteins that tag the cell for phagocytosis. For example, phosphatidylserine is an ‘eat-me’ signal that, when exposed on the surface of a cell, triggers phagocytes (i.e., cells that eat other cells) to eat that cell. Phosphatidylserine is normally found on the inside of healthy cells but can become exposed on the surface of dying, activated or stressed cells.

Fibrosis
is the formation of extracellular matrix connective tissue in an organ or tissue in a reparative or reactive process. Defined by the pathological accumulation of extracellular matrix proteins results in scarring and thickening of the affected tissue, it is an exaggerated wound healing response which interferes with normal organ function.

Myocardial infarction
(MI) is commonly known as a heart attack and occurs when blood flow decreases or stops to a part of the heart, causing damage to the heart muscle.

Non-resolving inflammation
Inflammation which did not let injury to recover back to the physiological levels that lead to organ (heart) dysfunction and failure.

Nonsteroidal anti-inflammatory drugs (NSAIDs)
are a drug class that reduces pain, decreases fever, prevents blood clots and, in higher doses, decreases inflammation.

Neutrophilia
is a high number of neutrophils in the blood.

Neutrophil extracellular traps (NETs)
are networks of extracellular fibers, primarily composed of DNA from neutrophils, which bind pathogens.

Polymorphonuclear neutrophils (PMNs)
are granulocytes which fall in the category of white blood cells characterized by the presence of granules in their cytoplasm. They are also called polymorphonuclear leukocytes or polymorphonuclear neutrophils (PMN, PML, or PMNL) because of the varying shapes of the nucleus, which is usually lobed into three segments.

Polyunsaturated fatty acids (PUFAs)
are fatty acids that contain more than one double bond in their backbone.

Zeitgeber
is any external or environmental cue that entrains or synchronizes an organism’s biological rhythms to the Earth’s 24-hour light/dark cycle and 12-month cycle.

References:

Adrover JM, del Fresno C, Crainiciuc G, Cuartero MI, Casanova-Acebes M, Weiss LA, Huerga-Encabo H, Silvestre-Roig C, Rossaint J, Cossío I, Lechuga-Vieco AV, García-Prieto J, Gómez-Parrizas M, Quintana JA, Ballesteros I, Martin-Salamanca S, Aroca-Crevillen A, Chong SZ, Evrard M, Balabanian K, López J, Bidzhekov K, Bachelerie F, Abad-Santos F, Muñoz-Calleja C, Zarbock A, Soehnlein O, Weber C, Ng LG, Lopez-Rodriguez C, Sancho D, Moro MA, Ibáñez B, & Hidalgo A. (2019). A Neutrophil Timer Coordinates Immune Defense and Vascular Protection. Immunity, 50, 390–402.e310. [PubMed: 30709741]

Akpek M, Kaya MG, Lam YY, Sahin O, Elcik D, Celik T, Ergin A, & Gibson CM. (2012). Relation of neutrophil/lymphocyte ratio to coronary flow to in-hospital major adverse cardiac events in patients with ST-elevated myocardial infarction undergoing primary coronary intervention. Am J Cardiol, 110, 621–627. [PubMed: 22608360]

Aydin M, Yilmaz A, Donma MM, Tulubas F, Demirkol M, Erdogan M, & Gurel A. (2015). Neutrophil/lymphocyte ratio in obese adolescents. North Clin Istanb, 2, 87–91. [PubMed: 28058347]

Balabanian K, Lagane B, Pablos JL, Laurent L, Planchenault T, Verola O, Lebbe C, Kerob D, Dupuy A, Hermine O, Nicolas JF, Latger-Cannard V, Bensoussan D, Bordigoni P, Baleux F, Le Deist F, Virelizier JL, Arenzana-Seisdedos F, & Bachelerie F. (2005). WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12. Blood, 105, 2449–2457. [PubMed: 15536153]

Bartlett DB, Fox O, McNulty CL, Greenwood HL, Murphy L, Sapey E, Goodman M, Crabtree N, Thogersen-Ntoumani C, Fisher JP, Wagenmakers AJ, & Lord JM. (2016). Habitual physical activity is associated with the maintenance of neutrophil migratory dynamics in healthy older adults. Brain Behav Immun, 56, 12–20. [PubMed: 26928196]

Basu S, Hodgson G, Katz M, & Dunn AR. (2002). Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation. Blood, 100, 854–861. [PubMed: 12130495]

Berezow AB, & Darveau RP. (2011). Microbial shift and periodontitis. Periodontol 2000, 55, 36–47. [PubMed: 21134227]

Borregaard N, Kjeldsen L, Lollike K, & Sengelov H. (1995). Granules and secretory vesicles of the human neutrophil. Clin Exp Immunol, 101 Suppl 1, 6–9. [PubMed: 7606861]

Bourke CD, Berkley JA, & Prendergast AJ. (2016). Immune Dysfunction as a Cause and Consequence of Malnutrition. Trends in immunology, 37, 386–398. [PubMed: 27237815]

Brandes Ralf P, Rezende F, & Schröder K. (2018). Redox Regulation Beyond ROS. Circulation Research, 123, 326–328. [PubMed: 30026378]

Bratton DL, & Henson PM. (2011). Neutrophil clearance: when the party is over, clean-up begins. Trends Immunol, 32, 350–357. [PubMed: 21782511]

