MONTANA STATE UNIVERSITY

Topological mapping of neutrophil cytochrome b epitopes with phage-display libraries
by James Barton Burritt

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Microbiology
Montana State University
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Abstract:
Cytochrome b (Cyt b) of human neutrophils is the central component of the microbicidal NADPH-oxidase system. However, the folding topology of this integral membrane protein remains undetermined. Topological features of Cyt b were obtained by determining the epitopes of monoclonal antibodies (mAbs) 44.1 and 54.1, specific for the p22-phox and gp9l-phox Cyt b chains, respectively. Two random-sequence bacteriophage peptide libraries were used as sources of surrogate epitopes for each of the mAbs. Recombinant DNA technology was used to construct the M13KBst phage vector used in the production of one such library. M13KBst offers the benefits of high copy replicative form, large plaque formation, and a kanamycin resistance gene. The vector also has a pair of noncomplimentary BstXI restriction sites positioned near the 5' end of the PIII gene. Insertion of foreign DNA between the BstXI sites allows phage surface-display of the protein corresponding to the foreign DNA. The J404 nonapeptide library produced with the M13KBst vector contains 5 x 10^8 unique phage, each expressing a different peptide sequence fused to the amino terminus of the pIII capsid protein. Phage selected by mAh 44.1 displayed the consensus peptide sequence, GGPQVXPI, which is nearly identical to 181GGPQVNPI188 of p22-phox. A second method of antibody-mediated peptide selection using mAh 44.1 suggested the epitope bound by this mAh may contain both the 181GGPQVNPI188 and 29TAGRF33 regions of p22-phox and suggests phage-display library analysis may be used to identify discontinuous segments of Cyt b juxtaposed by tertiary structure as well as simple linear epitopes. Phage selected by mAb 54.1 displayed the consensus sequence, PKXAVDGP, which resembles 382PKIAVDGP389 of gp91-phox. Western blotting demonstrated specific binding of each mAb to the respective Cyt b subunit and selected phage peptides. In flow cytometric analysis, mAb 44.1 bound only permeabilized neutrophils, while 54.1 did not bind intact or permeabilized cells. However, mAb 54.1 immunosedimented detergent solubilized Cyt b in sucrose gradients. These results suggest the 181GGPQVNPI188 segment of p22-phox is accessible on its intracellular surface, but the 382PKIAVDGP389 region on gp9l-phox is not accessible to antibody, and probably not on the protein surface.

TOPOLOGICAL MAPPING OF NEUTROPHIL CYTOCHROME B

EPITOPES WITH PHAGE-DISPLAY LIBRARIES

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James Barton Burritt

A thesis submitted in partial fulfillment
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APPROVAL

of a thesis submitted by

James Barton Burritt

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

_5/25/95_
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Date   Graduate Dean

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Advisor: Algirdas J. Jesaitis, Ph.D.

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ABSTRACT

Cytochrome *b* (Cyt *b*) of human neutrophils is the central component of the microbicidal NADPH-oxidase system. However, the folding topology of this integral membrane protein remains undetermined. Topological features of Cyt b were obtained by determining the epitopes of monoclonal antibodies (mAbs) 44.1 and 54.1, specific for the p22-*phox* and gp91-*phox* Cyt *b* chains, respectively. Two random-sequence bacteriophage peptide libraries were used as sources of surrogate epitopes for each of the mAbs. Recombinant DNA technology was used to construct the M13KBst phage vector used in the production of one such library. M13KBst offers the benefits of high copy replicative form, large plaque formation, and a kanamycin resistance gene. The vector also has a pair of noncomplimentary *Bst*XI restriction sites positioned near the 5' end of the PIII gene. Insertion of foreign DNA between the *Bst*XI sites allows phage surface-display of the protein corresponding to the foreign DNA. The J404 nonapeptide library produced with the M13KBst vector contains 5×10^8 unique phage, each expressing a different peptide sequence fused to the amino terminus of the pIII capsid protein. Phage selected by mAb 44.1 displayed the consensus peptide sequence, GGPQVXPI, which is nearly identical to ^{181}GGPQVNPI^{188} of p22-*phox*. A second method of antibody-mediated peptide selection using mAb 44.1 suggested the epitope bound by this mAb may contain both the ^{181}GGPQVNPI^{188} and ^{29}TAGRF^{33} regions of p22-*phox* and suggests phage-display library analysis may be used to identify discontinuous segments of Cyt b juxtaposed by tertiary structure as well as simple linear epitopes. Phage selected by mAb 54.1 displayed the consensus sequence, PKXAVDGP, which resembles ^{382}PKIAVDGP^{389} of gp91-*phox*. Western blotting demonstrated specific binding of each mAb to the respective Cyt *b* subunit and selected phage peptides. In flow cytometric analysis, mAb 44.1 bound only permeabilized neutrophils, while 54.1 did not bind intact or permeabilized cells. However, mAb 54.1 immunosedimented detergent solubilized Cyt *b* in sucrose gradients. These results suggest the ^{181}GGPQVNPI^{188} segment of p22-*phox* is accessible on its intracellular surface, but the ^{382}PKIAVDGP^{389} region on gp91-*phox* is not accessible to antibody, and probably not on the protein surface.

