REVIEW ARTICLE

Granules of the Human Neutrophilic Polymorphonuclear Leukocyte

By Niels Borregaard and Jack B. Cowland

POLYMORPHONUCLEAR leukocytes were discovered by Paul Ehrlich,[1] when fixation and staining techniques made it possible to identify the lobulated nucleus and the granules that have given name to these cells and allowed for their classification as eosinophils, basophils, and neutrophils. Neutrophilic granulation refers to staining by a mixture of basic and acid, ie, neutral, dyes,[1] whereas the term specific granules was used by Paul Ehrlich to distinguish true granules from artifacts with a granular appearance.[2] It was later realized that two types of granules could be distinguished in neutrophils on the basis of their affinity for dye: azurophil granules, which take up the basic dye azure A at the promyelocytic stage, due to their content of acid mucopolysaccharide,[3]and specific granules, which do not. A clear distinction between these two types of granules was established when the peroxidase staining method for electron microscopy was adopted to identify myeloperoxidase (MPO), which is present only in azurophil granules.[4,5] This distinction was corroborated by the development of subcellular fractionation techniques for separation of the granules.[6-9] It has become dogma that these two types of granules are fundamentally different. Specific granules have been characterized as secretory granules that play important roles in initiating the inflammatory response,[10] whereas azurophil granules are often viewed as lysosomes that are particularly active in the digestion of phagocytosed material.[11-14]

This simplistic view of granules has been challenged by results from subcellular fractionation experiments in which granule proteins were found in more than two peaks on density gradients.[9,13,15,16] In addition, the existence of a tertiary granule type, identified by its late appearance during myeloid maturation, was indicated by electron microscopy.[3] During the last 15 years, high-resolution subcellular fractionation techniques, alone or in combination with immune-electron microscopy and flow cytometry, have shown a bewildering heterogeneity of the neutrophil's granules[17-22] and have furthermore identified an additional regulated exocytotic storage organelle, the secretory vesicle.[23-26]

A novel aspect of the physiology of granules was unraveled by the discovery that these regulated storage organelles (granules and secretory vesicles) are not just simple bags of proteolytic or bactericidal proteins that are kept in store until liberated either to the outside of the cell or to the phagocytic vacuole, but are also important reservoirs of membrane proteins that become incorporated into the surface membrane of the neutrophils when these organelles fuse with the plasma membrane and exocytose their content.[17,27] In this way, granules and secretory vesicles may fundamentally change the ability of the neutrophil to interact with its environment.

Realizing this, a number of important questions immediately appear. Why this heterogeneity? Does it provide the neutrophil with the ability to differentially exocytose (or, in case of membrane proteins, translocate) proteins stored in different granules, and if so, how is this differential exocytosis controlled? Or, is the reason for the granule heterogeneity simply that some proteins must be segregated to survive inside the neutrophil, or for the neutrophil to survive, much as two-component glue has to be stored in two separate tubes, although both will be used at the same time? Whether one or the other reason applies, both raise the question of how the neutrophil can target proteins into different granule subsets.

The purpose of this review is to present the heterogeneity of granules and discuss the functional importance of this and, furthermore, to address how the neutrophil controls the structure and mobilization of these different granules.

BASIC ASPECTS OF GRANULOGENESIS

Granules start to form at the stage of neutrophil maturation marked by transition from myeloblast to promyelocyte.[4,5] From here on, formation of granule proteins continues even up to the stage of segmented cells.[28]

In general, granules are believed to be formed by aggregation of immature transport vesicles that bud off from the *trans*-Golgi network (TGN), in which sorting takes place between constitutively secreted proteins and proteins that are routed into the regulated secretory pathway, ie, go to granules.[29-31] The original study by Bainton et al[4,5] showed that such vesicles bud off from *cis*-Golgi to form storage granules at the promyelocyte stage, but from the *trans*-Golgi at the myelocyte stage, where specific granules are formed. This implies that the sorting apparatus (if existing) is localized in the *cis*-Golgi in promyelocytes and moves to the TGN in more mature cells. Unlikely as this may seem, it agrees with the finding that MPO, a major protein of azurophil granules, does not contain complex carbohydrate side chains,[32,33] but it is contradicted by the finding that several other azurophil granule proteins, including elastase, cathepsin G, and proteinase 3, acquire complex oligosaccharide side chains.[34,35] Refining of carbohydrate side chains from simple to complex is a feature of the intermediate- and *trans*-Golgi stacks.[36]

Several regulatory steps will be required if granules are formed by fusion from smaller unit transport vesicles that bud off from the Golgi and undergo homotypic fusion until they achieve the size of a granule. It must be secured that such transport vesicles do not fuse with the plasma membrane, as do transport vesicles that mediate constitutive secretion. Furthermore, it must be secured that such transport vesicles do not fuse with already formed granules, because this would lead to mixing of granule proteins destined for different types of granules.

From The Granulocyte Research Laboratory, The Finsen Center, Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark.

Submitted November 6, 1996; accepted January 29, 1997.

Address reprint requests to Niels Borregaard, MD, PhD, Rigshospitalet L-4042, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark.

0006-4971/97/8910-0049$3.00/0

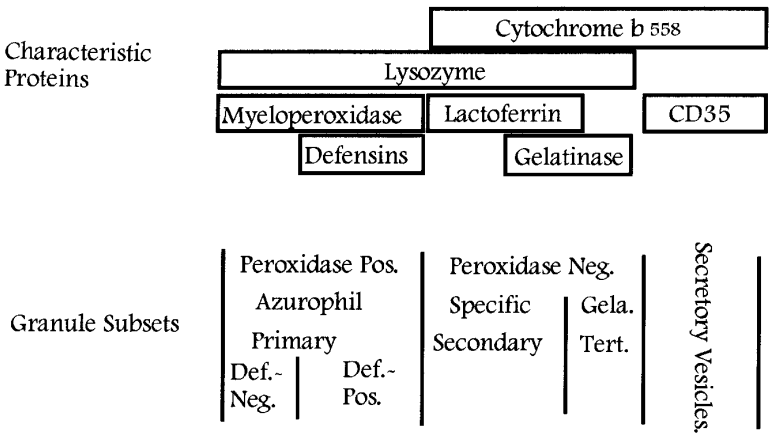

Fig 2. Classification of granules in neutrophils. Peroxidase-positive (azurophilic or primary) granules are characterized by the content of MPO and may be further divided based on their content of defensins into large, defensin-rich granules and the smaller defensin-poor granules. The peroxidase-negative granules may be divided into specific (secondary) granules and gelatinase (tertiary) granules on the basis of their relative content of lactoferrin and gelatinase. All granules contain lysozyme. Secretory vesicles share some of their membrane proteins with peroxidase-negative granules, whereas others are unique to secretory vesicles. Def., defensins; Gela., gelatinase; Tert., tertiary.

Aggregation of smaller unit vesicles to form mature granules creates a surface to volume problem because the surface area of the final granule is the sum of the surface area of the transport vesicles that fused to create the mature granule, and the volume of the mature granule is the sum of the volume of cargo carried by the transport vesicles (Fig 1). Azurophil granules are generally described as spherical or football shaped, whereas specific granules are known to adopt more irregular and elongated forms.[5] This might be a reflection of volume adjustment in azurophil granules, which are known to proteolytically process a significant part of the proteins that are targeted to these.[32,37,38] In this respect, azurophil granules resemble lysosomes.[39] In contrast, no processing and therefore no increase in osmotic activity due to proteolysis has been observed in specific granules, with one possible exception.[40]

The question of whether granules of neutrophils are formed by fusion of unit vesicles has not been addressed, but patch-clamp capacitance studies that accurately quantitate the size of granules that are incorporated into the plasma membrane have been performed on horse eosinophils and rat basophils. These studies have shown that individual granules differ in size by the size of unit vesicles. This indicates that granules are formed by homotypic fusion of a finite but slightly varying number of smaller unit vesicles.[41,42]

CLASSIFICATION OF NEUTROPHIL GRANULES

Granules may be classified on the basis of their size, morphology, or electron density or with reference to a given protein.[5] The initial classification into two major types of granules was based on the content of MPO.[4] However, the granules can be further subdivided on the basis of other intragranular proteins,[21,43,44] as observed in Fig 2. It should be emphasized that, according to the targeting-by-timing hypothesis that will be discussed later, classification of granules is arbitrary, because granules form a continuum from azurophil granules to gelatinase granules, sharing some proteins, eg, lysozyme,[45] whereas other proteins can be chosen to serve as specific markers of one particular subset, eg, MPO, lactoferrin, and gelatinase. A primary question is whether classification of granules is physiologically meaningful. A secondary question is whether it is of use in clinical hematology, eg, for classification of myeloproliferative or myelodysplastic disorders.

It is well established that major differences exist between the different granule subsets regarding the extent to which these are mobilized both in vitro and in vivo.[46-50] Gelatinase granules (identified by gelatinase) are mobilized more readily than specific granules (identified by lactoferrin),[16,51,52] which again are exocytosed more readily than azurophil granules (identified by MPO). This hierarchy applies both when neutrophils isolated from peripheral blood are stimulated with various secretagogues and when exudate neutrophils collected in a skin window chamber are analyzed and further stimulated.[48,50] It therefore makes sense from a functional point of view to classify the neutrophil granules into peroxidase-positive (or azurophilic or primary) granules, defined by their content of MPO, and to further subdivide the peroxidase-negative granules into specific (or secondary) granules, defined by their content of lactoferrin, and gelatinase (or tertiary) granules, defined by their high concentration of gelatinase[20,21] (Fig 2). A number of proteins have been identified in these granules. Table 1 gives the content of the different types of granules. The localization of some of these proteins has been determined by electron microscopy, some by subcellular fractionation, and some by mobilization, assuming that proteins that are mobilized together also localize together.