Broadbent J, Sampson D, Sabapathy S, Haseler LJ, Wagner KH, Bulmer AC, Peake JM, & Neubauer O (2017). Gene networks in skeletal muscle following endurance exercise are coexpressed in blood neutrophils and linked with blood inflammation markers. J Appl Physiol (1985), 122, 752–766. [PubMed: 28104750]

Brotfain E, Hadad N, Shapira Y, Avinoah E, Zlotnik A, Raichel L, & Levy R. (2015). Neutrophil functions in morbidly obese subjects. Clin Exp Immunol, 181, 156–163. [PubMed: 25809538]

Buckley CD, Ross EA, McGettrick HM, Osborne CE, Haworth O, Schmutz C, Stone PC, Salmon M, Matharu NM, Vohra RK, Nash GB, & Rainger GE. (2006). Identification of a phenotypically and functionally distinct population of long-lived neutrophils in a model of reverse endothelial migration. J Leukoc Biol, 79, 303–311. [PubMed: 16330528]

Canturk NZ, Esen N, Vural B, Canturk Z, Kirkali G, Oktay G, & Solakoglu S. (2001). The relationship between neutrophils and incisional wound healing. Skin Pharmacol Appl Skin Physiol, 14, 108–116. [PubMed: 11316969]

Casanova-Acebes M, Nicolas-Avila JA, Li JL, Garcia-Silva S, Balachander A, Rubio-Ponce A, Weiss LA, Adrover JM, Burrows K, N, A. G., Ballesteros I, Devi S, Quintana JA, Crainiciuc G, Leiva M, Gunzer M, Weber C, Nagasawa T, Soehnlein O, Merad M, Mortha A, Ng LG, Peinado H, &

Hidalgo A. (2018). Neutrophils instruct homeostatic and pathological states in naive tissues. J Exp Med, 215, 2778–2795. [PubMed: 30282719]

Cauwe B, Martens E, Proost P, & Opdenakker G. (2009). Multidimensional degradomics identifies systemic autoantigens and intracellular matrix proteins as novel gelatinase B/MMP-9 substrates Integr Biol (Camb), 1, 404–426. [PubMed: 20023747]

Chakrabarti S, & Patel KD. (2005). Regulation of matrix metalloproteinase-9 release from IL-8-stimulated human neutrophils. J Leukoc Biol, 78, 279–288. [PubMed: 15831558]

Cuartero MI, Ballesteros I, Moraga A, Nombela F, Vivancos J, Hamilton JA, Corbi AL, Lizasoain I, & Moro MA. (2013). N2 neutrophils, novel players in brain inflammation after stroke: modulation by the PPARgamma agonist rosiglitazone. Stroke, 44, 3498–3508. [PubMed: 24135932]

Dancey JT, Deubelbeiss KA, Harker LA, & Finch CA. (1976). Neutrophil kinetics in man. J Clin Invest, 58, 705–715. [PubMed: 956397]

Daseke MJ 2nd, Valerio FM, Kalusche WJ, Ma Y, DeLeon-Pennell KY, & Lindsey M. (2019). Neutrophil proteome shifts over the myocardial infarction time continuum. Basic research in cardiology, 114, 37–37. [PubMed: 31418072]

de Oliveira S, Rosowski EE, & Huttenlocher A. (2016). Neutrophil migration in infection and wound repair: going forward in reverse. Nat Rev Immunol, 16, 378–391. [PubMed: 27231052]

Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P, Lysiak JJ, Harden TK, Leitinger N, & Ravichandran KS. (2009). Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature, 461, 282–286. [PubMed: 19741708]

Faurschou M, & Borregaard N. (2003). Neutrophil granules and secretory vesicles in inflammation. Microbes Infect, 5, 1317–1327. [PubMed: 14613775]

Felix KM, Jaimez I, Nguyen T-V, Ma H, Raslan W, Klinger C, Doyle K, & Wu H-JJ. (2018). Gut microbiota enhances neutrophil resolution in immunocompromised hosts to improve response to pneumococcal pneumonia. The Journal of Immunology, 200, 173.110–173.110.

Fosshaug LE, Colas RA, Anstensrud AK, Gregersen I, Nymo S, Sagen EL, Michelsen A, Vinge LE, Øie E, Gullestad L, Halvorsen B, Hansen TV, Aukrust P, Dalli J, & Yndestad A. (2019). Early increase of specialized pro-resolving lipid mediators in patients with ST-elevation myocardial infarction. EBioMedicine, 46, 264–273. [PubMed: 31345784]

Foxman EF, Campbell JJ, & Butcher EC. (1997). Multistep navigation and the combinatorial control of leukocyte chemotaxis. J Cell Biol, 139, 1349–1360. [PubMed: 9382879]

Frangogiannis NG. (2012). Regulation of the inflammatory response in cardiac repair. Circ Res, 110, 159–173. [PubMed: 22223212]

Frenette J, Cai B, & Tidball JG. (2000). Complement activation promotes muscle inflammation during modified muscle use. Am J Pathol, 156, 2103–2110. [PubMed: 10854231]

Fu H, Karlsson J, Bylund J, Movitz C, Karlsson A, & Dahlgren C. (2006). Ligand recognition and activation of formyl peptide receptors in neutrophils. J Leukoc Biol, 79, 247–256. [PubMed: 16365159]

Ganz T. (2002). Antimicrobial polypeptides in host defense of the respiratory tract. J Clin Invest, 109, 693–697. [PubMed: 11901174]