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CHAPTER ONE

INTRODUCTION

Phagocyte NADPH-Oxidase System

The NADPH-oxidase system of phagocytic cells is a plasma membrane redox system that produces reactive oxygen metabolites important for the destruction of invading microbes. A heterodimeric membrane-bound cytochrome b (Cyt b), comprised of the subunits gp91-*phox* and p22-*phox*, functions as the central component of the multi-subunit oxidase system. The result of this system is the transfer of singlet electrons from a reduced flavin moiety to an oxygen acceptor bound to Cyt b and accessible to the exterior aspect of the cell. On the external surface of the cell or within the phagocytic vacuole, the reduced oxygen, in the form of superoxide anion (O_2^-), undergoes subsequent redox conversions to form other microbicidal oxidants important for the destruction of the microbe (7).

Neutrophils are unique among phagocytic cells in their ability to respond to chemoattractant stimuli and deliver a microbicidal response within seconds. They are well equipped for this role by possessing elaborate mechanisms of sensory detection, adhesion, migration, and microbicidal potential. In the circulating neutrophil, the anti-microbial mechanisms are inactive, and the components of the NADPH-oxidase remain in a disassembled configuration. However, within seconds following appropriate

stimulation by a variety of stimuli including chemoattractants, rapid assembly of the oxidase promotes the immediate production of superoxide and other necessary compounds for neutralization of most pathogens.

When the neutrophil is stimulated by a defined chemical signal, the sequence of the microbicidal responses are sequentially orchestrated. For example, less than 1 nanomolar concentrations of the bacterial product, fMet-Leu-Phe (fMLF), is capable of stimulating chemoattractant migration. However, production of superoxide generally does not take place until this compound is present in concentrations greater than 10 nM. Concentrations of fMLF sufficient for superoxide generation are probably only encountered in the neutrophil following engulfment of a microorganisms. Therefore, deployment of the microbicidal ordnance does not occur before it can be applied directly to the pathogen.

Activation of the cell to produce superoxide can be initiated by a number of events, and leads to a rapid increase of phagocyte oxygen consumption, known as the respiratory burst (7). The burst occurs immediately following assembly of several oxidase components of the oxidase on the cytosolic aspect of the cell membrane. Translocated factors include p47-*phox*, p67-*phox*, and Rac (45,49,121,147). The NADPH-oxidase components are tabulated in Table 1, and the assembled NADPH-oxidase is schematically represented in Figure 1. Exposure of the neutrophil to a variety of extracellular substances is known to activate the oxidase; cytokines, bacterial products, chemoattractants, aggregated immunoglobulins, activated complement factors, and inflammatory products are known examples. These substances cause signal

transduction activity of the cytosol by way of membrane transduction mechanisms (13), resulting in the production of superoxide. Direct activation of the oxidase with arachidonic acid (32,102), sodium dodecyl sulfate (21), and phosphatidic acid has been shown in cell free systems suggesting additional mechanisms of NADPH-oxidase activation.

Table 1.   Known NADPH-oxidase Subunit Proteins

Protein	Location[a]	Deficiency in CGD[b]	Relative Molec. Wt[c].	Chromosome Location	(Ref.)
p22-*phox*	plasma membrane	yes	22 kDa	16 q24	(40,115)
gp91-*phox*	plasma membrane	yes	91 kDa	X p 21.1	(130)
p47-*phox*	cytosol	yes	47 kDa	7 q 11.23	(160)
p67-*phox*	cytosol	yes	67 kDa	1 q 25	(93)
Rap1A	plasma membrane	unknown	22 kDa	?	(123)
Rac	cytosol	unknown	24 kDa	?	(38)

[a] Location in unactivated human neutrophils.
[b] Chronic Granulomatous Disease
[c] Determined by SDS-PAGE (88).

