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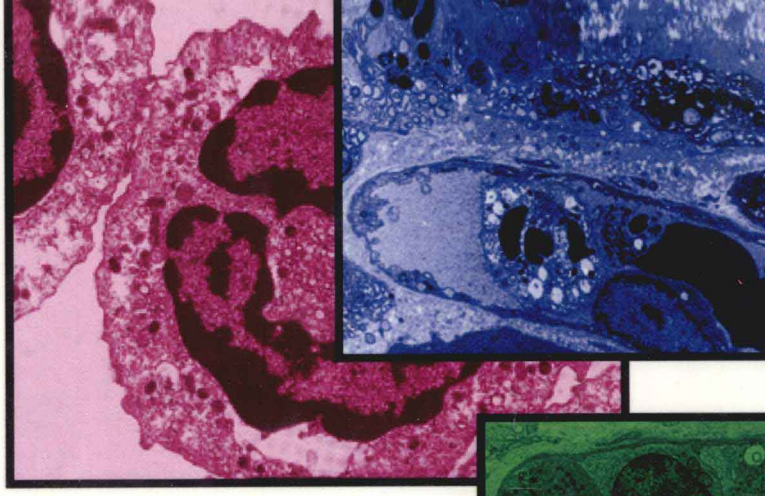
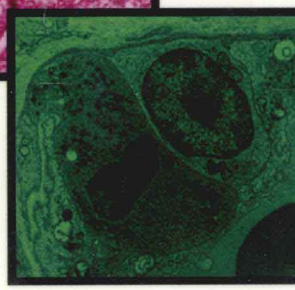
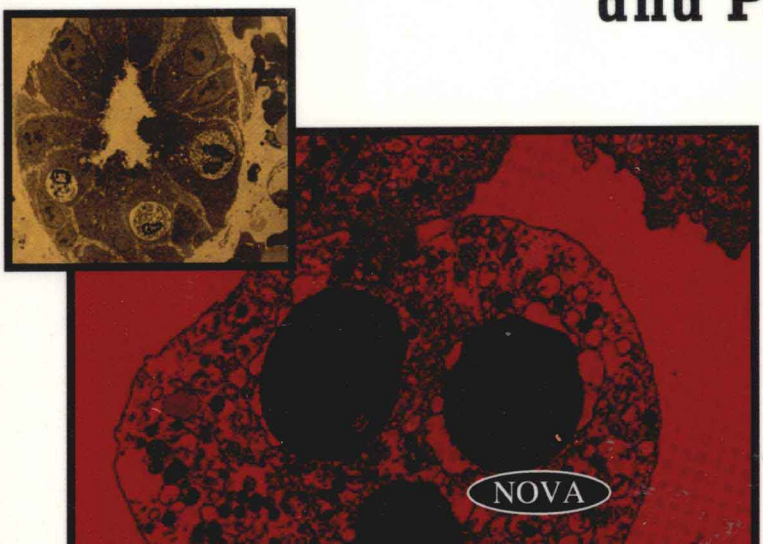




Reuben Hägg
Soren Kohlund
Editors

Handbook of Granulocytes

Classification, Toxic Materials Produced and Pathology



NOVA

HUMAN ANATOMY AND PHYSIOLOGY SERIES

HANDBOOK OF GRANULOCYTES: CLASSIFICATION, TOXIC MATERIALS PRODUCED AND PATHOLOGY

REUBEN HÄGG
AND
SOREN KOHLUND
EDITORS

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Preface

Granulocytes are a category of white blood cells characterized by the presence of granules in their cytoplasm. This book examines the characteristics of eosinophil granulocytes, its biological functions, its role in inflammation and its clinical significance in children. This book also reviews the role of granulocytes on the onset of tissue-destructive diseases when exposed to stress. In addition to the cases of disease, some physiological phenomena are also responsible for the stress-induced granulocytosis. Thus this book will also expands upon the role of granulocytes associated with various diseases and some physiological responses. Other topics discussed in this book include the role of basophil, the minute group of granulocyte, in tropical infections and in immune regulations; polymorphonuclear neutrophilic granulocytes (neutrophils), which are the most dangerous cells in the organism and their role in transient states of autoimmunity, and the effects of nucleoli change during differentiation and maturation of granulocytes.