Garner P, & Brabin B. (1994). A review of randomized controlled trials of routine antimalarial drug prophylaxis during pregnancy in endemic malarious areas. Bull World Health Organ, 72, 89–99. [PubMed: 8131256]

Gay JC. (1993). Mechanism and regulation of neutrophil priming by platelet-activating factor. J Cell Physiol, 156, 189–197. [PubMed: 8391006]

Gibbs J, Ince L, Matthews L, Mei J, Bell T, Yang N, Saer B, Begley N, Poolman T, Pariollaud M, Farrow S, DeMayo F, Hussell T, Worthen GS, Ray D, & Loudon A. (2014). An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. Nat Med, 20, 919–926. [PubMed: 25064128]

Halade GV, Jin Y-F, & Lindsey ML. (2013). Matrix metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. Pharmacology & therapeutics, 139, 32–40. [PubMed: 23562601]

Halade GV, & Kain V. (2017). Obesity and Cardiometabolic Defects in Heart Failure Pathology. Comprehensive Physiology, 7, 1463–1477. [PubMed: 28915332]

Halade GV, Kain V, Black LM, Prabhu SD, & Ingle KA. (2016). Aging dysregulates D- and E-series resolvins to modulate cardiosplenic and cardiorenal network following myocardial infarction. Aging (Albany NY), 8, 2611–2634. [PubMed: 27777380]

Halade GV, Kain V, Wright GM, & Jadapalli JK. (2018). Subacute treatment of carprofen facilitate splenocardiac resolution deficit in cardiac injury. J Leukoc Biol, 104, 1173–1186. [PubMed: 30145840]

Halade GV, & Ma Y. (2016). Neutrophils: Friend, foe, or contextual ally in myocardial healing. J Mol Cell Cardiol, 97, 44–46. [PubMed: 27133770]

Halade GV, Norris PC, Kain V, Serhan CN, & Ingle KA. (2018). Splenic leukocytes define the resolution of inflammation in heart failure. Science signaling, 11, eaao1818.

Hansson GK. (2005). Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med, 352, 1685–1695. [PubMed: 15843671]

Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, Klotman ME, & Diaz GA. (2003). Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet, 34, 70–74. [PubMed: 12692554]

Hirano Y, Aziz M, & Wang P. (2016). Role of reverse transendothelial migration of neutrophils in inflammation. In Biological Chemistry (Vol. 397, pp. 497). [PubMed: 26872312]

Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M, Weber C, Soehnlein O, & Steffens S. (2017). Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. Eur Heart J, 38, 187–197. [PubMed: 28158426]

Hu S, Wang L, Yang D, Li L, Togo J, Wu Y, Liu Q, Li B, Li M, Wang G, Zhang X, Niu C, Li J, Xu Y, Couper E, Whittington-Davies A, Mazidi M, Luo L, Wang S, Douglas A, & Speakman JR. (2018). Dietary Fat, but Not Protein or Carbohydrate, Regulates Energy Intake and Causes Adiposity in Mice. Cell Metabolism, 28, 415–431.e414. [PubMed: 30017356]

Jadapalli JK, Wright GW, Kain V, Sherwani MA, Sonkar R, Yusuf N, & Halade GV. (2018). Doxorubicin triggers splenic contraction and irreversible dysregulation of COX and LOX that alters the inflammation-resolution program in the myocardium. Am J Physiol Heart Circ Physiol, 315, H1091–H1100. [PubMed: 30074834]

Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN, Joshi M, & Halade GV. (2015). Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. J Mol Cell Cardiol, 84, 24–35. [PubMed: 25870158]

Kain V, Ingle KA, Kabarowski J, Barnes S, Limdi NA, Prabhu SD, & Halade GV. (2018). Genetic deletion of 12/15 lipoxygenase promotes effective resolution of inflammation following myocardial infarction. Journal of molecular and cellular cardiology, 118, 70–80. [PubMed: 29526491]

Kain V, Jadapalli JK, Tourki B, & Halade GV. (2019). Inhibition of FPR2 impaired leukocytes recruitment and elicited non-resolving inflammation in acute heart failure. Pharmacological Research, 146, 104295.

Kain V, Liu F, Kozlovskaya V, Ingle KA, Bolisetty S, Agarwal A, Khedkar S, Prabhu SD, Kharlampieva E, & Halade GV. (2017). Resolution Agonist 15-epi-Lipoxin A4 Programs Early Activation of Resolving Phase in Post-Myocardial Infarction Healing. Sci Rep, 7, 9999. [PubMed: 28855632]

Kain V, Prabhu SD, & Halade GV. (2014). Inflammation revisited: inflammation versus resolution of inflammation following myocardial infarction. Basic Res Cardiol, 109, 444. [PubMed: 25248433]

Kain V, Van Der Pol W, Mariappan N, Ahmad A, Eipers P, Gibson DL, Gladine C, Vigor C, Durand T, Morrow C, & Halade GV. (2019). Obesogenic diet in aging mice disrupts gut microbe composition and alters neutrophil:lymphocyte ratio, leading to inflamed milieu in acute heart failure. FASEB J, 33, 6456–6469. [PubMed: 30768364]

Kawamura T, Suzuki K, Takahashi M, Tomari M, Hara R, Gando Y, & Muraoka I. (2018). Involvement of Neutrophil Dynamics and Function in Exercise-Induced Muscle Damage and Delayed-Onset Muscle Soreness: Effect of Hydrogen Bath. Antioxidants (Basel), 7, 127.