In the absence of oxygen, which normally functions as the terminal electron acceptor for this system, an alternative mechanism(s) appears to ensure the maintenance of electron flow. This oxygen-independent activity is not dependent on the availability of p47-*phox* and can be measured with diaphorase, a dye reductase (31). In this case, assembly of the NADPH-oxidase components does not result in the

production of superoxide but may ensure other microbicidal neutrophil functions linked to the oxidase. Interleukin-4 has been shown to affect the oxidase in porcine macrophages and manifests an inhibitory effect on the mRNA level of the gp91-*phox*, but not p22-*phox* (164). Though the role of membrane carbohydrate groups in the activation of the oxidase is unclear, 20% membrane desialylation in neutrophils prevents activation of oxidase activity despite normal phagocytosis and hexose monophosphate shunt (HMP) activity associated with the active oxidase (152).

Following ingestion of a microbe by a neutrophil, the respiratory burst and degranulation constitute the hallmark of the neutrophil immune response. The two processes act independently, but are complimentary in the microbicidal response.

Diseases of an Ineffective NADPH-oxidase

Several diseases result from genetic anomalies which affect components of the NADPH-oxidase. The frequency of these diseases is about one in one million and familial patterns suggest classic Mendelian inheritance. Conditions associated with the diseases manifest primarily in the skin, lungs, lymph nodes, bone, and liver. The etiology of each disease is a mutation of the gene encoding a subunit of the NADPH-oxidase system, and diseases characterized at this time have involved either the gp91-*phox*, p22-*phox*, p47-*phox*, or p67-*phox* subunits. The genetic defect that affects gp91-*phox* accounts for about 55% of cases of disease, and is X-linked. The locus for this gene maps in close proximity to the locus for Duchenne muscular dystrophy, and some cases have been reported of unfortunate individuals affected by both diseases.
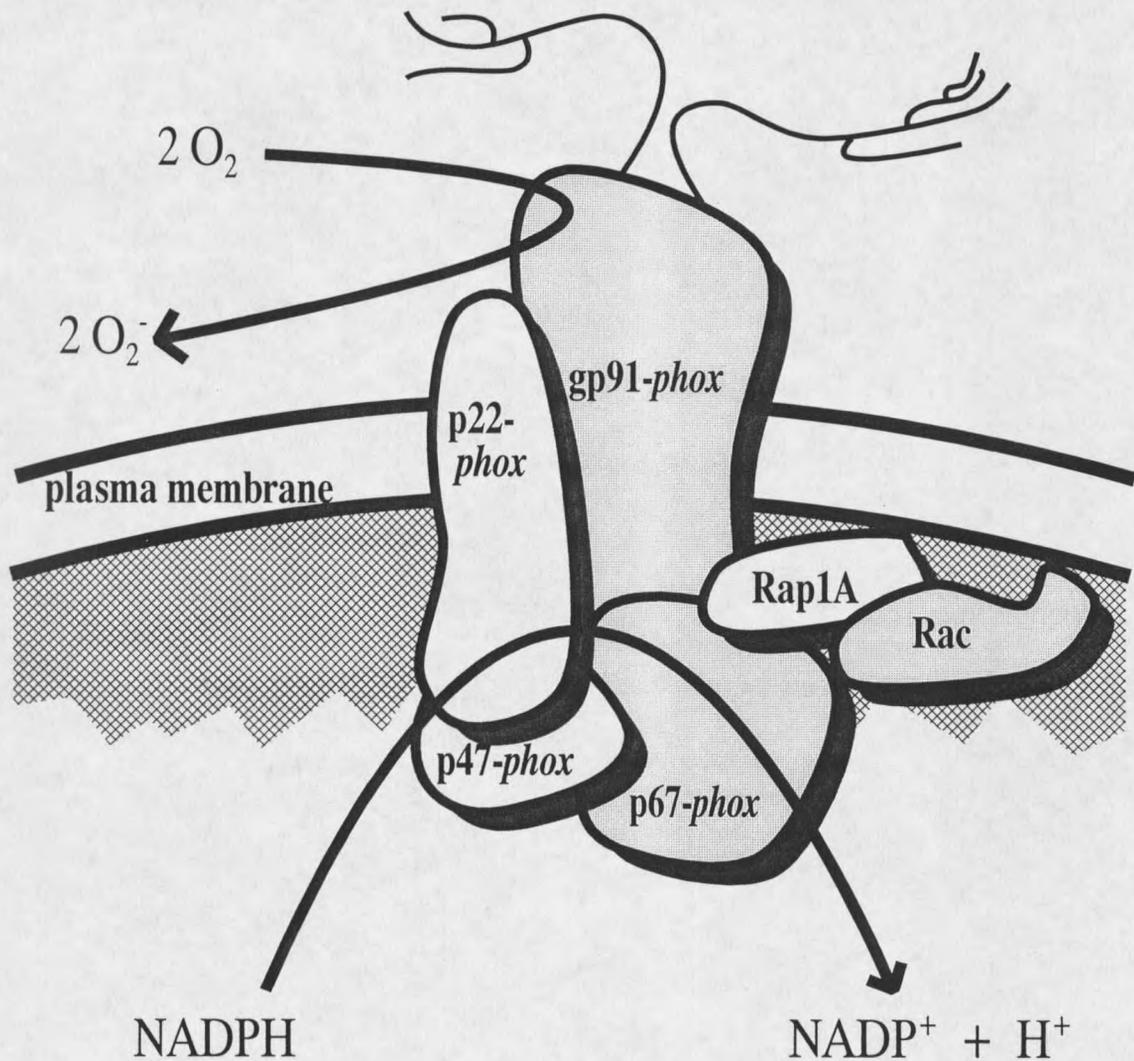
Figure 1. Components of the assembled NADPH-oxidase System. Cytochrome b_{558} is comprised of p22-phox and gp91-*phox*. Cytosolic factors are Rac, p47-*phox* and p67-*phox*. Rap 1A is associated with the plasma membrane. This model reflects the activated complex as it exists during the oxidation of NADPH and subsequent reduction of molecular oxygen which generates the superoxide anion.