Chapter 1 - The *Helicobacter pylori*-infected gastric mucosa of patients with active chronic inflammation is characterized by the infiltration of various inflammatory cells, including neutrophils and eosinophils which belong to granulocytes. In this chapter, we have investigated the role of neutrophils and eosinophils in the pathogenesis of *H. pylori* infection. When neutrophils were stimulated with *H. pylori* water extracts, the mobilization of intracellular free calcium, the expression of lymphocyte function-associated antigen-1β, and the secretion of myeloperoxidase (MPO) were enhanced in the neutrophils. In addition, transendothelial and transepithelial migration of neutrophils were observed by electron microscopy and mucosal MPO levels were elevated in the gastric mucosa of *H. pylori*-infected patients. *H. pylori* water extracts also upregulated the expression of interleukin-8 (IL-8) and growth-related oncogenes (GROs; GRO-α, GRO-β and GRO-γ) in neutrophils, suggesting that *H. pylori*-induced neutrophil recruitment may be mediated by CXC chemokines which are expressed by neutrophils activated by *H. pylori* water-soluble surface proteins. Aged neutrophils are rapidly removed from the circulation and are replaced by the release of mature neutrophils from the bone marrow. This process is essential for maintaining fully functional neutrophils in the circulation. We found that the water-soluble surface proteins of *H. pylori* could significantly inhibited the apoptosis of neutrophils through the suppression of Fas, FasL and TNF-R1 expression on the surface of neutrophils. Considering that the mechanism whereby recruited neutrophils are removed from the inflamed foci is an

important factor in the resolution or progression of inflammatory responses, the prolongation of neutrophil life-span may contribute to the pathogenesis of *H. pylori* infection.

Since *H. pylori*-infected gastric mucosa is also characterized by infiltration of eosinophils, we also investigate the role of vacuolating cytotoxin (VacA) in chemokine expression from human eosinophils. $VacA^+$ *H. pylori* water-soluble proteins (HPWP) induced higher expression of chemokines than Vac^- HPWP in human eosinophils. Purified VacA not only increased chemokine expression but also activated p65/p50 NF-κB heterodimers and phosphorylated IκB kinase (IKK)-α/β/ signals in human eosinophils. Inhibition of NF-κB and IKK significantly decreased the chemokine expression in VacA-stimulated eosinophils. Furthermore, VacA-induced NF-κB activation and chemokine release from eosinophils were dependent on Ca^{2+} influx and mitochondrial generation of reactive oxygen intermediates (ROI). These results suggest that NF-κB and IKK signals via Ca^{2+} influx and mitochondrial ROI play a role in the upregulation of chemokine expression in eosinophils stimulated with *H. pylori* VacA. These findings indicate that granuocytes such as neutrophils and eospinophils in gastric mucisal layer play an important role in the involvement of inflammatory responses to *H. pylori* infection.

Chapter 2 - Neutrophil granulocytes (neutrophils) play a central role in the innate immune response to Gram negative bacterial infection. In this chapter we discuss the immune response generated in response to *Burkholderia pseudomallei*, the causative agent of the tropical infection melioidosis. In more detail we examine changes that occur in the phagocytic function of human neutrophils in response to this infection, concentrating on work performed in our unit.

Neutrophils and monocytes/macrophages internalise particulate matter by phagocytosis. In the 1950's it was recognised that phagocytosis could be assessed by measuring cellular uptake of radioactive inorganic colloids. Susequent clarification of underlying cellular mechanisms has allowed a better understanding of what radiocolloid uptake techniques can tell us about phagocytic function.

[99mTc]technetium stannous colloid (TcSnC) labels neutrophils and monocytes by phagocytosis. Labelled cells accumulate at inflammatory foci, enabling Nuclear Medicine imaging of these sites. We have established the clinical usefulness of this technique in melioidosis. During these studies we found neutrophil TcSnC uptake was reduced in melioidosis. It was also reduced when whole blood from healthy volunteers was exposed to *B pseudomalle*i cell products in the absence of live bacteria. This counterintuitive result, that infection with *B pseudomallei* is associated with reduced neutrophil phagocytosis, may well have pathophysiological significance. This concept is supported by reports that G-CSF treatment can improve prognosis in patients with severe melioidosis. However treatment response is variable. It is possible it could be more effective if targeted at individuals with proven neutrophil phagocytic dysfunction.

These results led us to examine the mechanisms underlying neutrophil TcSnC uptake and effects of bacterial products on this process. We found that neutrophil TcSnC uptake is mediated by Complement Receptor 3 (CR3), a receptor central to phagocytosis and neutrophil migration. Furthermore we confirmed that neutrophils which have taken up TcSnC are not activated, nor are they significantly primed. However their response to a priming

stimulus (low dose LPS) is increased. This indicates that TcSnC uptake is not mediated by other phagocytic receptors (such as FcR) that activate cells.

Reduced neutrophil TcSnC uptake in melioidosis therefore suggests alteration in CR3 function in this infection. We have attempted to identify the causative bacterial factors. LPS has a similar effect on TcSnC uptake. Other *B pseudomallei* virulence factors (eg rhamnolipids, proteinaceous compounds) may also affect neutrophil function, and we have identified one potential causative protein. TNF alpha and GM-CSF have an effect on TcSnC uptake similar to LPS, while G-CSF and IFN-gamma alone do not. In addition our observations indicate that CR3 function is affected by gender and oestrogen levels. Further investigation of these relationships is warranted.