Kolaczkowska E, & Kubes P. (2013). Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol, 13, 159–175. [PubMed: 23435331]

Li Q, Wang C, Tang C, Zhao X, He Q, & Li J. (2018). Identification and Characterization of Blood and Neutrophil-Associated Microbiomes in Patients with Severe Acute Pancreatitis Using Next-Generation Sequencing. Front Cell Infect Microbiol, 8, 5. [PubMed: 29423379]

Liu M, Chen K, Yoshimura T, Liu Y, Gong W, Wang A, Gao JL, Murphy PM, & Wang JM. (2012). Formylpeptide receptors are critical for rapid neutrophil mobilization in host defense against Listeria monocytogenes. Sci Rep, 2, 786. [PubMed: 23139859]

Liu X, Ma B, Malik AB, Tang H, Yang T, Sun B, Wang G, Minshall RD, Li Y, Zhao Y, Ye RD, & Xu J. (2012). Bidirectional regulation of neutrophil migration by mitogen-activated protein kinases. Nat Immunol, 13, 457–464. [PubMed: 22447027]

Lopez EF, Kabarowski JH, Ingle KA, Kain V, Barnes S, Crossman DK, Lindsey ML, & Halade GV. (2015). Obesity superimposed on aging magnifies inflammation and delays the resolving response after myocardial infarction. Am J Physiol Heart Circ Physiol, 308, H269–280. [PubMed: 25485899]

Ma Y, Yabluchanskiy A, Iyer RP, Cannon PL, Flynn ER, Jung M, Henry J, Cates CA, Deleon-Pennell KY, & Lindsey ML. (2016). Temporal neutrophil polarization following myocardial infarction. Cardiovasc Res, 110, 51–61. [PubMed: 26825554]

Ma Y, Yabluchanskiy A, & Lindsey ML. (2013). Neutrophil roles in left ventricular remodeling following myocardial infarction. Fibrogenesis Tissue Repair, 6, 11. [PubMed: 23731794]

Mantovani A, Cassatella MA, Costantini C, & Jaillon S. (2011). Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol, 11, 519–531. [PubMed: 21785456]

Mayadas TN, Cullere X, & Lowell CA. (2014). The multifaceted functions of neutrophils. Annu Rev Pathol, 9, 181–218. [PubMed: 24050624]

McAlpine CS, Kiss MG, Rattik S, He S, Vassalli A, Valet C, Anzai A, Chan CT, Mindur JE, Kahles F, Poller WC, Frodermann V, Fenn AM, Gregory AF, Halle L, Iwamoto Y, Hoyer FF, Binder CJ, Libby P, Tafti M, Scammell TE, Nahrendorf M, & Swirski FK. (2019). Sleep modulates haematopoiesis and protects against atherosclerosis. Nature.

Mestas J, & Hughes CCW. (2004). Of Mice and Not Men: Differences between Mouse and Human Immunology. The Journal of Immunology, 172, 2731–2738. [PubMed: 14978070]

Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J, & Devarajan P. (2005). Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet, 365, 1231–1238. [PubMed: 15811456]

Mydel P, Takahashi Y, Yumoto H, Sztukowska M, Kubica M, Gibson FC 3rd, Kurtz DM Jr., Travis J, Collins LV, Nguyen KA, Genco CA, & Potempa J (2006). Roles of the host oxidative immune response and bacterial antioxidant rubrerythrin during Porphyromonas gingivalis infection. PLoS Pathog, 2, e76.

Nathan C. (2006). Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol, 6, 173–182. [PubMed: 16498448]

Nunez J, Nunez E, Bodi V, Sanchis J, Minana G, Mainar L, Santas E, Merlos P, Rumiz E, Darmofal H, Heatta AM, & Llacer A (2008). Usefulness of the neutrophil to lymphocyte ratio in predicting long-term mortality in ST segment elevation myocardial infarction. Am J Cardiol, 101, 747–752. [PubMed: 18328833]

O'Connell KE, Mikkola AM, Stepanek AM, Vernet A, Hall CD, Sun CC, Yildirim E, Staropoli JF, Lee JT, & Brown DE. (2015). Practical murine hematopathology: a comparative review and implications for research. Comp Med, 65, 96–113. [PubMed: 25926395]

Opie EL. (1907). Experimental Pleurisy--Resolution of a Fibrinous Exudate. Journal of Experimental Medicine, 9, 391–413. [PubMed: 19867099]

Oyama Y, Bartman CM, Bonney S, Lee JS, Walker LA, Han J, Borchers CH, Buttrick PM, Aherne CM, Clendenen N, Colgan SP, & Eckle T. (2019). Intense Light-Mediated Circadian Cardioprotection via Transcriptional Reprogramming of the Endothelium. Cell Reports, 28, 1471–1484.e1411. [PubMed: 31390562]

Papayannopoulos V, Metzler KD, Hakkim A, & Zychlinsky A. (2010). Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol, 191, 677–691. [PubMed: 20974816]

Piccard H, Muschel RJ, & Opdenakker G. (2012). On the dual roles and polarized phenotypes of neutrophils in tumor development and progression. Crit Rev Oncol Hematol, 82, 296–309. [PubMed: 21798756]