Inheritance for all other currently known diseases associated with the oxidase is autosomal recessive. These heterogeneous diseases of the NADPH-oxidase, regardless of the underlying genetic lesion, are known as chronic granulomatous disease (CGD) (12,33,39,91,94,140,147).

Patients with diseases of the NADPH-oxidase exhibit an abnormal inflammatory response when challenged by bacterial and fungal pathogens. The functional result in each case is a failure of the respiratory burst and attendant microbicidal activity, predisposing the affected individual to repeated life-threatening infections and granulomatous lesions. Because of the inability of neutrophils in these patients to kill, but not ingest microbes, persistence of microorganisms in endocytic vesicles of accumulated phagocytic cells appears to be associated with the granulomatous lesions which typify the disease. The granulomas themselves contain giant multinucleated cells, resulting from the fusion of macrophages with monocytes. These cells are sometimes distended with lipid material, reminiscent of some lipid storage diseases. The granulomas may become enlarged to the point that strictures in the digestive and urinary tracts result, precluding the normal passage of waste.

Microorganisms most often associated with infections in CGD patients belong to the genera, *Staphylococcus*, *Pseudomonas*, *Aspergillus*, some *Candida*, *Nocardia*, and many Gram negative rods belonging to the Enterobacteriaceae group. Catalase-negative organisms such as the lactobacilli and streptococci are generally not associated with infections of CGD patients. These organisms lack heme synthesis and are unable to produce heme proteins which reduce molecular oxygen to water. The

metabolic systems of these organisms reduce oxygen to hydrogen peroxide and release it into the medium. The inability of these organisms to produce catalase prevents them from degrading the self-generated hydrogen peroxide, which then is able to complement the deficiency in cells of CGD patients. Therefore, hydrogen peroxide produced by the streptococci is available to the microbicidal machinery of the neutrophil, and is ultimately used in destruction of the organism.

Despite the morbidity and mortality associated with CGD (12,91), molecular and genetic analysis in human cases has allowed discovery and examination of several components required for the functional oxidase complex.

Gene therapeutic measures which address deficiencies in CGD patients are limited at this time. However, some progress toward this goal has been reported recently, primarily by the use of retroviral gene delivery systems (95,98,119).