SECRETORY VESICLES

When considering exocytosis of granules, it should be kept in mind that exocytosis of granule content is a consequence of fusion of granule membrane with the plasma membrane and incorporation of granule membrane into the plasma membrane. In many cells, this membrane is rapidly retrieved for re-use (adipocytes, neurons),[53-57] but in neutrophils, the membrane of mobilized granules largely remains part of the plasma membrane.[58-61] In this way, membrane proteins located to the membrane of granules translocate to the surface membrane and furnish the cell with new receptors and other functional proteins.[17]

The observation that the β_2-integrin Mac-1 ($\alpha_m\beta_2$, CD11b/CD18) became incorporated into the plasma membrane without corresponding exocytosis of granule content led to the discovery of the most rapidly mobilizable intracellular structure in neutrophils, the secretory vesicle.[23,24,26,58] Secretory vesicles are

Table 1. Content of Human Neutrophil Granules and Secretory Vesicles

Azurophil Granules	Specific Granules	Gelatinase Granules	Secretory Vesicles
Membrane	Membrane	Membrane	Membrane
CD63[59,289]	CD11b[64,104]	CD11b[64,109,327-330]	Alkaline phosphatase[23-26,66]
CD68[290]	CD15 antigens[305]	Cytochrome b_{558}[20]	CR1[65]
V-type H^+-ATPase[291]	CD66[306]	Diacylglycerol-deacylating enzyme[331]	Cytochrome b_{558}[62,333]
	CD67[306]	fMLP-R[109,131]	CD11b[62,64]
	Cytochrome b_{558}[17,52,105]	SCAMP[170]	CD14[334]
	fMLP-R[131,307,308]	Urokinase-type plasminogen activator-R[319]	CD16[63,334] *
	Fibronectin-R[309]	VAMP-2[170]	fMLP-R[131]
	G-protein$_\alpha$-subunit[310,311]	V-type H^+-ATPase[291]	SCAMP[170]
	Laminin-R[309]		Urokinase-type plasminogen activator-R[319]
	NB 1 antigen[312]		V-type H^+-ATPase[291]
	19-kD protein[313]		VAMP-2[170]
	155-kD protein[314]		CD10, CD13, CD45[335] *
	Rap1, Rap2[315,316]		C1q-receptor[336] *
	SCAMP[170]		DAF[60] *
	Thrombospondin-R[317]		
	TNF-R[318]		
	Urokinase-type plasminogen activator-R[319]		
	VAMP-2[170]		
	Vitronectin-R[309]		
Matrix	Matrix	Matrix	Matrix
Acid β-glycerophosphatase[11]	β_2-Microglobulin[320]	Acetyltransferase[332]	Plasma proteins[24,25] (including tetranectin)
Acid mucopolysaccharide[292]	Collagenase[321]	β_2-Microglobulin[20]	
α_1-Antitrypsin[293]	Gelatinase[21,322]	Gelatinase[16,21,107]	
α-Mannosidase[11]	hCAP-18[117]	Lysozyme[45]	
Azurocidin/CAP37/heparin binding protein[294-296]	Histaminase[323]		
Bactericidal permeability increasing protein[297]	Heparanase[324]		
β-Glycerophosphatase[7]	Lactoferrin[301]		
β-Glucuronidase[7,298]	Lysozyme[7,8,298]		
Cathepsins[11]	NGAL[126]		
Defensins[43,299]	Urokinase-type plasminogen activator[319,325]		
Elastase[300]	Sialidase[303]		
Lysozyme[7,8,298]	SGP28[40]		
Myeloperoxidase[301]	Vitamin B_{12}-binding protein[326]		
N-Acetyl-β-glucosaminidase[7]			
Proteinase-3[302]			
Sialidase[303]			
Ubiquitin-protein[304]			

* This localization is based on kinetics of upregulation in response to stimulation with inflammatory mediators, but has not yet been demonstrated by subcellular localization by immunocytochemistry.

important because of their membrane, which is particularly rich in receptors.[62-65] The only known intravesicular content of secretory vesicles is plasma. Albumin therefore currently serves as marker of these vesicles.[25] This indicates that secretory vesicles are endocytic in origin. It should be noted that secretory vesicles are not part of a constitutive endocytosis/exocytosis organelle. Once mobilized, secretory vesicles are not reformed, either in vitro or in vivo.[66-70]

SPECIFIC FUNCTIONS OF GRANULES AND SECRETORY VESICLES

When combining the known content of the different granules and secretory vesicles with their order of mobilization, it becomes clear that these serve different and significant functions (Fig 3). Our view on secretory vesicles is that these are mobilized when the neutrophil establishes the primary rolling contact with activated endothelium, which is mediated primarily by selectins and their ligands, eg, PSGL-1.[71-74] The mobilization of secretory vesicles may be mediated by signaling through the selectins[75,76] or by inflammatory mediators liberated from the endothelium.[77,78] This view is in agreement with the observation that secretory vesicles have been completely mobilized in neutrophils that are collected in plasma in a skin window chamber.[50] Thus, mobilization of secretory vesicles transforms the neutrophil to a β_2-integrin presenting cell[79,80] and in this way changes the cell from a generally passive cell, well suited for

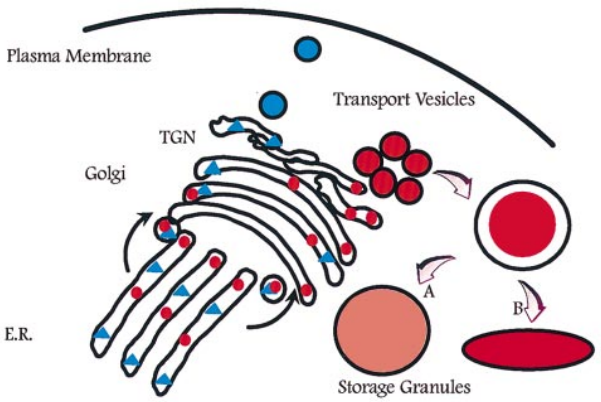

Fig 1. Intracellular routing of newly synthesized proteins. Newly synthesized proteins are sorted into pathways for constitutive (triangles) and regulated (circles) exocytosis. If formation of storage granules occurs by homotypic aggregation of transport vesicles, a surface to volume problem will arise that can be solved only by either adjusting (expanding) the volume to fit the surface of a spherical particle (A) or by adjusting the shape so that the surface fits the volume (B). E.R., endoplasmic reticulum; TGN, *trans*-Golgi network.

circulation, to a highly responsive cell, primed for migration into tissues.[81-93]

Type IV collagen, a major constituent of basement membranes, and type V collagen of interstitial tissues, are substrates of gelatinase.[94-96] It is likely that exocytosis of gelatinase from gelatinase granules is essential for migration of neutrophils through basement membranes.[97,98] Although evidence for disruption of the basement membrane has not been obtained by in vivo experiment,[99] in vitro experiments support the need for gelatinase activity in migration of neutrophils through Matrigel and amnion membranes,[100] but the appropriate gene knock-out experiment has not been performed to address this. A human gene knock-out is provided by the rare condition, specific granule deficiency,[101,102] although the functional defects in this condition, which include inability of neutrophils to infiltrate into tissues, may be caused by the lack of expression of other granule proteins than gelatinase.[103]

Although subject to much previous debate,[104-109] the membrane of specific granules has not definitively been shown to be different from that of gelatinase granules, except by quantitative measures (total area of specific granules is larger than the total area of gelatinase granules).[20,21] The membranes of specific-and gelatinase granules relate to phagocytosis and intracellular killing, because these are the main stores of Mac-1 ($\alpha_m\beta_2$, CD11b/CD18) and of the flavocytochrome b_{558} ($gp91^{phox}/p22^{phox}$), an essential component of the NADPH oxidase.[20,104,105]

With respect to intragranular proteins, specific granules are dominated by lactoferrin,[8,110] the function of which is still unknown. Of the constituents listed in Table 1, the zymogen collagenase should be mentioned because this, like gelatinase of gelatinase granules, most likely is important for the ability of the neutrophil to make its way through tissues.[111-113] Azurophil granules are characterized by their content of hydrolytic and bactericidal proteins such as elastase, bactericidal permeability-increasing protein, defensins, and MPO.[5,7,13] Azurophil granules have been further subdivided on the basis of several proteins.[114] Defensins are the dominating protein in a major subset of azurophil granules.[43] Three novel proteins of specific granules that may turn out to contribute significantly to the function of neutrophils have recently been identified. These are NGAL,[115,116] hCAP-18,[117-119] and SGP28.[40]

NGAL. NGAL is a member of the lipocalin family of

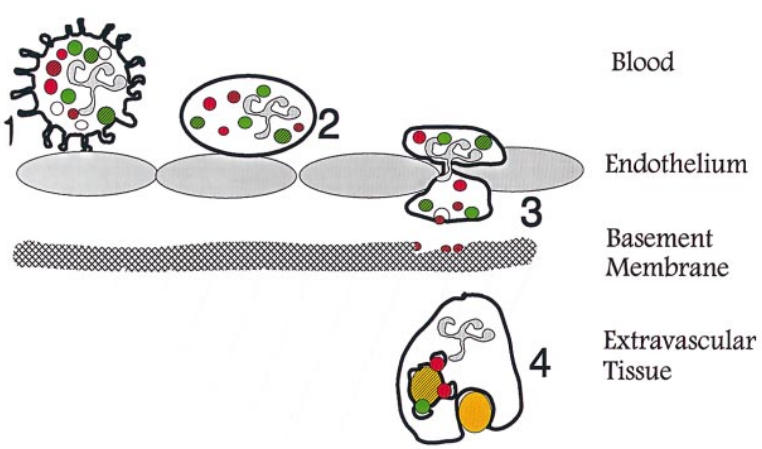

Fig 3. Specific functions of neutrophil granules and secretory vesicles in relation to diapedesis and phagocytosis. (1) Primary contact between endothelium and circulating neutrophils is established via selectins and their ligands, which causes the neutrophils to roll along the activated endothelium. This contact may transduce signals in the neutrophils that mobilize secretory vesicles. (2) Integration of the membrane of secretory vesicles and its associated CD11b/CD18 enhances the potential of the neutrophil for firm adhesion to endothelium. (3) Exocytosis of gelatinase from gelatinase granules may help degradation of type IV collagen in basement membranes. (4) Mobilization of specific granules to the surface membrane may enhances the phagocytic potential of the neutrophils by providing CR3 (CD11b/CD18). Fusion of azurophil and specific granules with the phagosome creates conditions for oxygen-dependent and -independent bactericidal activity. (Modified and reprinted with permission.[200]).

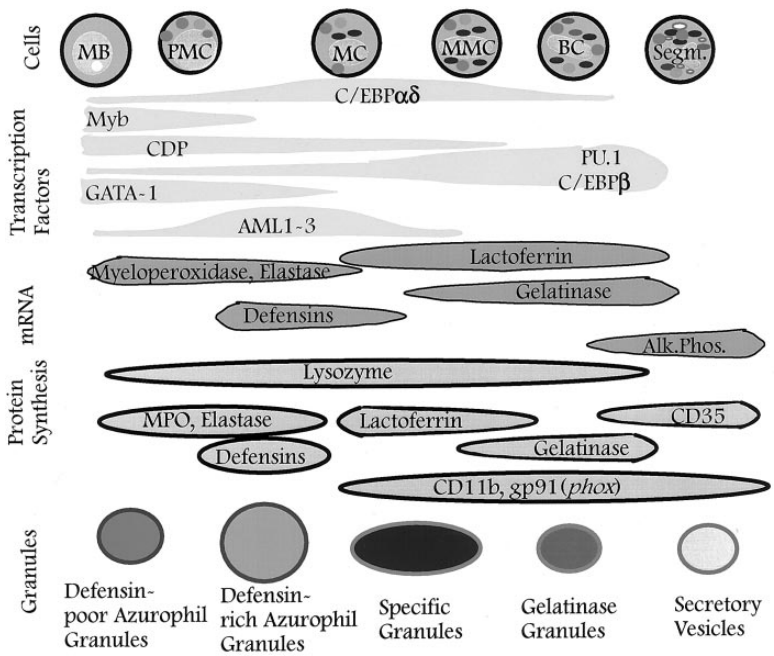

Fig 4. Granules defined by timing of biosynthesis of their characteristic proteins. The granules formed at any given stage of maturation of neutrophil precursors will be composed of the granule proteins synthesized at that time. The different subsets of granules identified are the result of differences in the biosynthetic windows of the various granule proteins during maturation and not the result of specific sorting between individual granule subsets. When the formation of granules ceases, secretory vesicles will form[337] (this point has not yet been proven). The control of biosynthesis is exerted by transcription factors that control the expression of genes for the various granule proteins. It cannot be ruled out that posttranscriptional control occurs so that biosynthesis of proteins is not a precise reflection of the corresponding mRNA levels. MB, myeloblast; PMC, promyelocyte; MC, myelocyte; MMC, metamyelocyte; BC, band cell; Segm., segmented cell.