Puklo M, Guentsch A, Hiemstra PS, Eick S, & Potempa J. (2008). Analysis of neutrophil-derived antimicrobial peptides in gingival crevicular fluid suggests importance of cathelicidin LL-37 in the innate immune response against periodontogenic bacteria. Oral Microbiol Immunol, 23, 328–335. [PubMed: 18582333]

Rankin SM. (2010). The bone marrow: a site of neutrophil clearance. J Leukoc Biol, 88, 241–251. [PubMed: 20483920]

Rashidi F, Rashidi A, Golmohamadi A, Hoseinzadeh E, Mohammadi B, Mirzajani H, Kheiri M, & Jamshidi P. (2008). Does absolute neutrophilia predict early congestive heart failure after acute myocardial infarction? A cross-sectional study. South Med J, 101, 19–23. [PubMed: 18176286]

Ravichandran KS. (2010). Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. J Exp Med, 207, 1807–1817. [PubMed: 20805564]

Ravichandran KS. (2011). Beginnings of a good apoptotic meal: the find-me and eat-me signaling pathways. Immunity, 35, 445–455. [PubMed: 22035837]

Roos D. (1998). Neutrophils In Encyclopedia of Immunology (pp. 1854–1858). Oxford: Elsevier.

Sapey E, Greenwood H, Walton G, Mann E, Love A, Aaronson N, Insall RH, Stockley RA, & Lord JM. (2014). Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. Blood, 123, 239–248. [PubMed: 24191150]

Scheiermann C, Kunisaki Y, & Frenette PS. (2013). Circadian control of the immune system. Nat Rev Immunol, 13, 190–198. [PubMed: 23391992]

Schloss MJ, Horckmans M, Nitz K, Duchene J, Drechsler M, Bidzhekov K, Scheiermann C, Weber C, Soehnlein O, & Steffens S (2016). The time-of-day of myocardial infarction onset affects healing through oscillations in cardiac neutrophil recruitment. EMBO Mol Med, 8, 937–948. [PubMed: 27226028]

Serhan CN. (2010). Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? Am J Pathol, 177, 1576–1591. [PubMed: 20813960]

Serhan CN, Chiang N, & Van Dyke TE. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol, 8, 349–361. [PubMed: 18437155]

Simon SI, & Kim MH. (2010). A day (or 5) in a neutrophil's life. Blood, 116, 511–512. [PubMed: 20671133]

Soehnlein O, Steffens S, Hidalgo A, & Weber C. (2017). Neutrophils as protagonists and targets in chronic inflammation. Nat Rev Immunol, 17, 248–261. [PubMed: 28287106]

Sørensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, & Borregaard N. (2001). Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood, 97, 3951–3959. [PubMed: 11389039]

Souto Padron de Figueiredo A, Salmon AB, Bruno F, Jimenez F, Martinez HG, Halade GV, Ahuja SS, Clark RA, DeFronzo RA, Abboud HE, & El Jamali A. (2015). Nox2 mediates skeletal muscle insulin resistance induced by a high fat diet. J Biol Chem, 290, 13427–13439. [PubMed: 25825489]

Spite M, Clària J, & Serhan CN. (2014). Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. Cell metabolism, 19, 21–36. [PubMed: 24239568]

Syu GD, Chen HI, & Jen CJ. (2012). Differential effects of acute and chronic exercise on human neutrophil functions. Med Sci Sports Exerc, 44, 1021–1027. [PubMed: 22130467]

Syu GD, Chen HI, & Jen CJ. (2013). Acute severe exercise facilitates neutrophil extracellular trap formation in sedentary but not active subjects. Med Sci Sports Exerc, 45, 238–244. [PubMed: 22903136]

Tak T, Tesselaar K, Pillay J, Borghans JA, & Koenderman L. (2013). What's your age again? Determination of human neutrophil half-lives revisited. J Leukoc Biol, 94, 595–601. [PubMed: 23625199]

Takele Y, Adem E, Getahun M, Tajebe F, Kiflie A, Hailu A, Raynes J, Mengesha B, Ayele TA, Shkedy Z, Lemma M, Diro E, Toulza F, Modolell M, Munder M, Muller I, & Kropf P. (2016). Malnutrition in Healthy Individuals Results in Increased Mixed Cytokine Profiles, Altered Neutrophil Subsets and Function. PLoS One, 11, e0157919.

Theilgaard-Monch K, Porse BT, & Borregaard N. (2006). Systems biology of neutrophil differentiation and immune response. Curr Opin Immunol, 18, 54–60. [PubMed: 16343884]

Toumi H, F'Guyer S, & Best TM. (2006). The role of neutrophils in injury and repair following muscle stretch. Journal of anatomy, 208, 459–470. [PubMed: 16637872]

Tourki B, & Halade G. (2017). Leukocyte diversity in resolving and nonresolving mechanisms of cardiac remodeling. FASEB J, 31, 4226–4239. [PubMed: 28642328]

Tracchi I, Ghigliotti G, Mura M, Garibaldi S, Spallarossa P, Barisione C, Boasi V, Brunelli M, Corsiglia L, Barsotti A, & Brunelli C. (2009). Increased neutrophil lifespan in patients with congestive heart failure. Eur J Heart Fail, 11, 378–385. [PubMed: 19276127]

Truman LA, Ford CA, Pasikowska M, Pound JD, Wilkinson SJ, Dumitriu IE, Melville L, Melrose LA, Ogden CA, Nibbs R, Graham G, Combadiere C, & Gregory CD. (2008). CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. Blood, 112, 5026–5036. [PubMed: 18799722]