We have developed a relatively simple quantitative functional test of CR3 related phagocytosis that can be performed in whole blood, avoiding changes in neutrophil CR3 function that occur during standard cell separation procedures. We have used it to investigate neutrophil functional abnormalities in *B pseudomallei* infection. The opportunity now exists to investigate the role of neutrophil CR3 function in other conditions.

Chapter 3 - *Polymorphonuclear neutrophilic granulocytes (neutrophils)* are the most *dangerous cells* in the organism due to their ability to produce excessive amounts of *reactive oxygen species* and to release lysosomal enzymes like proteases, lipases etc. Regulation of these cell functions is the crucial for normal functioning of the surrounding cells and the granulocytes itself. Moreover in consequence of neutrophil mobility the cells could be exposed to the treatment with varying microenvironmental factors. Besides the stimulus specific for the immune cells like antigens, cytokines and chemokines neutrophils interact with stimuli unrelated to immune system like hormones or neuromediators. For the last decades it became clear that neutrophils possess with receptors to almost all ligands existed in an organism, e.g. *insulin receptor (IR),* or opiate receptors, or acetylcholine receptors, or adrenoceptors.

In this chapter we will discuss *the neutrophil behavior strategy* available by the interplay of the different signalling pathways triggered by various stimuli. One of the known phenomena termed *"priming"* is the preparation of the cells for a faster and stronger response to stimulus by acting with the same stimulus in subthreshold dosage or with different one. Insulin modifies different functions of the neutrophils and leukocyte-endothelial cell adhesion. The neutrophils from healthy donors exposed to high dosage of insulin demonstrate increased chemotaxis, phagocytosis and bactericidal capacity, whereas the density of the certain neutrophil receptors (CD11b, CD15, CD62L, and CD89) on the plasma membrane was down regulated in insulin clamp [Walrand et al., 2004]. We will focus on insulin priming of the neutrophil respiratory burst initiated by chemotactic peptide fMLF. Binding of the low and high affinity *fMLF receptors* leads to activation of the alternative signalling pathways. Cross talking of the signalling pathways of the IR and fMLF receptors forms the basis for priming. Our findings suggest that the high and low affinity fMLF receptors are coupled to *NADPH oxidase* via distinct signalling pathways that are subjected to insulin modulation.

Such a complicated signalling network ensures the activity of the NADPH oxidase to provide optimal cytotoxity, and aberrations inside the net could transform the normal cell functioning to pathological.

Chapter 4 - Phagocytes are classically known to utilize the NADPH-oxidase dependent Reactive Oxygen Species (ROS) release during host defense for clearance of pathogenic organisms. Insufficient activation of the oxidase, as in Chronic Granulomatous Disease, can lead to inadequate elimination of pathogens and can cause severe, life threatening infections. In this review, we present and discuss non-classical functions of the NADPH oxidase and ROS in phagocytes. We describe mechanisms by which the NADPH oxidase and ROS play regulatory roles in intracellular signal transduction, affecting various immune cell functions such as migration, survival, cytokine / chemokine secretion and cell adhesion. Specifically, we discuss redox dependent post-translational protein modifications such as glutathionylation and oxidation, which affect function of various proteins including actin, protein tyrosine kinases and phosphatases, Ras, and transcription factors such as NFκB, and hence can modulate cell signaling and function. In addition, we describe novel mechanisms by which the electrogenic function of the NADPH oxidase can regulate phagocyte signaling via modulating membrane charge potential and controlling membrane localization of charge sensitive enzymes and proteins. Finally, implications of these mechanisms on the pathogenesis of Chronic Granulomatous Disease are discussed.

Chapter 5 - Neutrophils are instrumental in innate immunity by mediating the removal of pathogens by phagocytosis and reactive oxygen species production, but they fulfill other roles that shape the adaptive immune response. This chapter will first describe our present knowledge concerning the novel functions of neutrophils: antigen presentation; interactions with other leukocytes, such as lymphocytes and dendritic cells (DCs); and, the assistance they provide to DCs in the induction of adaptive immunity. Then, we will review the alterations observed in the functions of neutrophils with aging, and link these alterations to defects in signal transduction pathways induced by fMLP, GM-CSF or TLR and TREM-1 ligands. We will then explore in further details when and where the described low-grade inflammatory condition in the neutrophils of the elderly could take part in the development of age-related pathologies. In particular, we will focus on age-associated pathologies where neutrophils could play a role in the activation of adaptive immunity by mechanisms that potentially include antigen presentation. As the elderly population continues to grow, we need to know how aging potentially affects the functions of human neutrophils in a physiological context. This could lead to the instigation of novel strategies to develop potent means to prevent or treat age-associated diseases and increase the quality of life of the elderly individuals.

Chapter 6 - Eosinophil granulocytes (eosinophils) are white blood cells that develop from bone marrow precursor cells. Their major morphological characteristic is eosinophilic granules within the cytoplasm, which are divided into specific and small granules. Four cationic proteins have been recognized within the eosinophil specific granules that include eosinophil cationic protein (ECP). In the present chapter the biochemical characteristics of ECP, its biological functions, its role in inflammation and its clinical significance in wheezy children are reviewed.