proteins, which were recently reviewed by Flower.[120] Lipocalins, the archetype of which is retinol-binding protein, are 25- to 30-kD proteins that have a three-dimensional structure[121] that has been likened to a coffee filter.[122] Lipocalins are known to bind small lipophilic substances (eg, retinol). NGAL, so named because of its ability to complex with gelatinase (neutrophil gelatinase associated lipocalin),[115,116] is the human homologue of the mouse protein 24p3 that was identified in SV40 transformed kidney cells.[123,124] A closely homologous protein was recently identified as the protein whose expression is increased the most in *neu*-transformed rat mammary carcinomas. This protein was termed *neu*-related lipocalin.[125] NGAL is normally found only in the specific granules of human neutrophils,[126,127] but its production is significantly induced in colon epithelial cells during inflammation (Crohn's disease, ulcerative colitis, appendicitis, and diverticulitis), as evidenced both by immune-histochemistry and by in situ hybridization.[128] The synthesis of NGAL has been shown to be induced in peripheral blood neutrophils treated with granulocyte-macrophage colony-stimulating factor (GM-CSF).[129] Two studies on the subcellular localization of the fMLP receptor by photo-affinity labeling identified NGAL as a 25-kD fMLP-binding protein of specific granules.[130,131] Our hypothesis is that NGAL may participate in regulating the inflammatory response by binding small lipophilic inflammatory mediators such as fMLP, platelet activating factor (PAF), leukotriene B_4 (LTB_4), and lipopolysaccharide (LPS).

hCAP-18. hCAP-18, also named FALL-39, was recently identified independently by three groups.[117-119] It is the only human member of the cathelicidin family of bactericidal peptides[132] (see Zanetti et al[133] and Levy[134] for recent reviews). These peptides were first discovered in neutrophils of ruminants.[135] Cathelicidins are proteins that share a high level of homology in the N-terminal 14-kD part of the protein that is homologous to cathelin, a protein purified from porcine neutrophils,[136] whereas the C-terminal parts of cathelicidins are highly diverse, ranging from 12[135] to 100 amino acids.[137] The C-termini are highly cationic. Some are extremely rich in proline and arginine,[135,138,139] whereas others, including the C-terminal 39 aminoacids of hCAP-18, form an amphipatic α-helix.[119] Indeed, we discovered hCAP-18 as a protein highly enriched in the Triton-phase during Triton X-114 phase separation of proteins from specific granules.[117]

Cathelicidins are stored as intact proteins in specific granules (or large granules of ruminant neutrophils), but the C-termini may be cleaved off when the proteins are exposed to elastase[140,141] during concomitant degranulation of specific and azurophil granules, and the bactericidal activity of the C-terminal peptides is unleashed.[118,141] The C-terminal peptides are not only microbicidal, but may also be toxic to eucaryotic cells.[142]

SGP28. SGP28 is a glycoprotein of specific granules with a molecular weight of 28 kD, hence its name.[40] It is homologous to two other proteins: *Tpx-1*, which is a human testis specific protein,[143] and sperm-coating glycoproteins of rat epididymis.[144] These proteins constitute a family of cystein-rich proteins termed CRISPs (cysteine-rich secretory proteins).[145] SGP28 was independently cloned from a salivary gland cDNA library and termed CRISP-3 and was found expressed (Northern blotting) also in pancreas and epithelial cells of the large bowel.[145] Part of SGP28 shows high amino acid sequence homology to plant proteins known as pathogenesis-related proteins. There are proteins that are believed to be important for resistance to infections caused by both virus, bacteria, and fungi.[146]

FUNCTIONAL INTERPLAY OF GRANULES

The generation of reactive oxygen metabolites that is essential for proper microbicidal activity of neutrophils is de-

pendent on components of both peroxidase-negative granules (which harbor the flavocytochrome b_{558}, an essential component of the NADPH oxidase) and of azurophil granules (which contain MPO that transforms the relatively innocuous product of the NADPH oxidase, H_2O_2, to hypochlorous acid[147]). As mentioned above, proteases from azurophil granules may activate the cathelicidin present in specific granules by proteolytically removing the inhibitory (and protective) N-terminal cathelin-like part of the protein. Likewise, gelatinase and collagenases may be converted from their proform to active proteases by elastase liberated from azurophil granules[97] or by reactive oxygen metabolites generated by the NADPH oxidase.[112,148]

CONTROL OF GRANULE EXOCYTOSIS

As alluded to above, secretory vesicles and the different kinds of granules are mobilized in a hierarchy, which seems adjusted to the different roles these organelles play during the journey of the neutrophil from the circulation to the inflammatory focus. Because all granules and secretory vesicles appear randomly distributed throughout the cytosol in the circulating cell[21,25,62,63] and localized towards the lamellipod in the (fMLP-) activated cell,[70] this hierarchy must rely on mechanisms that discriminate between the different granule subsets. However, hierarchic mobilization excludes independent mobilization, ie, mobilization of specific granules without concomitant and more extensive mobilization of gelatinase granules. This indicates that differences in the mobilization of the granule subsets are due to quantitative differences and not to qualitative differences in the machinery that controls exocytosis of the individual granule subsets.

Elevation of intracellular Ca^{2+} is known to elicit exocytosis of storage granules in a variety of cells,[47,149-152] but the molecular mechanism by which this occurs is unknown. Differences in sensitivity toward intracellular Ca^{2+} as a signal to elicit mobilization were observed among the different granule subsets[49,51,153] and secretory vesicles, in correspondence with the hierarchy of their mobilization. It has been shown that a few cytosolic proteins translocate to granules in a Ca^{2+}-dependent way (annexins I, II, III, IV, VI, and XI).[154-160] No clear-cut qualitative differences were observed between binding of different annexins to the different granule subsets, although annexin III was suggested to bind preferentially to specific granules.[156] Furthermore, the Ca^{2+} concentrations that elicited binding of annexins were found to differ among the different granule subsets.[155] A non-annexin Ca^{2+}-binding protein, later identified as grancalcin,[161] was found to be translocated selectively to secretory vesicles.[154] However, the role, if any, of these proteins in regulation of exocytosis is unclear.

Recently, the studies of intracellular transport (from ER to Golgi and within Golgi) have been extended to exocytosis, and a unifying hypothesis called the SNAP/SNARE-hypothesis has been forwarded according to which specific targeting of granules and vesicles is provided by specific interaction of v-SNAREs (present on the membrane of donor organelles) with their cognate t-SNAREs (present on the target membrane). According to this hypothesis, fusion is mediated by the combined action of the cytosolic factors NSF (N-ethyl maleimide sensitive factor) and the attachment proteins for NSF, the SNAPs (soluble NSF-attachment proteins), in further combination with Ca^{2+}, synaptotagmin, and other membrane proteins.[30,31,162,163] The experimental evidence for the relevance of this mechanism to control exocytosis is derived mainly from studies on neuronal tissue.[164-166] One of the strongest indications to support this hypothesis is the observation that the neurotoxins botulinus and tetanus toxins specifically cleave SNARE proteins.[167-169]

In neutrophils, the t-SNARE protein, syntaxin-4, is found exclusively in the plasma membrane, whereas the cognate v-SNARE, VAMP-2, is present in the membrane of the mobilizable granules and secretory vesicles, with the highest density on the membrane of secretory vesicles and gelatinase granules followed by specific granules. VAMP-2 could not be detected on azurophil granules.[170] This could indicate that exocytosis of the neutrophil granules and of secretory vesicles is stochastic, ie, that the likelihood that any given granule will be exocytosed, is determined entirely by the density of fusion-proteins in the membrane of that granule (as recently observed in sea urchin eggs[171]) and that no qualitative differences exist among fusion proteins associated with the different types of granules.

The signal transduction pathways that lead to granule exocytosis are not completely known. Involvement of G-proteins is indicated by the ability of the nonhydrolyzable GTP-γ-S to induce exocytosis in permeabilized neutrophils[172,173] and by localization of distinct G-proteins to granules.[174] Patch clamp capacitance measurements provide a powerful tool to analyze granule exocytosis. Using this technique, incorporation of granule membrane into the plasma membrane is quantitated as increase in the electrical capacitance of the plasma membrane.[175] This technique has documented the role of GTP and Ca^{2+} in control of granule exocytosis and holds promise for further delineation of the molecular mechanism of exocytosis.[176]

The differences among different granule subsets in their ability to become exocytosed have been shown to extent also to orientation of exocytosis. Whereas peroxidase-negative granule are often characterized as secretory granules that mainly mediate extracellular release of their proteins,[177,178] peroxidase-positive granules have been characterized as specialized lysosomal structures[4,11] that do not participate significantly in extracellular release, but form a digestive organelle for phagocytosed particles.[178,179]

The notion that azurophil granules are specialized lysosomes has recently been challenged by the observation that the membrane of azurophil granules is devoid of the characteristic lysosomal membrane proteins (LAMPs),[180,181] which instead are found in multilamellar and multivesicular bodies in the neutrophil, probably defining these organelles as the true lysosomal structures of neutrophils.[181,182]

TARGETING OF PROTEINS TO INDIVIDUAL GRANULE SUBSETS

The mechanism for controlling the protein profile of the individual granule subsets, including the proteins that determine their later exocytosis, must be highly efficient to account for the differences in granule composition and for the

hierarchy in exocytosis presented above. Two essentially unrelated steps are involved in targeting of proteins to granules. The first step is sorting between transport vesicles that mediate constitutive exocytosis of secretory proteins and insertion of membrane proteins into the plasma membrane and vesicles that will form storage granules capable of undergoing regulated exocytosis. The second step is sorting between different subsets of storage granules.