Vulesevic B, Sirois MG, Allen BG, de Denus S, & White M. (2018). Subclinical Inflammation in Heart Failure: A Neutrophil Perspective. Can J Cardiol, 34, 717–725. [PubMed: 29801737]

Wan E, Yeap XY, Dehn S, Terry R, Novak M, Zhang S, Iwata S, Han X, Homma S, Drosatos K, Lomasney J, Engman DM, Miller SD, Vaughan DE, Morrow JP, Kishore R, & Thorp EB. (2013). Enhanced efferocytosis of apoptotic cardiomyocytes through myeloid-epithelial-reproductive tyrosine kinase links acute inflammation resolution to cardiac repair after infarction. Circ Res, 113, 1004–1012. [PubMed: 23836795]

Watanabe K, Gilchrist CA, Uddin MJ, Burgess SL, Abhyankar MM, Moonah SN, Noor Z, Donowitz JR, Schneider BN, Arju T, Ahmed E, Kabir M, Alam M, Haque R, Pramoonjago P, Mehrad B, & Petri WA Jr. (2017). Microbiome-mediated neutrophil recruitment via CXCR2 and protection from amebic colitis. PLoS Pathog, 13, e1006513.

Winterbourn CC, Kettle AJ, & Hampton MB. (2016). Reactive Oxygen Species and Neutrophil Function. Annu Rev Biochem, 85, 765–792. [PubMed: 27050287]

Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, & Halbwachs-Mecarelli L. (2000). Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest, 80, 617–653. [PubMed: 10830774]

Yndestad A, Landro L, Ueland T, Dahl CP, Flo TH, Vinge LE, Espevik T, Froland SS, Husberg C, Christensen G, Dickstein K, Kjekshus J, Oie E, Gullestad L, & Aukrust P. (2009). Increased systemic and myocardial expression of neutrophil gelatinase-associated lipocalin in clinical and experimental heart failure. Eur Heart J, 30, 1229–1236. [PubMed: 19329498]

Yu T, Robotham JL, & Yoon Y. (2006). Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci U S A, 103, 2653–2658. [PubMed: 16477035]

Zahorec R. (2001). Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy, 102, 5–14. [PubMed: 11723675]

Outstanding Question Box

- A recent discovery confirmed that neutrophils enter two different ways at the site of injury or infection: intra-endothelial migration and trans-endothelial migration (‘get-in’signal). Studies are required to determine time line of neutrophils at the entry and exit at the site of injury and when they should leave in HF.
- Future research is required to define the fate and lifespan of neutrophils: whether neutrophils leave the infected or injured organ at a specific time, or if they are phagocytosed by macrophages at pre-determined time.
- The circumstances neutrophil infiltration is accelerated and exfiltration is delayed, leading to non-resolving inflammation and neutrophil swarming.
- The ability of activated and overactive neutrophils to eat macrophages and delay the resolution of inflammation and the markers they express.
- The mechanism that will facilitate on time-infiltration and clearance without altering the phagocytic activity and residual time of neutrophils to aid in resolving inflammation.

Highlights

Neutrophils have emerged as a surrogate marker of cardiovascular risk. Plasma neutrophil elastase levels have been shown to be correlated with severity of coronary artery diseases. MPO plasma levels in coronary angiography patients could predict cardiovascular mortality. Clinical trials have confirmed high NLR (neutrophil/ lymphocyte ratio) values are accurate and strong predictors of the in-hospital mortality of patients with acute HF.

- Neutrophil swarming has been reported in several inflammatory diseases. The CALIBER (Clinical Research Using Linked Bespoke Studies and Electronic Health Records) trial revealed high neutrophils are strongly correlated with HF, PAD, and nonfatal myocardial infarction (MI).
- Neutrophil count and activation could be considered as a means of monitoring a patient’s progress or as a surrogate endpoint in trials investigating anti-inflammatory interventions to reduce cardiovascular risk.
- Not only the timely activation of neutrophils in precise space but lack of overly activation is essential to resolve post-MI inflammation