The Respiratory Burst

The respiratory burst of neutrophils is the sudden increase in oxidative metabolism that occurs during the ingestion of microorganisms. This event takes place as molecular oxygen is consumed and reactive oxygen metabolites are produced. Under anaerobic conditions, both the respiratory burst and microbicidal effect are abrogated. The burst can result from any of a variety of appropriate stimuli in functional neutrophils. These stimuli activate the NADPH-oxidase system in an event which is not perturbed by classic inhibitors of mitochondrial metabolism, such as cyanide and azide. These unusual properties of neutrophils were first reported in 1933

(8), when they were described as the "extra respiration of neutrophils." It was concluded at that time that the event represented an unusual mechanism of microbial killing which involved oxygen metabolites.

Once triggered, the respiratory burst is fueled by energy derived from the hexose monophosphate shunt. A diffusible proton/electron carrier such as $NADP^+$ delivers the metabolic electron-proton pair from the HMP to the cytochrome system and electron transport chain on the cytosolic aspect of the cell membrane. The protons are deposited in the periplasmic space to establish and maintain the proton motive force (PMF), and the electrons are similarly displaced to the external surface of the cell by the NADPH-oxidase, where it reduces molecular oxygen to form superoxide. The overall reactions of the electron transport can be summarized as:

$$NADPH + 2\ O_2 \longrightarrow NADP^+ + H^+ + 2\ O_2^-$$
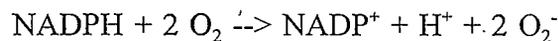
The result of this event is the production of superoxide anion on the external surface of the cell which neutralizes the positively charged PMF, causing a membrane depolarization which is greatest at 40 seconds following maximal cell stimulation. This burst-associated depolarization is not found to occur in neutrophils of CGD patients, supporting the belief that superoxide is at least partially responsible for depolarization.

A significant limitation to the study of the NADPH-oxidase system has been the difficulty with which components of this system can be extracted in a functional

form from the neutrophils. In 1985, it was reported that superoxide production could be studied in cell-free systems when using anionic detergents such as arachidonic acid or sodium dodecyl sulfate when combined with cytosolic and membrane fractions extracted form neutrophils (21,32,102). By many indications, these *in vitro* reactions closely parallel events of the oxidase in the cell, and have therefore made the cell-free assay a useful representation of the system. This technique has led to the understanding of several features of component localization and interaction. Furthermore, because components can be introduced into a system which occurs naturally within the cell membrane, the influence of novel compounds and conditions can easily be tested. Recently, the production of recombinant proteins in baculovirus or bacterial expression systems has proven to be an advancement in the study of this and other multiprotein systems. An active NADPH-oxidase in a cell-free system has been constructed solely from cloned and expressed proteins (128).

Molecular oxygen is the obligate electron acceptor of the NADPH-oxidase when reactive oxygen intermediates are produced. Under normal conditions, molecular oxygen may be regarded as being kinetically (but not thermodynamically) inert, and therefore, unlikely to form compounds without participation of a catalyst (81). This property of oxygen results because electrons in molecular oxygen occur in paired spin configurations of opposite polarity. However, if oxygen is reduced by a low-potential oxidant, the addition of an additional electron results in the superoxide free radical with unusual properties.

<u>Superoxide Anion</u> Superoxide anion is capable of dismutation (oxidation-reduction) which results in the formation of a number of highly reactive oxygen species. Substances such as singlet oxygen, hydrogen peroxide, hydroxyl radical, and water are formed as shown below:

$$\begin{array}{l} \rightarrow \text{(electron excitation) singlet oxygen} \\ \hline \\ O_2 + e^- \rightarrow \quad O_2^- + e^- \rightarrow \quad H_2O_2 + e^- \rightarrow \qquad OH + e^- \rightarrow \qquad H_2O \end{array}$$

| Oxygen | Super-
oxide | Hydrogen
peroxide | Hydroxyl
Radical | Water |

In an aqueous environment, superoxide lacks the necessary toxicity to be significantly microbicidal by itself. However, much of the reactivity of superoxide may take place in hydrophobic microenvironments within the cell membrane. In this setting, superoxide can act as a strong base and is capable of reactive nucleophilic attack. However, microorganisms which produce the enzyme superoxide dismutase are able to neutralize this substance, and are therefore protected from its toxic properties.