Sorting between regulated and constitutive exocytotic pathways. Very little is known with respect to mechanisms that sort proteins into the constitutive or the regulated exocytotic pathways in general, let alone in myeloid cells. Sorting of insulin and neuropeptides is (partly) controlled by pH and Ca^{2+}-mediated aggregation,[183-185] without involvement of carrier proteins or membrane attachments.[186] The well-known targeting of mannose 6-phosphate containing glycoproteins to lysosomes via the cation-dependent and cation-independent mannose 6-phosphate receptors[187] has not been shown to be important for targeting of proteins to granules in neutrophils. Although MPO is decorated with mannose 6-phosphate that is recognized by the cation-independent mannose 6-phosphate receptor,[33,181] this is not important for sorting to azurophil granules.[33,188] In addition, azurophil granules contain proteins that do not have the mannose 6-phosphate label (eg, lysozyme[189] and defensins[190]). Recently, MPO was found associated with calreticulin as a chaperone,[191] but the significance of this for sorting is unknown. No common primary protein structure has been identified in proteins that are retained versus those that are constitutively secreted. N-terminal hydrophobic domains have been suggested as a sorting signal in some neuropeptides.[192]

An essential question is whether retention signals are common for all cells, ie, is a protein that in neutrophils is retained in granules also retained if transfected to other cell types? If so, is this dependent on whether the cells have the capacity for formation of granules? This has been studied with defensins, the constituents of azurophil granules, as probe. It has been observed that defensins can localize to granules in nonmyeloid cells, indicating that sorting to storage granules occurs by mechanisms common to all cells.[193] This is also supported by transfection of other azurophil granule proteins to other myeloid cells.[194,195] Yet, to prove the point, it is necessary to show active sorting between two proteins synthesized in the same cell, but handled differently by the sorting machinery. Because granules are so abundant in granulocytes and appear to be formed rapidly, at least as evaluated in eosinophils,[196] there may be little need for a sorting mechanism if the bulk of cargo will go to granules anyway. This question must be addressed by examining whether all proteins that are routed through the Golgi go to granules with the same efficiency or whether some are more efficiently retained than others.

No studies have been presented to address the route of transport of membrane proteins that eventually localize to granules in granulocytes. In neuroendocrine cells, the targeting of peptidylglycyl α-amidating monooxygenase seems to be mediated by two targeting signals: a carboxyterminal cytoplasmic domain that retains the protein in the TGN (as opposed to being routed to the plasma membrane) and a lumenal domain that is essential for routing to secretory granules.[197] P-selectin, as opposed to E-selectin, is targeted to its intracellular localization in endothelial cells by specific signals present in the cytosolic carboxy-terminal tail.[198,199] Such have not been reported regarding the localization of flavocytochrome b_{558} and CD11b/CD18, proteins that are localized to the membrane of peroxidase-negative granules. Thus, it has not been addressed whether there is need for specialized targeting or whether sufficient amounts of the membrane proteins going through the Golgi will go passively to the granules at the time of their formation.

Sorting between individual granule subsets. In view of the considerable heterogeneity of neutrophil granules, sorting of proteins into different granules would require a very complex sorting mechanism if it was dependent on sorting information present in the individual proteins. We have forwarded the hypothesis that there is no specific sorting of proteins to individual granule subsets and that all granule proteins that are synthesized at the same time will localize to the same granules.[28,200,201] This implies that the differences in protein content that define the different subsets of granules result from differences in the biosynthetic window of the various granule proteins in relation to maturation, as depicted in Fig 4. This extends the original observation of Dorothy F. Bainton that peroxidase-positive granules are formed at the promyelocyte stage, whereas peroxidase-negative granules are formed at the myelocyte stage.[5] We were able to show that the differences among peroxidase-negative granules with regard to their content of gelatinase and lactoferrin could be explained by differences in the time of biosynthesis of these proteins, ie, lactoferrin synthesis occurred in cells at the myelocyte/metamyelocyte stage, whereas maximal gelatinase synthesis occurred at the metamyelocyte/band cell stage.[28]

Although the available information regarding the localization of proteins and their time of biosynthesis is in agreement with this hypothesis,[37,202-204] formal proof that no targeting is required for sorting into individual granule subsets was achieved by our analysis of the granule protein NGAL. NGAL is synthesized at the same stage as lactoferrin in normal neutrophil precursors[28] and colocalizes with lactoferrin in specific granules.[126] When NGAL was transfected to HL-60 cells under control of a constitutively active cytomegalovirus promoter, NGAL became synthesized at the same time as the endogenous granule protein MPO. NGAL was efficiently retained in granules of these HL-60 cells and colocalized with MPO in azurophil granules.[201] However, NGAL, so targeted to azurophil granules by changing its timing of expression, was slowly degraded in the proteolytic milieu of the azurophil granules or their precursors.[201] This illustrates that, in addition to the need for sequential mobilization of different granule proteins, the need for segregating proteins that cannot coexist may be another reason for the development of neutrophil granule heterogeneity.

The degradation of NGAL in azurophil granules or their precursors is in line with a number of observations that point to the azurophil granule as the place of terminal processing of many of its proteins. A variety of proteins localized to azurophil granules (MPO,[32] defensins,[37] cathepsin G, and elastase[38]) are proteolytically modified to their mature form after exit from the TGN. This is supported by the observation

that defensins and MPO are not proteolytically modified when targeted to storage granules of cells of nonmyeloid origin.[193,205] Signals present in the N-terminus of defensins have been shown to be important for correct targeting to granules.[206] This most likely illustrates that the N-terminal pro-piece neutralizes the highly cationic C-terminus[190] and prevents the protein from becoming stuck to membranes en route through the ER and Golgi and underscores the importance of final processing occurring in azurophil granules and not before. The fact that pro-segments are described for a variety of azurophil granule proteins, but not for granule proteins localized to peroxidase-negative granules, may be a matter of semantics. A variety of the specific granule proteins are only active after N-terminal trimming of a propiece has occurred (cathelicidin, gelatinase, and collagenase, as previously discussed), but this only takes place after exposure to azurophil granule proteins or to external proteases.[97,113,140,141,207] Thus, it is possible that pro-segments play a role for correct transport through the biosynthetic machinery, but maybe not for specific sorting, as was recently suggested.[208] As mentioned in the legend and text to Fig 1, the proteolytic processing in granules may even affect the shape of the granules by generating osmotically active substances.

GRANULE-DEFICIENT GRANULOCYTES

HL-60 cells share with other human promyelocytic cell lines, eg, NB_4 cells, the inability to express endogenous proteins normally localized to specific granules, despite the fact that the gene structure, including the 5′-untranslated regions assumed to control transcription of these genes, are intact, where investigated.[209-211] Yet, these cells retain the ability to express proteins normally localized in the membrane of specific granules when driven into maturation by retinoic acid or dimethyl sulfoxide (DMSO).[204,212] We therefore investigated the fate of the transfected specific granule protein NGAL in HL-60 cells when these were forced to maturation by retinoic acid and DMSO and had stopped the synthesis of endogenous azurophil granule proteins.[213] In these cells, newly synthesized NGAL was not retained, but was routed to the extracellular medium, and the endogenous specific granule membrane protein, the flavocytochrome b_{558} was localized to the plasma membrane and not to granules. This is in contrast to the undifferentiated transfected cells, in which NGAL was localized to azurophil granules, as previously discussed. This illustrates that a shift of routing from the regulated secretory pathway to the constitutive secretory pathway takes place, when HL-60 cells mature. This indicates that the ability to maintain a regulated secretory pathway is dependent on continued synthesis of proteins that are needed for formation of regulated storage granules. This is consistent with the observation that formation of transport vesicles that mediate constitutive secretion continues after cycloheximide treatment, whereas formation of transport vesicles that mediate regulated secretion is blocked.[214] It has recently been observed that transfection of fibroblasts with synaptotagmin is able to transform constitutive secretion to regulated secretion.[215] It appears, therefore, that the difference between regulated and constitutive secretion may rely on a few proteins and is not due to fundamental differences in the budding and targeting mechanisms of the TGN. The proteins that are needed for formation of regulated storage granules have not been identified in myeloid cells.

The rare condition termed specific granule deficiency is characterized by defects in expression of defensins and of matrix proteins of specific granules, but preservation of the ability to form some membrane proteins that are normally localized to specific granules.[103] These membrane proteins colocalize with the plasma membrane in specific granule deficient neutrophils[216] and, in this respect, these cells resemble differentiated HL-60 cells.

If, as we believe, targeting of proteins to granules is entirely dependent on the time of biosynthesis, then control mechanisms must exist to secure that vesicles, budding off the TGN and carrying newly synthesized proteins, do not fuse with already formed granules. One such mechanism could be that granules bud off from Golgi at their final size with no further homotypic aggregation, although the observations that have been made on the size of granules do not support this, as previously mentioned. On the other hand, all cells that contain regulated storage granules must have a mechanism to control the size of the granules, otherwise the cells would continue to put all proteins into an ever-growing granule. The targeting-by-timing hypothesis therefore does not invoke further control mechanisms than are already needed in all cells that form granules. An indication that factors exist that limit the size of storage vesicles is given by the observation that such factors seem to be absent from cells of the promyelocytic mouse cell line 32D cl3, which accumulate granule proteins into large intracellular sacs,[206] and by the large granules of neutrophils, eosinophils, and other cells from patients with the Chédiak-Higashi syndrome,[22,217,218] now shown to be defective in *Lyst* protein.[219]

CONTROL OF GRANULE PROTEIN BIOSYNTHESIS

The control of granule protein biosynthesis can be on either the transcriptional or the translational level. Whereas the transcriptional control is exerted by transcription factors, translational control is more elusive.[220,221] Although growth factors may exert translational control, translational control has not been shown to be relevant for expression of myeloid granule proteins.

The stage of maturation of neutrophil precursors at which biosynthesis of granule proteins starts is in agreement with the start of transcription of the relevant genes in the cases in which this has been investigated (MPO,[202,211,222-224] defensins,[211,224-228] lactoferrin,[211,224,229,230] vitamin B_{12}-binding protein,[211,224] CD11b,[204] and alkaline phosphatase[202]), although no distinct temporal difference between gelatinase and lactoferrin mRNA expression was observed during maturation in a mouse myeloid cell.[231] It is not quite clear whether there is a complete correlation between stop of protein biosynthesis and downregulation of mRNA. This indicates that start of protein synthesis is controlled by transcription, whereas the termination of protein synthesis, at least for some granule proteins, may be under translational control.