ECP displays a variety of biological activities, most notably cytotoxicity that is due to its ability to make pores in cell membranes. ECP exerts this action on parasites, viruses, bacteria, tumor cells, and even respiratory epithelial cells. Other biological functions include stimulation of mucus production in the airway, interaction with the coagulation and complement systems, and immunomodulation.

The role of ECP as a marker of eosinophil activity has been investigated in a variety of body fluids and diseases, particularly in wheezing children The role of ECP has been investigated in acute viral bronchiolitis of infants and preschool wheezing in reference to both pathogenesis and prognosis of the disease. There is evidence that ECP can be used in asthma to evaluate severity, patient compliance to anti-asthma treatment, and to step-down inhaled corticosteroid dosage in stabilized patients. Further studies are deemed necessary in order to elucidate its role as a diagnostic and prognostic tool in childhood wheezing.

Chapter 7 - Severe trauma and hemorrhage renders the patient more susceptible to a second, seemingly trivial, inflammatory stimulus, the so-called "two-hit" hypothesis. The post-trauma sepsis, which involves activation of innate immunity, can lead to severe multi-organ failure (MOF) or systemic inflammatory response syndrome (SIRS), and death. Studies have suggested that inflammatory cell priming caused by a first hit is the mechanism for enhanced response of the cell to a second hit.

Polymorphonuclear neutrophils (PMN) are essential effector cells of the innate immune system. The accumulation of PMN in tissue is considered a critical event in organ inflammation and injury, and has been the target of preventative strategies. PMN migration is a result of a cascade of cellular events, in which PMN, endothelial cells (EC), and macrophages (Mϕ) act in concert. Recent studies explored interrelated novel findings indicating that receptor cross-talk mechanisms occurring in PMN, EC, and Mϕ are important determinants for priming PMN migration in a sepsis setting. This chapter will focus on the detailed receptor cross-talk mechanisms underlying PMN priming. In Mϕ and EC, lipopolysaccharide (LPS) acts through Toll-like receptor (TLR)4 signaling to up-regulate TLR2. Oxidant signaling derived from PMN NAD(P)H oxidase enhances the TLR2 upregulation through PMN-Mϕ or PMN-EC interaction, resulting in an amplified release of cytokines and chemokines from the Mϕ and expression of adhesion molecules in the EC in response to TLR2 ligands, thereby promoting PMN migration. Furthermore, hemorrhagic shock is potent in activating PMN NAD(P)H oxidase through high-mobility group box 1 (HMGB1)-TLR4-p38 MAPK signaling, and therefore initiates the mechanisms of cell priming. On the other hand, LPS through TLR4 on PMN and phosphoinositide 3-kinase γ (PI3Kγ) signaling down-regulates the expression of G protein-coupled receptor kinases (GRK)2 and GRK5 in response to chemokine, thereby decreases chemokine receptor desensitization and augments the PMN migration. Taken together, receptor cross-talk mechanisms are important determinants for cell priming and subsequent augmented PMN infiltration in sepsis.

Chapter 8 - Myeloperoxidase (MPO), a heme protein is expressed only in myeloid cells. MPO is one of the principal enzymes released from azurophil granules of neutrophils and plays a central role in innate immune defense. Elevated plasma MPO levels are a hallmark of microbe-related diseases, such as sepsis and pneumonia. Increased plasma MPO also predicts risks for major adverse cardiovascular events. MPO catalyzes the formation of hypochlorous acid (HOCl), a potent oxidant and antimicrobial agent. MPO through formation of secondary oxidants and nitration of protein tyrosine residues could modulate intercellular signaling pathways, including activation of MAP kinases, induction of nuclear translocation of transcription factors, activation of matrix metalloproteinases and the tumor suppressor p53. MPO-derived oxidants and HOCl in particular are also capable of inflicting tissue destruction

through induction of necrosis and apoptosis. Emerging evidence indicate that MPO binds to the β_2 integrin Mac-1 (CD11b/CD18) and signals to affect the activation state of neutrophils and to suppress the constitutive apoptotic machinery, events that are critical in the generation as well as in the resolution of the inflammatory response. Thus, inhibition of MPO's catalytic activity and/or MPO signaling through Mac-1 represents novel therapeutic approaches to counter the deleterious actions of MPO, and ultimately to prevent neutrophil-mediated tissue injury.

Chapter 9 - Proteases are a group of enzymes with the capacity to degrade proteins to either smaller-sized proteins or to peptides. These enzymes are found in abundant concentrations in immune cells, most notably polymorphonuclear leukocytes (PMNs or neutrophils), and have been implicated in chronic inflammatory pulmonary diseases. In non-activated neutrophils, most proteases are maintained in discrete granules. However, upon stimulation, there is degranulation and the proteases are released to impact the extracellular environment.