<u>Hydrogen Peroxide</u> Hydrogen peroxide is produced rapidly by dismutation of superoxide in stimulated neutrophils (81). Hydrogen peroxide is less reactive with many biological compounds than the hydroxyl radical and hypochlorous acid. Therefore, hydrogen peroxide demonstrates a greater penetrating ability than the more reactive compounds which tend to be consumed rapidly upon diffusion. For this reason, hydrogen peroxide may be the most microbicidal of the reactive oxygen compounds found in inflamed joint fluids and exudates where large amounts of

proteinaceous fluids occur. Sensitivity of bacteria to superoxide is usually dictated by the lack of microbial catalase and the glutathione reductase system, both of which act as peroxide scavengers. Neutrophils themselves are protected from the toxic effects of hydrogen peroxide by these mechanisms. The availability of hydrogen peroxide is crucial for the activity of myeloperoxidase (below).

Hydroxyl Radical The hydroxyl radical is one of the most reactive oxygen radicals known, and may be utilized in the burst oxidase repertoire of microbicidal compounds (81). It is also possible that the hydroxyl radical is produced as a result of the activity of myeloperoxidase (below). At this time, however, convincing demonstration of the importance of the hydroxyl radical in a microbicidal response has not been reported.

Myeloperoxidase

Neutrophils contain all of their myeloperoxidase (MPO) within azurophilic storage granules. Therefore, the action of myeloperoxidase upon ingested microorganisms constitutes a union of degranulation and burst oxidase mechanisms. The activity of MPO can also be exerted beyond the periplasm, as diffusion, direct application, or cell lysis may present this enzyme outside the cell membrane (81). MPO constitutes as much as five percent of the neutrophil dry weight, and its inherent green color produces the characteristic green appearance of purulent material. The primary MPO transcript contains a 41 residue leader sequence and undergoes cleavage

by a signal peptidase and other post-translational modifications to produce subunits of 12 and 57 kDa (109). The native enzyme is a heterotetramer, comprised of two protomers, each of which contains a heavy and light subunit. Both heme and carbohydrate groups are coordinated by the large subunits.

As myeloperoxidase is released by the azurophilic granules into the phagosome during degranulation, it reacts with hydrogen peroxide and halide ions to form primarily hypochlorous acid, but other hypohalogenous acids, chloramine, aldehydes, and possibly singlet oxygen and hydroxyl radical (81). The toxic activity of these compounds to biological material in the phagosome generally belongs to either of the groups; oxidation or halogenation. The effect of these processes includes tyrosine and unsaturated fatty acid halogenation; oxidation of sulfhydryl groups, iron-sulfur centers and heme proteins; oxidative decarboxylation, deamination, and peptide cleavage; and lipid peroxidation. Surprisingly, individuals with nonfunctional MPO systems do not appear to be predisposed to infectious conditions. Perhaps this fact demonstrates the complimentarity of various facets of the immune response, and the ability to rely on compensatory mechanisms in some instances.

Degranulation

The granulocytes are comprised of three groups of leukocytes: neutrophils, eosinophils, and basophils. All three cell types harbor vesicular organelles or storage granules which contain materials important in the inflammatory response. The vesicles give these cells the typical granular appearance and the descriptive term for this group

of cells. Each of the granulocytes contains several different types of granules, but each contains a different predominant type. The cell types can be distinguished by Wright stain, according to the appearance of the specific (dominating) granule. On Wright stained blood smears, the specific granules of eosinophils stain strongly with the acidic stain eosin, and appear red. Those of basophils stain with a basic dye in the Wright stain, producing a dark blue color; and those of neutrophils show neutral staining, and assume a light pink color.

Degranulation refers to the redistribution of granule contents as the granule membranes fuse with other cellular membranes. Target membrane surfaces usually include either the phagosomal vacuoles or the plasma membrane. During this process, contents are deposited either within target phagosomes, or into the periplasmic space. Degranulation of the neutrophil usually takes place concomitantly with activation of the NADPH-oxidase, as pre-packaged microbicidal factors are introduced either into the phagosome containing ingested microbes, or into the periplasmic space in order to act upon adjacent target structures. Trafficking of the cellular granules to the phagosome or the plasma membrane may depend upon events associated with phagocytosis or activation of the neutrophil in the absence of phagocytosis, respectively.