TRANSCRIPTION FACTORS INVOLVED IN CONTROL OF GRANULE PROTEIN EXPRESSION

Of the largely myeloid-specific granule proteins listed in Table 1, the following have been cloned at the genomic

level and their 5′-upstream or flanking sequences have been characterized: MPO,[232] elastase,[233,234] cathepsin G,[233,235,236] proteinase 3,[237] lysozyme,[238] defensin,[239] lactoferrin,[210] gp91^{phox},[240-243] p22^{phox},[244] CD11b,[245-247] CD18,[248-250] vitamin B_{12}-binding protein,[251] hCAP-18/FALL-39,[132] NGAL (Cowland and Borregaard, EMBL database accession no. X99133), gelatinase,[252,253] and alkaline phosphatase.[254] Putative binding sites for a variety of transcription factors have been identified in these promoter regions, but only a few promoter regions have been characterized by functional analysis (expression and gel shift analysis, and experiments of nature [CGD states[241,255]]). These include MPO,[256,257] cathepsin G,[236] lysozyme,[238] elastase,[234,258] CD11b,[246,247,259,260] CD18,[248-250] gp91^{phox},[242,243,261] and gelatinase.[253]

GATA-1. The transcription factor GATA-1 is upregulated in the very early stages of commitment of the hematopoietic progenitor cells as a result of induction of cell division.[262] This results in a gradual increase in the concentration of GATA-1 over a period of 1 to 2 days,[263] followed by an abrupt downregulation and the disappearance of GATA-1 within 4 to 5 days from cells committed to the myelomonocytic lineage.[262] A GATA-1 site capable of binding the GATA-1 factor is found in the genes for lactoferrin and for the Mac-1 subunit CD11b, but it has not been shown that these sites are of any importance for the control of expression of these genes.[210,247]

AML (PEBP2/CBP). The AML (PEBP2/CBP) transcription factors are a family of heterodimeric proteins consisting of a common β-subunit and a specific DNA-binding α-subunit, of which three different types are known in humans.[264] This is further varied by different splice variants of the individual α-unit transcripts.[264,265] A functionally important PEBP2/CBP site has been identified in the promoters of the human GM-CSF[266] and in the mouse MPO and elastase genes.[267,268] PEBP2/CBP is upregulated in the murine 32D cl3 cells upon induction of differentiation (days 1 to 3) and downregulated upon exit from the promyelocytic stage (days 5 to 6).[267] Besides the regulatory effects of upregulation and downregulation of the AML (PEBP2/CBP) factors and the need for co-operativity with other transcription factors for optimal function, a positive and negative regulatory effect of two differently spliced AML-1 transcripts has been observed on differentiation of murine 32D cl3 cells.[265]

c-Myb. c-Myb is found to be highly expressed in immature hematopoietic cells and completely absent from mature granulocytes.[269-271] Overexpression of *c-myb* causes continued proliferation of progenitor cells and blocks differentiation of the cells.[272] In humans, only genes expressed at early stages of granulocyte differentiation, such as elastase[213,267] and MPO,[268] have been shown to carry a functional important Myb site. Potential c-Myb sites have also been identified in the early expressed proteinase-3 and azurocidin genes. Co-operativity with C/EBP has been shown for the *min-1* gene[273,274] and with both C/EBP and PU.1 for the elastase gene.[213]

PU.1. PU.1 is specific for the hematopoietic system and highly expressed in B-lymphocytic, granulocytic, and monocytic cells.[275,276] PU.1 is present at all stages of granulocyte differentiation.[276,277] Its expression increases gradually up to the myelocytic stage of differentiation and then remains constant.[276,277] This is reflected by the observation that many genes contain a functionally important PU.1 site, both some with an early onset of transcription (elastase,[234] PU.1,[278] GM-CSF receptor,[279] and G-CSF receptor[280]) and some with a later onset of transcription during granulocytic differentiation (CD11b,[247,281] CD18,[249,282] and FcγRI[283,284]). Based on its pattern of expression, it can be concluded that PU.1 is likely to confer hematopoietic specificity to gene expression, but by itself is unable to exert a granulocytic- or stage-specific expression of the target genes. The latter has in some instances been shown to be achieved by co-operativety with other lineage- and/or stage-restricted transcription factors, as in the case of the elastase[213] and GM-CSF receptor promoters.[279]

C/EBPα. The C/EBP family of transcription factors contains at least 6 members, of which three (C/EBPα, -β, and -δ) are restricted to the myeloid cells within the hematopoietic lineages.[285] C/EBPα and C/EBPδ expression is intense in immature myeloid cells and increases until the onset of the promyelocytic stage of differentiation and then declines rapidly, whereas the expression of C/EBPβ is low in immature cells, but increases steadily during the course of granulocytic maturation to reach a maximum in the mature granulocytic cell.[285] A number of genes have been shown to carry a functionally important C/EBP site such as the elastase,[213] *min-1,*[273,274] G-CSF receptor,[286] and GM-CSF receptor genes.[279] Co-operativity with c-Myb has been shown for the elastase,[213] GM-CSF,[279] and *min-1* promoters.[273,274] For the elastase promoter, additional co-operativity has also been observed with PU.1.[213]

CCAAT displacement protein (CDP). CDP is a repressor of the ubiquitous CCAAT-binding transcription factor CP1. CDP has been shown to be involved in the regulation of the gp91^{phox} gene[240,242] and to play a regulatory role in the transcriptional regulation of proteins localized to the matrix of specific granules.[287] These granule proteins are all expressed at the myelocytic stage of neutrophil development. This may indicate that de-repression of gene expression, due to downregulation of CDP over the course of granulocytic differentiation, is important for the stage-specific expression of some granule proteins.

It is likely that it is the appearance (activation) or disappearance (suppression) of one or more lineage- and developmental-specific transcription factors that, alone or in combination, determines the developmental expression pattern of neutrophil-specific genes in concert with ubiquitous transcription factors (Fig 4). No single transcription factor has been shown to confer myeloid-specific expression of genes, but PU.1, which is specific for hematopoietic cells, should be able to determine myeloid-specific gene expression in combination with the C/EBPs, which are found in many different cell types but, within the hematopoietic lineages, only in myeloid cells. Early myeloid expression can then be achieved by the requirement of c-Myb and/or a PEBP2/CBP binding for gene expression. Late granulocytic gene expression can be achieved by the disappearance of one or more repressors, such as CDP, or the appearance of a yet unidentified positive regulatory protein during differentia-

tion. Another possibility is that the C/EBP sites of some of the genes expressed at the early stages of granulocytic differentiation have a high affinity for C/EBPα and a low affinity for C/EBPβ and that the opposite is the case for genes expressed at later stages. The expression patterns of these granulocyte-specific genes may then simply reflect the expression patterns of C/EBPα and C/EBPβ. Although no difference in specificity towards the recognized DNA core sequence has been ascribed for the two C/EBP transcription factors,[288] a difference in binding affinity and specificity might be achieved by subtle differences in the surrounding sequences. Activation of transcription factors as a result of ligand binding to the GM-CSF and G-CSF receptors may also contribute to the regulation of late gene expression.

CONCLUSION

The granules of human neutrophils should be viewed as a continuum that reflects the differences in the time of biosynthesis of the various proteins that characterize the granules. With the growing knowledge about the stages of neutrophil maturation at which the different granule proteins are synthesized and about transcription factors involved in controlling this process, the subsets of neutrophil granules that may now be identified not only provide the basis for understanding essential parts of neutrophil physiology and pathophysiology, but also give a more detailed framework on which to put the defects in neutrophil maturation that are characteristic of myeloproliferative and myelodysplastic disorders.

ACKNOWLEDGMENT

The authors express their sincere appreciation of the collaboration with the previous and present staff at the Granulocyte Research Laboratory who contributed with much of the work that forms the basis of this review (Charlotte Horn, Ole Bjerrum, Lars Kjeldsen, Henrik Sengeløv, Karsten Lollike, Ole Sørensen, Sanne Christiansen, Veronique Le Cabec, Tomas Bratt, and Kristina Arnljots). We also thank Dr John K. Spitznagel for helpful information.

REFERENCES

1. Ehrlich P, Lazarus A: Histology of the Blood. Normal and Pathological. (Myers W [ed and trans]: Cambridge, MA, Cambridge, 1900), reprinted in Himmelweit F (ed): The Collected Papers of Paul Ehrlich. Vol 1. Histology, Biochemistry, and Pathology. New York, NY, Pergamon, 1956, p 181

2. Ehrlich P: Über die specifischen granulationen des blutes. Archiv für anatomie und physiologie, Physiologische abteilung 571 Supplementum. Leipzig, Germany, Veit und comp, 1879

3. Spicer SS, Hardin JH: Ultrastructure, cytochemistry, and function of neutrophil leukocyte granules. Lab Invest 20:488, 1969

4. Bainton DF, Farquhar MG: Origin of granules in polymorphonuclear leukocytes. J Cell Biol 28:277, 1966

5. Bainton DF, Ullyot JL, Farquhar M: The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. J Exp Med 143:907, 1971

6. Baggiolini M, Hirsch JG, de Duve C: Resolution of granules from rabbit heterophil leukocytes into distinct populations by zonal sedimentation. J Cell Biol 40:529, 1969

7. Bretz U, Baggiolini M: Biochemical and morphological characterization of azurophil and specific granules of human neutrophilic polymorphonuclear leukocytes. J Cell Biol 63:251, 1974

8. Leffell MS, Spitznagel JK: Association of lactoferrin with lysozyme in granules of human polymorphonuclear leukocytes. Infect Immun 6:761, 1972

9. West BC, Rosenthal AS, Gelb NA, Kimball HR: Separation and characterization of human neutrophil granules. Am J Pathol 77:41, 1974

10. Gallin JI: Neutrophil specific granules: A fuse that ignites the inflammatory response. Clin Res 32:320, 1984

11. Baggiolini M: The enzymes of the granules of polymorphonuclear leukocytes and their functions. Enzyme 12:132, 1972

12. Spitznagel JK, Chi MS: Cationic proteins and antibacterial properties of infected tissues and leukocytes. Am J Pathol 43:697, 1963

13. Welsh IRH, Spitznagel JK: Distribution of lysosomal enzymes, cationic proteins, and bactericidal substances in subcellular fractions of human polymorphonuclear leukocytes. Infect Immun 4:97, 1971

14. Cohn ZA, Hirsch JG: The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. J Exp Med 112:983, 1960

15. Spitznagel JK, Dalldorf FG, Leffell MS, Folds JD, Welsh IRH, Cooney MH, Martin LE: Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. Lab Invest 30:774, 1974

16. Dewald B, Bretz U, Baggiolini M: Release of gelatinase from a novel secretory compartment of human neutrophils. J Clin Invest 70:518, 1982

17. Borregaard N, Heiple JM, Simons ER, Clark RA: Subcellular localization of the b-cytochrome component of the human neutrophil microbicidal oxidase: Translocation during activation. J Cell Biol 97:52, 1983

18. Rice WG, Kinkade JM, Parmley RT: High resolution of heterogeneity among human neutrophil granules: Physical, biochemical, and ultrastructural properties of isolated fractions. Blood 68:541, 1986

19. Nitsch M, Gabrijelcic D, Tschesche H: Separation of granule subpopulations in human polymorphonuclear leukocytes. Biol Chem Hoppe Seyler 371:611, 1990

20. Kjeldsen L, Sengeløv H, Lollike K, Nielsen MH, Borregaard N: Isolation and characterization of gelatinase granules from human neutrophils. Blood 83:1640, 1994

21. Kjeldsen L, Bainton DF, Sengeløv H, Borregaard N: Structural and functional heterogeneity among peroxidase-negative granules in human neutrophils: Identification of a distinct gelatinase containing granule subset by combined immunocytochemistry and subcellular fractionation. Blood 82:3183, 1993

22. Gilbert CS, Parmley RT, Rice WG, Kinkade JM Jr: Heterogeneity of peroxidase-positive granules in normal human and Chédiak-Higashi neutrophils. J Histochem Cytochem 41:837, 1993

23. Borregaard N, Miller L, Springer TA: Chemoattractant-regulated mobilization of a novel intracellular compartment in human neutrophils. Science 237:1204, 1987