Evidence suggests that the imbalance of proteases and their regulatory anti-proteases may lead to ongoing inflammation and changes in the immune response. Proteases seem to play an important role in recently-described pathways of extracellular matrix (ECM) remodeling associated with persistent pulmonary inflammation. Protease activity has also been found to affect several soluble components of the innate immune response. These include such integral proteins as interleukin-8 (IL-8) and other chemokines, components of the complement cascade, lung surfactant-associated proteins and other regulatory proteases/antiproteases. In addition, PMN-derived proteases also affect cellular components of the innate immune system including changes in toll-like receptor (TLR) activation, CXC chemokine receptors (CXCR), integrins and CD14 pattern recognition receptors.

This chapter will more specifically examine the role of neutrophil-derived proteases in the control of the innate immune response in the lung. The chapter will also address antiprotease therapies in chronic pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF). A better understanding of the pathways of protease dysregulation may lead to improved outcomes in these and other conditions.

Chapter 10 - Similarly as in other cells, nucleoli change during differentiation and maturation of granulocytes. At this occasion it should be mentioned that the incidence of various nucleolar types must be evaluated separately in each developmental stage of the granulocytic development. Nucleolar morphology, structure and cytochemistry reflect not only the nucleolar functional activities but also the cell state at the single cell level with a full respect of the cell differentiation and maturation stage including pathological abnormalities. The visualization of nucleoli and nucleolar functional compartments requires special methods, especially in advanced maturation stages. Nucleoli may be also considered as very useful markers of cell proliferation, resting state, aging and cell death. Nucleolar structural organization and cytochemistry may provide complementary information to molecular biology and biochemistry of granulocytes under physiological, experimental and pathological conditions.

Chapter 11 - Atomic force microscopy (AFM) has been used in the study of the neuthrophil granulocyte morphology changes under a lipopolysaccharide condition in real time. AFM is the method that permits observation of the change of morphological parameters

in a real-time regime. Lipopolysaccharide (LPS) is a powerful stimulator of many cells types (especially neutrophils) and can induce apoptosis in these cells. At this time there are no identical opinions about LPS as a cause of the process of apoptosis. Neutrophils exert key functions during the process of inflammation. However, they do not always play a positive role in the homeostasis of the immune system. Under some circumstances, neutrophils have a deleterious effect. Most investigations have studied the activities of the factors of inflammation (e.g., cytokines) as agents that cause apoptosis. However, observing the change in cell morphology during the deleterious effects of LPS is also very important. AFM is a capable of real time imaging in vitro of living cells under high resolution. The present chapter has established the change of rigid cell membranes and the change of volume and height of neutrophils after the addition of LPS from *P. mirabilis 210*. Young's module was used for estimating the elastic properties of the neutrophils' membranes. Young's module of the intact cells was 2.1 ± 0.7 kPa. It was changed significantly after addition of lipopolysaccharide.

Chapter 12 - Calcium mobilization plays an important role in the regulation of superoxide anion secretion by neutrophils. Intracellular calcium increase is predominantly a result of calcium influx from extracellular media through the opening of calcium-permeable channels, which is subsequent to the emptying of intracellular stores. This capacitative mechanism, referred as store-operated calcium entry (SOCE), implies that depletion of agonist-sensitive intracellular calcium stores generates a secondary signal that promotes plasma membrane calcium influx. Accumulating evidence indicates that three protein families (TRPC, STIM, and Orai) play obligatory roles in the activation of this pathway by forming a ternary heterologous complex on the plasma membrane. This complex might mediate communication between the endoplasmic reticulum and the plasma membrane, perhaps by facilitating coupling between TRPC and inositol 3,4,5-trisposphate receptors. STIM1 behaves as a sensor of Ca^{2+} level in the endoplasmic reticulum, while Orai 1, by interacting with TRPC and STIM1, might act as a regulatory subunit that operates the transduction of the signals to the calcium-permeable channels on the plasma membrane. The role of the microtubule network in the regulation of SOCE is still obscure. Experiments performed with microtubule inhibitors gave rise to contradictory results, since these compounds induced partial depletion of Ca^{2+} stores and influx of bivalent cations from extracellular medium in the cytosol, but at the same time they inhibited SOCE triggered by agonists that are known to deplete Ca^{2+} from the endoplasmic reticulum.

Chapter 13 - Basophils are the least abundant granulocytes in the blood, barely reaching 1% of the total leukocyte population. These cells have long been ignored and their function in health and disease has remained an enigma [1]. In fact, basophils have been viewed by many as "redundant circulating mast cells". Recent advances, however, towards understanding the development of both mast cells and basophils, has allowed identification of key factors leading to the production of these cell types [2]. In humans, it is known that basophils are derived from $CD34^+$ bone marrow hematopoietic progenitor cells that yield $Fc\varepsilon RI\alpha^{hi}$c-kit$^-$ cells [2]. In the mouse, a common spleen progenitor leading to basophils and mast cells has been described [2]. Basophils arise from this GATA2-expressing progenitor by up regulation of the transcription factor C/EBPα. In contrast, downregulation of C/EBPα together with expression of GATA2 leads this common precursor to generate mast cells [3].