It has long been recognized that exudate fluids associated with inflammatory processes contained many constituents of inflammatory cells. However, recent evidence suggests these products are actively deposited by degranulation and secretion, rather than by cellular lysis. Therefore, the process of degranulation may be regarded

as an important mechanism whereby cells which accumulate during the inflammatory response perform an active, rather than a passive role in presenting microbicidal compounds.

Neutrophils contain three types of granules: specific, azurophilic, and a group of less numerous heterogenic granules. Following phagocytosis, the fusion of each granule type with the phagosome appears to follow sequential order. This may allow granule contents to act in the phagolysosome in a manner which augments the pH optima for the presented enzymes. The compounds contained within each granule type and the prospective functions of the compounds have been the subject of considerable interest. Primary constituents of the azurophilic granules include defensin-like proteins, peroxidase, lysozyme, serine proteases such as elastase and cathepsin b, and acid phosphatase. Specific granules contain lactoferrin, histamine, collagenase, vitamin B binding proteins, and are a reservoir of plasma membrane proteins such as alkaline phosphatase, receptors such as fMLF and cytochrome b; and other granules contain acid phosphatases, glycolytic enzymes, acid proteinases, and gelatinase (64).

Because neutrophils can release the repertoire of antimicrobial compounds outside the cell membrane independently of phagosome formation, they are capable of destroying fungal elements and other organisms too large for ingestion. Unfortunately, normal tissue cells may also be damaged by inappropriately stimulated neutrophils in this way. In response to these non-ingestible targets, neutrophils attach to adjacent surfaces where they deposit the contents of the host-defensive granules and products of the respiratory burst. The term "frustrated phagocyte" has been used to describe these

cells which attack objects too large for ingestion by phagocytosis.

Oxygen-independent Microbicidal Factors

Elucidation of the deficiencies associated with the NADPH-oxidase in CGD

patients reduced the level of interest in other oxygen-independent microbicidal

constituents of neutrophil. However, the clinical heterogeneity of CGD patients and

observations that some pathogens were apparently destroyed by the cells of CGD

patients suggested that other mechanisms of neutrophil-associated immune surveillance

were involved. This led to the discovery of a number of compounds which exert the

microbicidal effect independently of the reactive oxygen compounds (50). These

include the cationic peptide-like compounds called defensins which share strong

similarity to constituents of the insect immune system (44). Collectively, these

oxidase-independent defensive factors constitute an important means of protection in

several situations. For example, the activity of oxygen-independent microbicidal

functions are critically important in cases where the NADPH-oxidase is impaired, as in

CGD, or when molecular oxygen is not available to the neutrophil. Such conditions

include sites of infection lacking adequate perfusion of oxygenated blood, and settings

in which microbial challenge occurs concomitantly with severe cyanosis such as

bacterial pneumonia.

Formylpeptide Receptor and Signal Transduction

An enigmatic process of cell signalling is the mechanism by which many

different extracellular stimuli can be received, resulting in the engagement of

appropriate effector systems, yet only limited avenues of communication exist between

molecular systems within the cell. The specificity by which varied signal transduction

systems are routed relies on exquisite linkage of involved cellular factors. Such

factors include specific enzyme systems, and on the timing, concentration, and species

of small substances mobilized or activated in response to the signal. These small

substances are referred to as second messengers, and include cAMP, diacyl glycerol,

Ca^{2+}, and inositol triphosphate (IP_3).

The chemical responses which begin with the exposure of the neutrophil to

bacterial products and result in cell adherence, aggregation, migration, the respiratory

burst, and degranulation have received significant attention (27,96). Many of the

signal transduction events associated with neutrophil activation have been revealed, yet

perhaps many more remain obscure. Information about signal transduction in

neutrophils has illuminated similar mechanisms in other cells.

The formylpeptide receptor (FPR) and associated signal transduction cascade is

a well-characterized receptor-mediated chemical signaling system in human neutrophils

and is involved in many microbicidal events (Figure 2). The gene encoding the FPR

was cloned and sequenced, and the transcript was deduced to contain 350 amino acids

and seven membrane-spanning domains predicted by hydropathy analysis (19). Two

other receptors, the C5a anaphylatoxin receptor and the platelet activating factor (PAF)

receptor, have also been characterized in neutrophils (107). These receptors have

sequence homology to the FPR and appear to share routes of neutrophil activation.