24. Borregaard N, Christensen L, Bjerrum OW, Birgens HS, Clemmensen I: Identification of a highly mobilizable subset of human neutrophil intracellular vesicles that contains tetranectin and latent alkaline phosphatase. J Clin Invest 85:408, 1990

25. Borregaard N, Kjeldsen L, Rygaard K, Bastholm L, Nielsen MH, Sengeløv H, Bjerrum OW, Johnsen AH: Stimulus-dependent secretion of plasma proteins from human neutrophils. J Clin Invest 90:86, 1992

26. Sengeløv H, Nielsen MH, Borregaard N: Separation of human neutrophil plasma membrane from intracellular vesicles containing alkaline phosphatase and NADPH oxidase activity by free flow electrophoresis. J Biol Chem 267:14912, 1992

27. Todd RF III, Arnaout MA, Rosin RE, Crowley CA, Peters

WA, Babior BM: Subcellular localization of the large subunit of Mo1 (Mo1α; formerly gp 110), a surface glycoprotein associated with neutrophil adhesion. J Clin Invest 74:1280, 1984

28. Borregaard N, Sehested M, Nielsen BS, Sengeløv H, Kjeldsen L: Biosynthesis of granule proteins in normal human bone marrow cells. Gelatinase is a marker of terminal neutrophil differentiation. Blood 85:812, 1995

29. Sossin WS, Fisher JM, Scheller RH: Sorting within the regulated secretory pathway occurs in the trans-Golgi network. J Cell Biol 110:1, 1990

30. Rothman JE, Orci L: Molecular dissection of the secretory pathway. Nature 355:409, 1992

31. Rothman JE, Wieland FT: Protein sorting by transport vesicles. Science 272:227, 1996

32. Strömberg K, Persson A-M, Olsson I: The processing and intracellular transport of myeloperoxidase. Modulation by lysosomotropic agents and monensin. Eur J Cell Biol 39:424, 1985

33. Nauseef WM, McCormick S, Yi H: Roles of heme insertion and the mannose-6-phosphate receptor in processing of the human myeloid lysosomal enzyme, myeloperoxidase. Blood 80:2622, 1992

34. Lindmark A, Persson A-M, Olsson I: Biosynthesis and processing of cathepsin G and neutrophil elastase in the leukemic myeloid cell line U-937. Blood 76:2374, 1990

35. Rao NV, Rao GV, Marshall BC, Hoidal JR: Biosynthesis and processing of proteinase 3 in U-937 cells—Processing pathways are distinct from those of cathepsin G. J Biol Chem 271:2972, 1996

36. Kornfeld R, Kornfeld S: Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 54:631, 1985

37. Valore EV, Ganz T: Posttranslational processing of defensins in immature human myeloid cells. Blood 79:1538, 1992

38. Lindmark A, Gullberg U, Olsson I: Processing and intracellular transport of cathepsin G and neutrophil elastase in the leukemic myeloid cell line U-937-modulation by brefeldin a, ammonium chloride, and monensin. J Leukoc Biol 55:50, 1994

39. Hasilik A, Neufeld EF: Biosynthesis of lysosomal enzymes in fibroblasts. Synthesis as precursors of higher molecular weight. J Biol Chem 255:4937, 1980

40. Kjeldsen L, Cowland JB, Johnsen AH, Borregaard N: SGP28, a novel matrix glycoprotein in specific granules of human neutrophils with similarity to a human testis-specific gene product and to a rodent sperm-coating glycoprotein. FEBS Lett 380:246, 1996

41. Hartmann J, Scepek S, Lindau M: Regulation of granule size: In human and horse eosinophils by number of fusion events among unit granules. J Physiol 483:201, 1995

42. De Toledo GA, Fernandez JM: Patch-clamp measurements reveal multimodal distribution of granule sizes in rat mast cells. J Cell Biol 110:1033, 1990

43. Rice WG, Ganz T, Kinkade JM, Selsted ME, Lehrer RI, Parmley RT: Defensin-rich dense granules of human neutrophils. Blood 70:757, 1987

44. Oren A, Taylor JMG: The subcellular localization of defensins and myeloperoxidase in human neutrophils: Immunocytochemical evidence for azurophil granule heterogeneity. J Lab Clin Med 125:340, 1995

45. Lollike K, Kjeldsen L, Sengeløv H, Borregaard N: Lysozyme in human neutrophils and plasma. A parameter of myelopoietic activity. Leukemia 9:159, 1995

46. Wright DG: Human neutrophil degranulation. Methods Enzymol 162:538, 1988

47. Wright DG, Bralove DA, Gallin JI: The differential mobilization of numan neutrophil granules. Am J Pathol 87:273, 1977

48. Wright DG, Gallin JI: Secretory responses of human neutrophils: Exocytosis of specific (secondary) granules by human neutrophils during adherence in vitro and during exudation in vivo. J Immunol 123:285, 1979

49. Sengeløv H, Kjeldsen L, Borregaard N: Control of exocytosis in early neutrophil activation. J Immunol 150:1535, 1993

50. Sengeløv H, Follin P, Kjeldsen L, Lollike K, Dahlgren C, Borregaard N: Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. J Immunol 154:4157, 1995

51. Lew PD, Monod A, Waldvogel FA, Dewald B, Baggiolini M, Pozzan T: Quantitative analysis of the cytosolic free calcium dependency of exocytosis from three subcellular compartments in intact human neutrophils. J Cell Biol 102:2179, 1986

52. Kjeldsen L, Bjerrum OW, Askaa J, Borregaard N: Subcellular localization and release of human neutrophil gelatinase, confirming the existence of separate gelatinase-containing granules. Biochem J 287:603, 1992

53. Burgoyne RD, Morgan A: Ca^{2+} and secretory-vesicle dynamics. Trends Neurosci 18:191, 1995

54. Berthiaume EP, Medina C, Swanson JA: Molecular size-fractionation during endocytosis in macrophages. J Cell Biol 129:989, 1995

55. Shibata H, Suzuki Y, Omata W, Tanaka S, Kojima I: Dissection of GLUT4 recycling pathway into exocytosis and endocytosis in rat adipocytes—Evidence that GTP-binding proteins are involved in both processes. J Biol Chem 270:11489, 1995

56. Robinson MS, Watts C, Zerial M: Membrane dynamics in endocytosis. Cell 84:13, 1996

57. Ryan TA, Smith SJ, Reuter H: The timing of synaptic vesicle endocytosis. Proc Natl Acad Sci USA 93:5567, 1996

58. Miller LJ, Bainton DF, Borregaard N, Springer TA: Stimulated mobilization of monocyte Mac-1 and p150,95 adhesion proteins from an intracellular vesicular compartment to the cell surface. J Clin Invest 80:535, 1987

59. Kuijpers TW, Tool ATJ, van der Schoot CE, Ginsel LA, Onderwater JJM, Roos D, Verhoeven AJ: Membrane surface antigen expression on neutrophils: A reappraisal of the use of surface markers for neutrophil activation. Blood 78:1105, 1991

60. Berger M, Medof E: Increased expression of complement decay-accelerating factor during activation of human neutrophils. J Clin Invest 79:214, 1987

61. Berger M, O'Shea J, Cross AS, Folks TM, Chused TM, Brown EJ, Frank MM: Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. J Clin Invest 74:1566, 1984

62. Calafat J, Kuijpers TW, Janssen H, Borregaard N, Verhoeven AJ, Roos D: Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b_{558} and the adhesion molecule CD11b/CD18. Blood 81:3122, 1993

63. Dehaas M, Kerst JM, Vanderschoot CE, Calafat J, Hack CE, Nuijens JH, Roos D, Vanoers RHJ, Vondemborne AEGK: Granulocyte colony-stimulating factor administration to healthy volunteers: analysis of the immediate activating effects on circulating neutrophils. Blood 84:3885, 1994

64. Sengeløv H, Kjeldsen L, Diamond MS, Springer TA, Borregaard N: Subcellular localization and dynamics of Mac-1 ($\alpha_m\beta_2$) in human neutrophils. J Clin Invest 92:1467, 1993

65. Sengeløv H, Kjeldsen L, Kroeze W, Berger M, Borregaard N: Secretory vesicles are the intracellular reservoir of complement receptor 1 in human neutrophils. J Immunol 153:804, 1994

66. Kobayashi T, Robinson JM: A novel intracellular compartment with unusual secretory properties in human neutrophils. J Cell Biol 113:743, 1991

67. Berger M, Wetzler EM, Welter E, Turner J, Tartakoff AM: Intracellular sites for storage and recycling of C3b receptors in human neutrophils. Proc Natl Acad Sci USA 88:3019, 1991

68. Paccaud J-P, Carpentier J-L, Schifferli JA: Exudation induces clustering of CR1 receptors at the surface of human polymorphonuclear leukocytes. Biochem Biophys Res Commun 172:1203, 1990

69. Fernandez-Segura E, Garcia JM, Campos A: Dynamic reorganization of the alkaline phosphatase-containing compartment during chemotactic peptide stimulation of human neutrophils imaged by backscattered electrons. Histochem Cell Biol 104:175, 1995

70. Fernandez-Segura E, Garcia JM, Campos A: Topographic distribution of CD18 integrin on human neutrophils as related to shape changes and movement induced by chemotactic peptide and phorbol esters. Cell Immunol 171:120, 1996

71. Moore KL, Patel KD, Bruehl RE, Li FG, Johnson DA, Lichenstein HS, Cummings RD, Bainton DF, McEver RP: P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. J Cell Biol 128:661, 1995

72. McEver RP, Moore KL, Cummings RD: Leukocyte trafficking mediated by selectin-carbohydrate interactions. J Biol Chem 270:11025, 1995

73. Norman KE, Moore KL, McEver RP, Ley K: Leukocyte rolling in vivo is mediated by p-selectin glycoprotein ligand-1. Blood 86:4417, 1995

74. Ley K, Bullard DC, Arbones ML, Bosse R, Vestweber D, Tedder TF, Beaudet AL: Sequential contribution of L- and P-selectin to leukocyte rolling in vivo. J Exp Med 181:669, 1995

75. Waddell TK, Fialkow L, Chan CK, Kishimoto TK, Downey GP: Potentiation of the oxidative burst of human neutrophils—A signaling role for l-selectin. J Biol Chem 269:18485, 1994

76. Crockett-Torabi E, Fantone JC: The selectins: Insights into selectin-induced intracellular signaling in leukocytes. Immunol Res 14:237, 1995

77. Jeannin P, Delneste Y, Gosset P, Molet S, Lassalle P, Hamid Q, Tsicopoulos A, Tonnel AB: Histamine induces interleukin-8 secretion by endothelial cells. Blood 84:2229, 1994

78. Patel KD, Modur V, Zimmerman GA, Prescott SM, McIntyre TM: The necrotic venom of the brown recluse spider induces dysregulated endothelial cell-dependent neutrophil activation—Differential induction of GM-CSF, IL-8, and E- selectin expression. J Clin Invest 94:631, 1994