However, whether these common spleen precursors lead to both kinds of cells *in vivo* remains to be established.

Chapter 14 - Chemokines activate and direct the migration of leukocytes, and are subdivided into two major classes: C-C and CXC. Interleukin (IL)-8, epithelial-neutrophil-activating peptide (ENA)-78, and growth-regulated oncogene (GRO)α are CXC chemokines that are known chemoattractants for neutrophils. We measured cytokines/chemokines (IL-6, IL-8, GROα and ENA-78), soluble molecules (sE-selectin and sVCAM-1) and platelet activation markers (sCD40L, sP-selectin, and platelet-derived microparticles [PDMP]) in patients with hematologic malignancies and febrile neutropenia with cefepime. We classified patients into two groups by the level of sP-selectin. There were no differences in the levels of clinical parameters (peripheral blood cells, GOT, GPT, LDH, BUN, CRTN and CRP) between the two groups. Patients in the high sP-selectin group (>161 ng/ml) exhibited a significant elevation of two chemokines (ENA-78 and GROα), soluble molecules, and platelet activation markers compared with those in the low sP-selectin group (≤160 ng/ml), but the levels of IL-6 and IL-8 did not show a significant difference. The high sP-selectin group exhibited a significant decrease in all markers compared with the low sP-selectin group. On the other hand, the low sP-selectin group did not exhibit significant changes in sCD40L, sP-selectin and PDMPs. Furthermore, the levels of CRP for the patients in the high sP-selectin group significantly decreased after cefepime treatment, although the low sP-selectin group exhibited a significant change only three weeks after treatment. These findings suggest that febrile neutropenia may modulate the vascular events in which some platelet-related chemokines are involved.

Chapter 15 - Similarly to other leukocytes, neutrophils are immune cells involved in the innate response against infections and other inflammatory triggers. In the presence of proinflammatory stimuli they release several inflammatory molecules, such as cytokines, proteases and free oxygen radicals. Thus, they are considered potent damage mediators. Recent studies have shown in both human and animal models that neutrophils are key players in inflammatory diseases. However, their contribution to atherogenesis has not been conclusively explored. Neutrophils are mainly involved in endothelial dysfunction and acute complications of atherosclerosis. In particular, neutrophil adhesion to endothelium increases vessel damage in early phases of atherosclerosis. Furthermore, neutrophil recruitment in atherosclerotic plaques favours plaque progression to rupture through the production of proteases and cytokines which reduce plaque stability. Among these mediators, a crucial role has been shown for neutrophil myeloperoxidase (MPO), elastase and the pro-inflammatory cytokine tumor necrosis factor (TNF)-α. In 2008, evidence from our research group has clearly showed that in the presence of certain proinflammatory soluble mediators, neutrophils also develop previously unknown functions. Insulin, TNF-α and granulocyte macrophage colony stimulating factor (GM-CSF) induce neutrophil migration in response to "not classical" chemoattractants, such as CC chemokines. These evidences suggest a possible new mechanism of neutrophil recruitment in atherosclerotic plaques. Although highly speculative, these novel pathways of neutrophil recruitment could represent a very promising pathophysiological target for future therapies against atherosclerosis and its dramatic acute consequences.

Chapter 16 - Acute stress induces generally adrenergic stimulation and secretion of glucocorticoids and results in hypothermia and hyperglycemia. If those stress-induced conditions are continued for a long time, we fall victims into various diseases such as general fatigue, diabetes mellitus, mental disorders, etc. In some cases, acute or chronic stress is also related to the onset of tissue-destructive diseases, including periodontitis, sudden difficulty of hearing, gastric ulcer, ulcerative colitis, pancreatitis, hemorrhoids, etc. At that time, we have to consider the role of granulocytes which bear adrenergic receptors on the cell surface. Indeed, acute and chronic stresses have a potential to induce granulocytosis in the circulation and specific sites of the tissues. Granulocytes are important for the defense against bacterial infections. However, the excess number of granulocytes often induces tissue damage due to their secretion of superoxides. In addition to the cases of disease, some physiological phenomena are also responsible for the stress-induced granulocytosis (e.g., neonatal granulocytosis). The commencement of pulmonary respiration acts as oxygen stress in neonates and then induces neonatal granulocytosis. This granulocytosis is associated with subsequent destruction of fetal hematopoiesis in the liver and physiological jaundice. We will review the role of granulocytes associated with various diseases and some physiological responses.