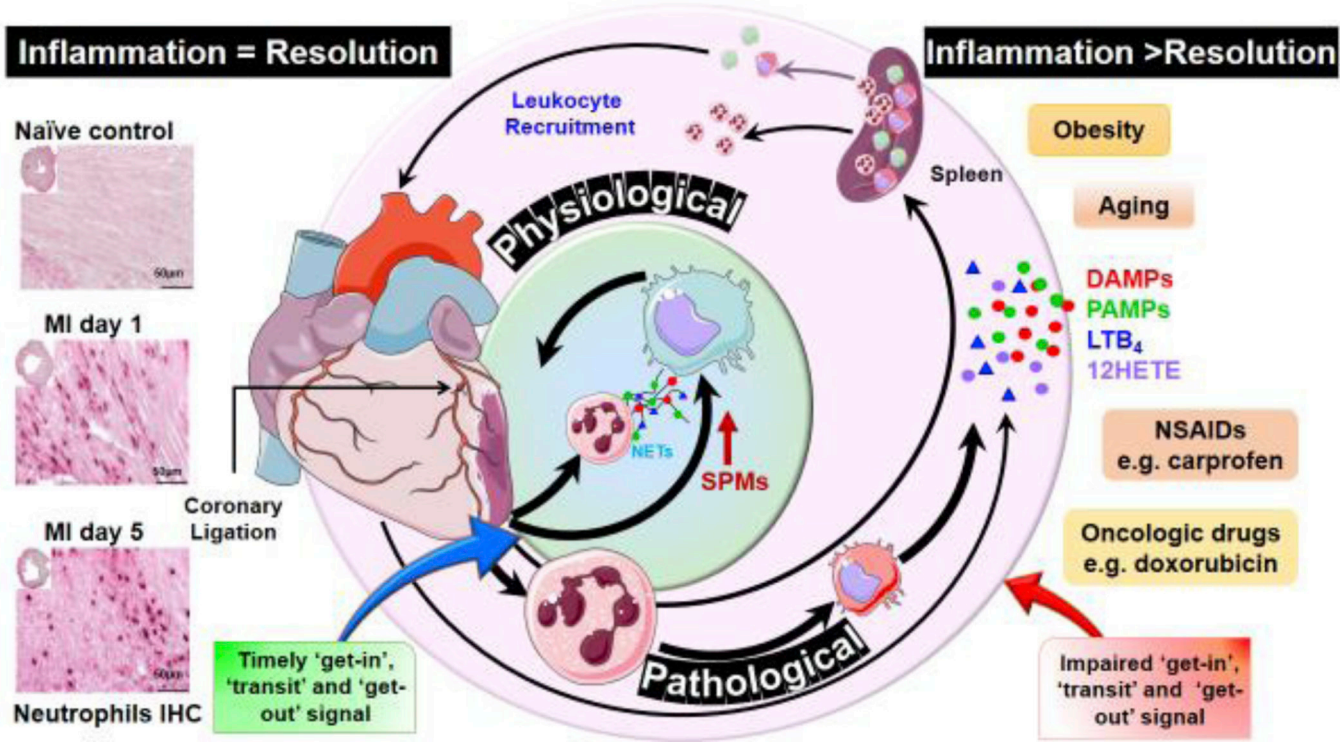

Figure 1:

Schematic design defines 'get-in' and 'get-out' signal that determines the safe clearance and transit time of neutrophils at the site of infection, injury, or inflammation. Overall, the figure indicates that innate responsive infiltration of neutrophil set the platform for physiological cardiac healing (in abesnce of risk factors like obesity or aging) post-myocardial infarction (MI). In physiological cardiac healing post-MI resolution is almost equal to inflammation, marked with the biosynthesis of specialized pro-resolving mediators (SPMs) at the site of infarction along with timely 'get-in', 'transit', and 'get-out' signal of immune cells that leads to effective resolution with balanced inflammation termed as physiological healing (Light green color –inner circle). However, in presence of risk factors like diet-induced obesity (Lopez, et al., 2015), aging (Halade, et al., 2016), co-medications like NSAIDs (nonsteroidal anti-inflammatory drugs) (G. V. Halade, et al., 2018), oncologic drugs e.g. doxorubicin (Jadapalli, et al., 2018) or combinations of these risk factors leads to impairment of 'get-in', 'transit', and 'get-out' signals leading to pathological and dysregulated inflammation that outweigh over the resoution. Neutrophils immunohistochemistry of heart tissue sections displaying infiltration of neutrophils in left ventricle at day 1 and day 5 post-myocardial infarction in comparison to naïve control showing absence of neutrophils (40X). The blue arrow and green shade denotes physiological cardiac healing and timely get-in and get-out signal. The red arow and and pink shade denotes the pathological healing with impared get- and get-out signal. NETs: Neutrophil extracellular traps ; SPMs: Specialized proresolving Mediators; DAMPs: Damage-associated molecular patterns, PAMPs:pathogen-associated molecular patterns, LTB_4: Leukotriene B_4; 12HETE: 12-hydroxyeicosatetraenoic acid.