79. Hughes BJ, Hollers JC, Crockett-Torabi E, Smith CW: Recruitment of CD11b/CD18 to the neutrophil surface and adherence-dependent cell locomotion. J Clin Invest 90:1687, 1992

80. Borregaard N, Kjeldsen L, Sengeløv H, Diamond MS, Springer TA, Anderson HC, Bainton DF, Kishimoto TK: Changes in the subcellular localization and surface expression of L-selectin, alkaline phosphatase, and Mac-1 in human neutrophils during stimulation with inflammatory mediators. J Leukoc Biol 56:80, 1994

81. von Andrian UH, Chambers DJ, McEnvoy LM, Bargatze RF, Arfors K-E, Butcher EC: Two-step model of leukocyte endothelial cell interaction in inflammation: Distinct roles for LECAM-1 and the leukocyte β_2 integrins in vivo. Proc Natl Acad Sci USA 88:7538, 1991

82. Petersen M, Williams JD, Hallett MB: Cross-linking of CD11b or CD18 signals release of localized Ca^{2+} from intracellular stores in neutrophils. Immunology 80:157, 1993

83. Pavalko FM, Laroche SM: Activation of human neutrophils induces an interaction between the integrin-beta-2-subunit (CD18) and the actin binding protein alpha-actinin. J Immunol 151:3795, 1993

84. Roberts PJ, Pizzey AR, Khwaja A, Carver JE, Mire-Sluis AR, Linch DC: The effects of interleukin-8 on neutrophil fMetLeuPhe receptors, CD11b expression and metabolic activity, in comparison and combination with other cytokines. Br J Haematol 84:586, 1993

85. Ng-Sikorski J, Anderson R, Patarroyo M, Andersson T: Calcium signalling of the CD11b/CD18 integrin of human neutrophils. Exp Cell Res 195:504, 1991

86. Gadd SJ, Eher R, Majdic O, Knapp W: Signal transduction via Fc gamma R and Mac-1 alpha-chain in monocytes and polymorphonuclear leucocytes. Immunology 81:611, 1994

87. Zhou MJ, Brown EJ: CR3 (mac-1, α(m)β(2), CD11b/CD18) and Fc gamma RIII cooperate in generation of a neutrophil respiratory burst: Requirement for Fc gamma RII and tyrosine phosphorylation. J Cell Biol 125:1407, 1994

88. Roth D, Burgoyne RD: SNAP-25 is present in a SNARE complex in adrenal chromaffin cells. FEBS Lett 351:207, 1994

89. Petersen MM, Steadman R, Williams JD: Human neutrophils are selectively activated by independent ligation of the subunits of the CD11b/CD18 integrin. J Leukoc Biol 56:708, 1994

90. Crockett-Torabi E, Sulenbarger B, Smith CW, Fantone JC: Activation of human neutrophils through L-selectin and mac-1 molecules. J Immunol 154:2291, 1995

91. Simon SI, Burns AR, Taylor AD, Gopalan PK, Lynam EB, Sklar LA, Smith CW: L-selectin (CD62l) cross-linking signals neutrophil adhesive functions via the mac-1 (CD11b/CD18) beta(2)-integrin. J Immunol 155:1502, 1995

92. Liles WC, Ledbetter JA, Waltersdorph AW, Klebanoff SJ: Cross-linking of CD18 primes human neutrophils for activation of the respiratory burst in response to specific stimuli: Implications for adhesion-dependent physiological responses in neutrophils. J Leukoc Biol 58:690, 1995

93. Zheng LM, Sjolander A, Eckerdal J, Andersson T: Antibody-induced engagement of beta(2) integrins on adherent human neutrophils triggers activation of p21(ras) through tyrosine phosphorylation of the protooncogene product Vav. Proc Natl Acad Sci USA 93:8431, 1996

94. Murphy G, Ward R, Hembry RM, Reynolds JJ, Kühn K, Tryggvason K: Characterization of gelatinase from pig polymorphonuclear leucocytes. A metalloproteinase resembling tumor type IV collagenase. Biochem J 258:463, 1989

95. Hibbs MS, Hasty KA, Seyer JM, Kang AH, Mainardi CL: Biochemical and immunological characterization of the secreted forms of human neutrophil gelatinase. J Biol Chem 260:2493, 1985

96. Murphy G, Reynolds JJ, Bretz U, Baggiolini M: Partial purification of collagenase and gelatinase from human polymorphonuclear leucocytes. Biochem J 203:209, 1982

97. Delclaux C, Delacourt C, d'Ortho MP, Boyer V, Lafuma C, Harf A: Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. Am J Respir Cell Mol Biol 14:288, 1996

98. Leppert D, Waubant E, Galardy R, Bunnett NW, Hauser SL: T cell gelatinases mediate basement membrane transmigration in vitro. J Immunol 154:4379, 1995

99. Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA: Effects of leukotriene B_4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. J Clin Invest 84:1609, 1989

100. Bakowski B, Tschesche H: Migration of polymorphonuclear leukocytes through human amnion membrane—A scanning electron microscopic study. Biol Chem Hoppe Seyler 373:529, 1992

101. Breton-Gorius J, Mason DY, Buriot D, Vilde J-L, Griscelli C: Lactoferrin deficiency as a consequence of a lack of specific granules in neutrophils from a patient with recurrent infections. Am J Pathol 99:413, 1980

102. Gallin JI: Neutrophil specific granule deficiency. Annu Rev Med 36:263, 1985

103. Johnston JJ, Boxer LA, Berliner N: Correlation of messenger RNA levels with protein defects in specific granule deficiency. Blood 80:2088, 1992

104. Bainton DF, Miller LJ, Kishimoto TK, Springer TA: Leukocyte adhesion receptors are stored in peroxidase-negative granules of human neutrophils. J Exp Med 166:1641, 1987

105. Jesaitis A, Buescher ES, Harrison D, Quinn MT, Parkos CA, Livesey S, Linner J: Ultrastructural localization of cytochrome b in

the membrane of resting and phagocytosing human granulocytes. J Clin Invest 85:821, 1990

106. Ginsel LA, Onderwater JJM, Fransen JAM, Verhoeven AJ, Roos D: Localization of the low-M_r subunit of cytochrome b_{558} in human blood phagocytes by immunoelectron microscopy. Blood 76:2105, 1990

107. Mollinedo F, Schneider DL: Subcellular localization of cytochrome b and ubiquinone in a tertiary granule of resting human neutrophils and evidence for a proton pump ATPase. J Biol Chem 259:7143, 1984

108. Lacal P, Pulido R, Sánchez-Madrid F, Mollinedo F: Intracellular location of T200 and Mo1 glycoproteins in human neutrophils. J Biol Chem 263:9946, 1988

109. Graves V, Gabig T, McCarthy L, Strour EF, Leemhuis T, English D: Simultaneous mobilization of Mac-1 (CD11b/CD18) and formyl peptide chemoattractant receptors in human neutrophils. Blood 80:776, 1992

110. Borregaard N, Lollike K, Kjeldsen L, Sengeløv H, Bastholm L, Nielsen MH, Bainton DF: Human neutrophil granules and secretory vesicles. Eur J Haematol 51:187, 1993

111. Hasty KA, Hibbs MS, Kang AH, Mainardi CL: Secreted forms of human neutrophil collagenase. J Biol Chem 261:5645, 1986

112. Weiss SJ, Peppin G, Ortiz X, Ragsdale C, Test ST: Oxidative autoactivation of latent collagenase by human neutrophils. Science 227:747, 1985

113. Knauper V, Murphy G, Tschesche H: Activation of human neutrophil procollagenase by stromelysin 2. Eur J Biochem 235:187, 1996

114. Egesten A, Breton-Gorius J, Guichard J, Gullberg U, Olsson I: The heterogeneity of azurophil granules in neutrophil promyelocytes: Immunogold localization of myeloperoxidase, cathepsin G, elastase, proteinase 3, and bactericidal permeability increasing protein. Blood 83:2985, 1994

115. Triebel S, Bläser J, Reinke H, Tschesche H: A 25 kDa $alpha_2$-microglobulin-related protein is a component of the 125 kDa form of human gelatinase. FEBS Lett 314:386, 1992

116. Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N: Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 268:10425, 1993

117. Cowland JB, Johnsen AH, Borregaard N: hCAP-18, a cathelin/bactenecin like protein of human neutrophil specific granules. FEBS Lett 368:173, 1995

118. Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC: Human CAP18: A novel antimicrobial lipopolysaccharide-binding protein. Infect Immun 63:1291, 1995

119. Agerberth B, Gunne H, Odeberg J, Kogner P, Boman HG, Gudmundsson GH: Fall-39, a putative human peptide antibiotic, as cysteine-free and expressed in bone marrow and testis. Proc Natl Acad Sci USA 92:195, 1995

120. Flower DR: The lipocalin protein family: Structure and function. Biochem J 318:1, 1996

121. Cowan SW, Newcomer ME, Jones TA: Crystallographic refinement of human serum retinol binding protein at 2Å resolution. Proteins 8:44, 1990

122. Åkerström B, Lögdberg L: An intriguing member of the lipocalin protein family: $Alpha_1$-microglobulin. Trends Biochem Sci 15:240, 1990

123. Hraba-Renevey S, Türler H, Kress M, Salomon C, Weil R: SV40-induced expression of mouse gene 24p3 involves a post-transcriptional mechanism. Oncogene 4:601, 1989

124. Flower DR, North ACT, Attwood TK: Mouse oncogene protein 24p3 is a member of the lipocalin protein family. Biochem Biophys Res Commun 180:69, 1991

125. Stoesz SP, Gould MN: Overexpression of neu-related lipocalin (NRL) in neu-initiated but not ras or chemically initiated rat mammary carcinomas. Oncogene 11:2233, 1995

126. Kjeldsen L, Bainton DF, Sengeløv H, Borregaard N: Identification of NGAL as a novel matrix protein of specific granules in human neutrophils. Blood 83:799, 1994

127. Xu SY, Varlson M, Engström Å, Peterson CGB, Venge P: Purification and characterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils. Scand J Clin Lab Invest 54:365, 1994

128. Nielsen BS, Borregaard N, Bundgaard JR, Timshel S, Sehested M, Kjeldsen L: Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel disease. Gut 38:414, 1996

129. Axelsson L, Bergenfeldt M, Ohlsson K: Studies of the release and turnover of a human neutrophil lipocalin. Scand J Clin Lab Invest 55:577, 1995

130. Allen RA, Erickson RW, Jesaitis AJ: Identification of a human neutrophil protein of M_r 24000 that binds N-formyl peptides: Co-sedimentation with specific granules. Biochim Biophys Acta 991:123, 1989

131. Sengeløv H, Boulay F, Kjeldsen L, Borregaard N: Subcellular localization and translocation of the receptor for N-formyl-methionyl-leucyl-phenylalanine in human neutrophils. Biochem J 299:473, 1994

132. Gudmundsson GH, Agerberth B, Odeberg J, Bergman T, Olsson B, Salcedo R: The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. Eur J Biochem 238:325, 1996