Chapter 17 - Myeloperoxidase is an important white blood cell enzyme. This intracellular enzyme plays an important role in destroying foreign bodies in the process of the defense mechanism of human beings. Myeloperoxidase can be seen in many types of white blood cells, especially those that function in phagocytosis. Neutrophils and monocytes are the two main groups of white blood cells that store myeloperoxidase. In addition, eosinophils, which play a role against parasitic infestation, also store myeloperoxidase. Deficiency of this intracellular enzyme is a significant problem in white blood cell pathology. Deficiencies of this enzyme can be seen in neutrophils, monocytes and eosinophils. In this work, the author will briefly review eosinophilic myeloperoxidase deficiency, which is uncommon.

Chapter 18 - White blood cells play a main role in the defense mechanism of human beings. Among the five kinds of white blood cells, neutrophils comprise the main group that reacts to foreign bodies. Neutrophils accumulate at sites of injury and initiate several response mechanisms. Fighting bacteria by phagocytosis is the main response in cases of severe bacterial infection. An important non-malignant white blood cell disorder is toxic granulation. This phenomenon is a finding in the abnormal granules within the neutrophils, and is detected in cases of severe bacterial infection. In this work, the author will focus on toxic granulation of neutrophils.

Chapter 19 - Basophils are granulocytes that constitute the fewest in number among white blood cells. Knowledge of basophils is limited. In this chapter, the author will briefly detail and discuss the role of basophils in tropical infections. The scenarios of malaria, dengue, tuberculosis and leprosy will be presented.

Chapter 20 - Until present, basophils have been less studied in comparison to other types of white blood cells. Medical scientists know that basophils play a main role relating to anaphylactic reaction. However, there are many other facets of the role of basophils. In this chapter, the author will briefly summarize the important reports on basophils in Thailand.

Contents

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Chapter 1

Immunological Responses of Granulocytes to *Helicobacter pylori* Infection

Jung Mogg Kim*[*1] and Joo Sung Kim[2]
[1] Hanyang University College of Medicine, Seoul, South Korea
[2] Seoul National University College of Medicine, Seoul, South Korea

Abstract

The *Helicobacter pylori*-infected gastric mucosa of patients with active chronic inflammation is characterized by the infiltration of various inflammatory cells, including neutrophils and eosinophils which belong to granulocytes. In this chapter, we have investigated the role of neutrophils and eosinophils in the pathogenesis of *H. pylori* infection. When neutrophils were stimulated with *H. pylori* water extracts, the mobilization of intracellular free calcium, the expression of lymphocyte function-associated antigen-1β, and the secretion of myeloperoxidase (MPO) were enhanced in the neutrophils. In addition, transendothelial and transepithelial migration of neutrophils were observed by electron microscopy and mucosal MPO levels were elevated in the gastric mucosa of *H. pylori*-infected patients. *H. pylori* water extracts also upregulated the expression of interleukin-8 (IL-8) and growth-related oncogenes (GROs; GRO-α, GRO-β and GRO-γ) in neutrophils, suggesting that *H. pylori*-induced neutrophil recruitment may be mediated by CXC chemokines which are expressed by neutrophils activated by *H. pylori* water-soluble surface proteins. Aged neutrophils are rapidly removed from the circulation and are replaced by the release of mature neutrophils from the bone marrow. This process is essential for maintaining fully functional neutrophils in the circulation. We found that the water-soluble surface proteins of *H. pylori* could significantly inhibited the apoptosis of neutrophils through the suppression of Fas, FasL and TNF-R1 expression on the surface of neutrophils. Considering that the mechanism

* Corresponding author: Jung Mogg Kim, M.D.,Department of Microbiology, Hanyang University College of Medicine, 17 Haengdang-dong, Sungdong-gu, Seoul 133-791, South Korea, Tel: +82-2-2220-0645; Fax: +82-2-2282-0645; E-mail: jungmogg@hanyang.ac.kr

whereby recruited neutrophils are removed from the inflamed foci is an important factor in the resolution or progression of inflammatory responses, the prolongation of neutrophil life-span may contribute to the pathogenesis of *H. pylori* infection.

Since *H. pylori*-infected gastric mucosa is also characterized by infiltration of eosinophils, we also investigate the role of vacuolating cytotoxin (VacA) in chemokine expression from human eosinophils. $VacA^+$ *H. pylori* water-soluble proteins (HPWP) induced higher expression of chemokines than Vac^- HPWP in human eosinophils. Purified VacA not only increased chemokine expression but also activated p65/p50 NF-κB heterodimers and phosphorylated IκB kinase (IKK)-α/β/ signals in human eosinophils. Inhibition of NF-κB and IKK significantly decreased the chemokine expression in VacA-stimulated eosinophils. Furthermore, VacA-induced NF-κB activation and chemokine release from eosinophils were dependent on Ca^{2+} influx and mitochondrial generation of reactive oxygen intermediates (ROI). These results suggest that NF-κB and IKK signals via Ca^{2+} influx and mitochondrial ROI play a role in the upregulation of chemokine expression in eosinophils stimulated with *H. pylori* VacA. These findings indicate that granuocytes such as neutrophils and eospinophils in gastric mucisal layer play an important role in the involvement of inflammatory responses to *H. pylori* infection.