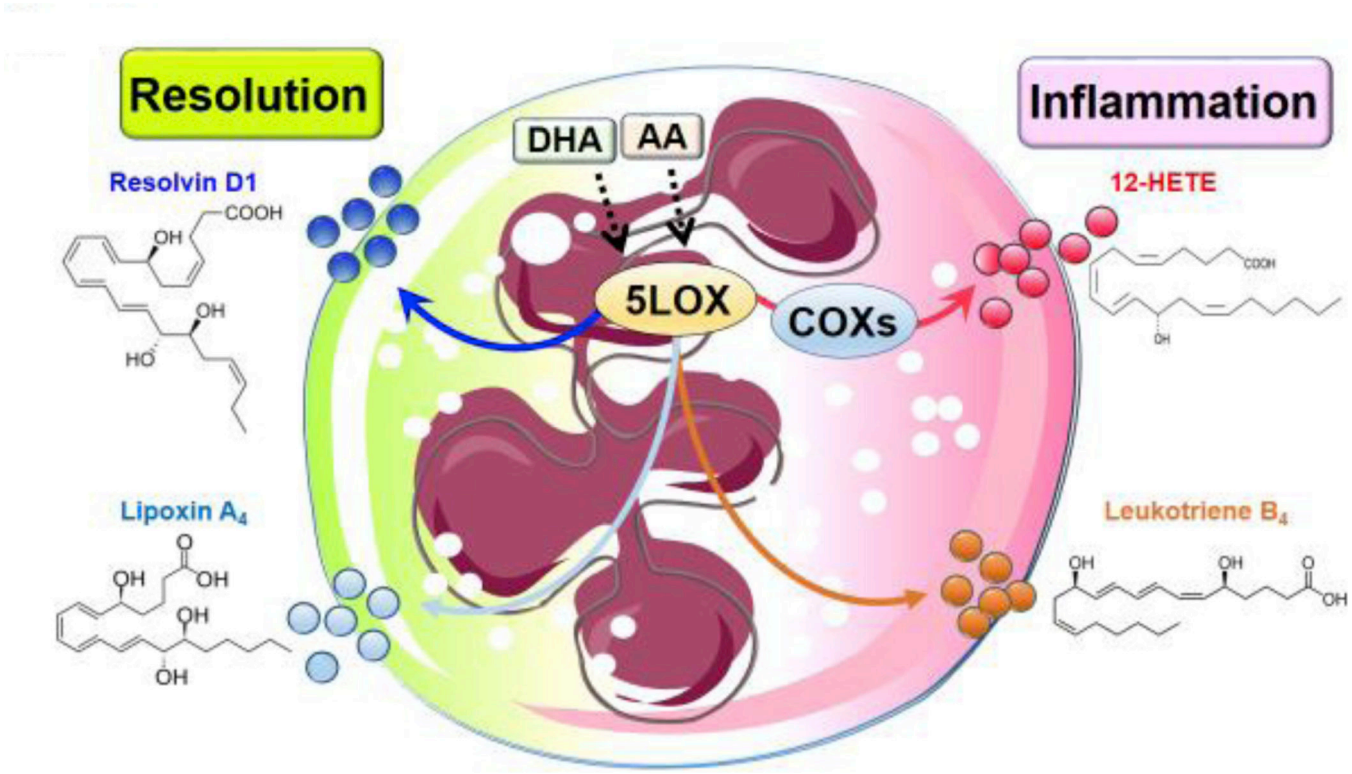

Figure 2:

Schematic illustration displaying pro-resolving immunoresolvent (resolvin D1 and lipoxin A_4) and pro-inflammatory lipid mediators (12-HETE and leukotriene B_4) biosysthesied from their respective substrate docohexaenoic acid (DHA) and arachidonic acid (AA) using immune responsive enzymes 5-lipoxygenas (5LOX) and cyclooxgenase-2 (COX-2) produced by neutrophils at the site of injury. The colour code at right signifies moieties produced by neutrophils to facilitate resolution of inaflammation (blue to green) while at left signiifies moieties produced during inflammtory resonse (pink to red and brown).

Table 1:

Traditional and novel paradigms of neutrophil activation and inactivation markers in the inflammation-resolution axis.

	Traditional paradigms	Novel paradigms
Function	Three phases: 1) Adhesion, Migration and diapedesis -E-, P-, L-selectins, ICAM-1, VCAM-1, integrins 2) Opsonization and recognition (PAMP, DAMPS, complement proteins, pentraxins, collectins, and ficolins 3) Phagocytosis (Fc receptors CD32 and CD16) (Theilgaard-Monch, Porse, & Borregaard, 2006)	**1) On-time entry (infiltration)**: Complement component 5a (C5a), formyl-Met-Leu-Phe (fMLP), platelet-activating factor and leukotriene B_4 (LTB_4), Mac-1 receptors (Fu, et al., 2006; Gay, 1993) **2) Residual time (phagocytic war time: NETosis)**: granule-derived antimicrobial peptides, neutrophil elastase, cathepsin G, and myeloperoxidase (MPO) (Papayannopoulos, Metzler, Hakkim, & Zychlinsky, 2010; Puklo, Guentsch, Hiemstra, Eick, & Potempa, 2008; Sørensen, et al., 2001) **3) On-time exit (clearance)**: Resolvins, Protectin, Maresins, Lipoxins, adiponectins, Maresin-1, PD1(Kain, et al., 2015; Kain, et al., 2017; Serhan, 2010; Serhan, et al., 2008)
Life span (circulation)	Short lived (8–12 hrs)	5.4 days humans and.75 day in mice
Phenotype	Homogeneous in nature. Based on same size, function and granule proteins (Theilgaard-Monch, et al., 2006)	Heterogeneous (N1: proinflammatory phenotype: $CD11b^+/Ly6G^+/CCL2^+/Arg^-$, N2: proresolving phenotype: $CD11b^+/Ly6G^+/Ly6C^{int}CCL3^+/Arg^+$, (Piccard, Muschel, & Opdenakker, 2012); $Ly6G^+/CD206^+$(Ganesh V. Halade, et al., 2018; Kain, et al., 2018)
Transmigration	Direct infiltration and migration (such as macrophage-inflammatory protein-2 (MIP-2), keratinocyte-derived chemokine (KC), and by other chemoattractants, such as bacteria-derived N-formyl-methionyl-leucyl-phenylalanine (fMLP), and the complement component (C5a) (Foxman, Campbell, & Butcher, 1997) (de Oliveira, Rosowski, & Huttenlocher, 2016)	Reverse Transmigration ($CD54^{hi}CXCR1^{lo}$ expression) cytokine-induced neutrophil chemoattractant-1, CXCR4, granulocyte-colony stimulating factor (G-CSF), and CXCL12. (Balabanian, et al., 2005; Buckley, et al., 2006; Hernandez, et al., 2003; Hirano, Aziz, & Wang, 2016; Rankin, 2010)
Interaction with microbiome	No interaction – understudied	Microbiota control neutrophil phenotype and function – evolving. (V. Kain, et al., 2019)