Introduction

Granulocytes are characterized by the presence of granules in their cytoplasm and three different types, including neutrophils, eosinophils and basophils, are existed [1]. *Helicobacter pylori* (*H. pylori*) is a major cause of chronic gastritis and recurrent peptic ulcer diseases [2-4]. It has also been associated with gastric carcinoma and low-grade gastric mucosa-associated lymphoid tissue lymphoma [5, 6]. Although this organism is known to be noninvasive, *H. pylori* infection can elicit gastric mucosal infiltration of inflammatory cells, especially neutrophils and eosinophils [7, 8]. Therefore, it is possible that these inflammatory granulocytes play a role in gastric mucosal inflammation in response to *H. pylori* infection.

Neutrophils, polymorphonuclear neutrophil granulocytes (PMN) contribute to the ''first line of defence'' against infectious agents or ''nonself'' substances that penetrate the body's physical barriers [9]. Morphologically, neutrophils are characterized by a lobulated chromatin-dense nucleus and their granules. The different types of granules – primary or azurophil granules, secondary or specific granules, gelatinase granules and secretory vesicles – are not merely containers of proteolytic and bactericidal substances [10]. They also serve as reservoirs of membrane receptors such as CD66b and Mac-1, a subunit of complement receptor 3 (CD11b). The bone marrow of a healthy adult produces up to 10^{11} neutrophils a day and increases their production to 10^{12} per day in acute inflammation. After release from the bone marrow, neutrophils enter the circulation and represent more than 50% of circulating leukocytes [11].

Once an inflammatory response is initiated by infectious microorganisms, neutrophils are the first cells to leave the circulation and to be recruited to the site of infection. They are primary antimicrobial effector cells and serve to destroy invading pathogens. To accomplish this, neutrophils possess an array of microbicidal mechanisms. As their initial mode of attack is to phagocytose the pathogen, neutrophils are known as "professional" phagocytes.

Investigations over the past two decades revealed two basic mechanisms of recognition of microorganisms by neutrophil granulocytes, opsonin-dependent and opsonin-independent [12, 13]. Opsonins are serum components that act by binding both to the surface of the microorganisms and to specific receptors on the phagocyte surface. Examples are immunoglobulins, the C3bi fragment of the C3 component of complement, and mannan-binding lectin (MBL). In addition to opsonin-mediated mechanisms, phagocytosis of microorganisms can be mediated by direct recognition of pathogen-associated molecular patterns (PAMP) via pattern recognition receptors (PRR) by a process referred to as non-opsonic phagocytosis [11, 12].

Usually, phagocytosis is followed by the fusion of phagosome with cytosolic granules converting the phagosome into a phagolysosome. Especially, azurophil granules release their hydrolytic enzymes and bactericidal proteins such as elastase, bactericidal permeability-increasing protein and defensins for the oxygen-independent killing. In addition, neutrophils possess an oxygen-dependent mechanism, the oxidative burst, to generate highly bactericidal reactive oxygen species (ROS). Both mechanisms work together to destroy the engulfed pathogens [11, 14]. Senescent neutrophils undergo apoptosis and are phagocytosed by macrophages via the vitronectin receptor [15]. The apoptotic program in neutrophils is very sensitive to the influence of factors in the local microenvironment of infection and immunity. Neutrophil apoptosis is delayed by neutrophil activating agents such as bacterial lipopolysaccharide (LPS) and proinflammatory cytokines [16-18].

Recently, a novel neutrophil-mediated antibacterial mechanism, the release of neutrophil extracellular traps (NETs), has been described [19]. NETs are extracellular structures produced by stimulated neutrophils containing DNA, histone proteins and antibacterial enzymes that bind and kill both bacteria and fungi. NETs are released by dying neutrophils. This cell death process is, however, distinct from apoptosis and necrosis and depends on the generation of ROS by NADPH oxidase [20, 21]. This novel ROS-dependent death allows neutrophils to fulfil their antimicrobial function, even beyond their life span [11].

Eosinophils are bone marrow-derived granulocytes with a richness of surface receptors for immunologic ligands [22, 23]. Eosinophils have capacity to express several chemokines and cytokines, which play an important role in mucosal inflammation. Moreover, eosinophils characteristically harbor specific granules which contain large amounts of the tissue-toxic cationic proteins major basic protein, eosinophil cationic protein (ECP), eosinophil peroxidase, and eosinophil-derived neurotoxin (EDN) [22]. Considering that the inflammatory granulocytes such as neutrophils and eosinophils may play important roles in gastric mucosal inflammation in response to *H. pylori* infection, it is necessary to review findings regarding the interaction between human inflammatory granulocytes and *H. pylori* infection.

H. pylori Activates Human Neutrophils and Upregulates the Expression of Chemokines

A high proportion of the neutrophils in gastric mucosal inflammatory cells showed high specificity and positive predictive values in the presence of *H. pylori* [24, 25]. Neutrophil