133. Zanetti M, Gennaro R, Romeo D: Cathelicidins: A novel protein family with a common proregion and a variable c-terminal antimicrobial domain. FEBS Lett 374:1, 1995

134. Levy O: Antibiotic proteins of polymorphonuclear leukocytes. Eur J Haematol 56:263, 1996

135. Romeo D, Skerlavaj B, Bolognesi M, Gennaro R: Structure and bactericidal activity of an antibiotic dodecapeptide purified from bovine neutrophils. J Biol Chem 263:9573, 1988

136. Ritonja A, Kopitar M, Jerala R, Turk V: Primary structure of a new cysteine proteinase inhibtor from pig leucocytes. FEBS Lett 255:211, 1989

137. Pungercar J, Strukelj B, Kopitar G, Renko M, Lenarcic B, Gubensek F, Turk V: Molecular cloning of a putative homolog of proline/arginine-rich antibacterial peptides from porcine bone marrow. FEBS Lett 336:284, 1993

138. Harwig SSL, Kokryakov VN, Swiderek KM, Aleshina GM, Zhao CQ, Lehrer RI: Prophenin-1, an exceptionally proline-rich antimicrobial peptide from porcine leukocytes. FEBS Lett 362:65, 1995

139. Agerberth B, Lee J-Y, Bergman T, Carlquist M, Boman HG, Mutt V, Jörnvall H: Amino acid sequence of PR-39. Isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides. Eur J Biochem 202:849, 1991

140. Zanetti M, Litteri L, Griffiths G, Gennaro R, Romeo D: Stimulus-induced maturation of probactenecins, precursors of neutrophil antimicrobial polypeptides. J Immunol 146:4295, 1991

141. Scocchi M, Skerlavaj B, Romeo D, Gennaro R: Proteolytic cleavage by neutrophil elastase converts inactive storage proforms to antibacterial bactenecins. Eur J Biochem 209:589, 1992

142. Radermacher SW, Schoop VM, Schleuesener HJ: Bactenecin, a leukocytic antimicribial peptide, is cytotoxic to neuronal and glial cells. J Neurosci Res 36:657, 1993

143. Kasahara M, Gutknecht J, Brew K, Spurr N, Goodfellow PN: Cloning and mapping of a testis-specific gene with sequence similarity to a sperm-coating glycoprotein gene. Genomics 5:527, 1989

144. Brooks DE, Means AR, Wright EJ, Singh SP, Tiver KK: Molecular cloning of the cDNA for androgen-dependent sperm-coat-

ing glycoprotein secreted by the rat epididymis. Eur J Biochem 161:13, 1986

145. Krätzschmar J, Haendler B, Eberspaecher U, Roosterman D, Donner P, Schleuning W-D: The human cysteine-rich secretory protein (CRISP) family. Primary structure and tissue distribution of CRISP-1, CRISP-2, and CRISP-3. Eur J Biochem 236:827, 1996

146. Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinogu S, Kauffmann S, Geoffroy P, Legrand M, Fritig B: Plant ''pathogenesis-related'' proteins and their role in defense against pathogens. Biochemie 78:687, 1993

147. Weiss SJ, Klein R, Slivka A, Wei M: Chlorination of taurine by human neutorphils. Evidence for hypochlorous acid generation. J Clin Invest 70:598, 1982

148. Peppin GJ, Weiss SJ: Activation of the endogenous metalloproteinase, gelatinase, by triggered human neutrophils. Proc Natl Acad Sci USA 83:4322, 1986

149. Cromwell O, Bennett JP, Hide I, Kay AB, Gomperts BD: Mechanism of granule enzyme secretion from permeabilized guinea pig eosinphils. J Immunol 147:1905, 1991

150. Regazzi R, Wollheim CB, Lang J, Theler JM, Rossetto O, Montecucco C, Sadoul K, Weller U, Palmer M, Thorens B: VAMP-2 and cellubrevin are expressed in pancreatic beta-cells and are essential for Ca^{2+}- but not for GTP gamma S-induced insulin secretion. EMBO J 14:2723, 1995

151. Jahn R, Südhof TC: Synaptic vesicles and exocytosis. Annu Rev Neurosci 17:219, 1994

152. Hay JC, Fisette PL, Jenkins GH, Fukami K, Takenawa T, Anderson RA, Martin TFJ: ATP-dependent inositide phosphorylation required for Ca^{2+}-activated secretion. Nature 374:173, 1995

153. Perez HD, Marder S, Elfman F, Ives HE: Human neutrophils contain subpopulations of specific granules exhibiting different sensitivities to changes in cytosolic free calcium. Biochem Biophys Res Commun 145:976, 1987

154. Borregaard N, Kjeldsen L, Lollike K, Sengeløv H: Ca^{2+}-dependent translocation of cytosolic proteins to isolated granule subpopulations and plasma membrane from human neutrophils. FEBS Lett 304:195, 1992

155. Sjölin C, Stendahl O, Dahlgren C: Calcium-induced translocation of annexins to subcellular organelles of human neutrophils. Biochem J 300:325, 1994

156. Le Cabec V, Maridonneau-Parini I: Annexin 3 is associated with cytoplasmic granules in neutrophils and monocytes and translocates to the plasma membrane in activated cells. Biochem J 303:481, 1994

157. Ernst JD: Annexin III translocates to the periphagosomal region when neutrophils ingest opsonized yeast. J Immunol 148:3110, 1991

158. Kaufman M, Leto T, Levy R: Translocation of annexin I to plasma membranes and phagosomes in human neutrophils upon stimulation with opsonized zymosan: Possible role in phagosome function. Biochem J 316:35, 1996

159. Sjölin C, Dahlgren C: Diverse effects of different neutrophil organelles on truncation and membrane-binding characteristics of annexin I. Bba Biomembranes 1281:227, 1996

160. Sjölin C, Dahlgren C: Isolation by calcium-dependent translocation to neutrophil-specific granules of a 42-kD cytosolic protein, identified as being a fragment of annexin XI. Blood 87:4817, 1996

161. Lollike K, Sørensen O, Bundgaard JR, Segal AW, Boyhan A, Borregaard N: An ELISA for grancalcin, a novel cytosolic calcium-binding protein present in leukocytes. J Immunol Methods 185:1, 1995

162. Söllner T, Whiteheart SW, Brunner M, Erdjument-Bromage H, Geromanos S, Tempst P, Rothman JE: SNAP receptors implicated in vesicle targeting and fusion. Nature 362:318, 1993

163. Rothman JE: The protein machinery of vesicle budding and fusion. Protein Sci 5:185, 1996

164. Söllner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE: A protein assembly-disassembly pathway invitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75:409, 1993

165. Südhof TC, De Camilli P, Niemann H, Jahn R: Membrane fusion machinery: Insights from synaptic proteins. Cell 75:1, 1993

166. Südhof TC: The synaptic vesicle cycle: A cascade of protein-protein interactions. Nature 375:645, 1995

167. McMahon HT, Ushkaryov YA, Edelmann L, Link E, Binz T, Niemann H, Jahn R, Südhof TC: Cellubrevin is a ubiquitous tetanus-toxin substrate homologous to a pupative synaptic vesicle fusion protein. Nature 364:346, 1993

168. Blasi J, Chapman ER, Link E, Binz T, Yamasaki S, De Camilli P, Südhof TC, Niemann H, Jahn R: Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25. Nature 365:160, 1993

169. Hayashi T, Mcmahon H, Yamasaki S, Binz T, Hata Y, Südhof TC, Niemann H: Synaptic vesicle membrane fusion complex: Action of clostridial neurotoxins on assembly. EMBO J 13:5051, 1994

170. Brumell JH, Volchuk A, Sengeløv H, Borregaard N, Cieutat AM, Bainton DF, Grinstein S, Klip A: Subcellular distribution of docking/fusion proteins in neutrophils, secretory cells with multiple exocytic compartments. J Immunol 155:5750, 1995

171. Vogel SS, Blank PS, Zimmerberg J: Poisson-distributed active fusion complexes underlie the control of the rate and extent of exocytosis by calcium. J Cell Biol 134:329, 1996

172. Bauldry SA, Wooten RE, Bass DA: Activation of cytosolic phospholipase a(2) in permeabilized human neutrophils. Bba Lipid Metab 1299:223, 1996

173. Nüsse O, Lindau M: The calcium signal in human neutrophils and its relation to exocytosis investigated by patch-clamp capacitance and Fura-2 measurements. Cell Calcium 14:255, 1993

174. Philips MR, Abramson SB, Kolasinski SL, Haines KA, Weissmann G, Rosenfeld MG: Low molecular weight GTP-binding proteins in human neutrophil granule membranes. J Biol Chem 266:1289, 1991

175. Nüße O, Lindau M: The dynamics of exocytosis in human neutrophils. J Cell Biol 107:2117, 1988

176. Lollike K, Borregaard N, Lindau M: The exocytotic fusion pore of small granules has a conductance similar to an ion channel. J Cell Biol 129:99, 1995

177. Bainton DF: Sequential degranulation of the two types of polymorphonuclear leukocyte granules during phagocytosis of microorganisms. J Cell Biol 58:249, 1973

178. Leffell MS, Spitznagel JK: Intracellular and extracellular degranulation of human polymorphonuclear azurophil and specific granules induced by immune complexes. Infect Immun 10:1241, 1974

179. Joiner KA, Ganz T, Albert J, Rotrosen D: The opsonizing ligand on Salmonella typhimurium influences incorporation of specific, but not azurophil, granule constituents into neutrophil phagosomes. J Cell Biol 109:2771, 1989

180. Dahlgren C, Carlsson SR, Karlsson A, Lundqvist H, Sjölin C: The lysosomal membrane glycoproteins lamp-1 and lamp-2 are present in mobilizable organelles, but are absent from the azurophil granules of human neutrophils. Biochem J 311:667, 1995

181. Cieutat A-M, Lobel P, August JT, Kjeldsen L, Sengeløv H, Borregaard N, Bainton DF: Azurophil granules of human neutrophilic leukocytes are deficient in lysosome-associated membrane proteins but retain the mannose 6-phosphate recognition marker. (submitted)

182. Berger M, Wetzler E, August JT, Tartakoff AM: Internaliza-

tion of type 1 complement receptors and de novo multivesicular body formation during chemoattractant-induced endocytosis in human neutrophils. J Clin Invest 94:1113, 1994

183. Yoo SH: pH- and Ca^{2+}-dependent aggregation property of secretory vesicle matrix proteins and the potential role of chromogranins a and b in secretory vesicle biogenesis. J Biol Chem 271:1558, 1996

184. Ma YH, Lores P, Wang J, Jami J, Grodsky GM: Constitutive (pro)insulin release from pancreas of transgenic mice expressing monomeric insulin. Endocrinology 136:2622, 1995

185. Kuliawat R, Arvan P: Distinct molecular mechanisms for protein sorting within immature secretory granules of pancreatic β-cells. J Cell Biol 126:77, 1992

186. Milgram SL, Eipper BA, Mains RE: Differential trafficking of soluble and integral membrane secretory granule-associated proteins. J Cell Biol 124:33, 1994

187. Dahms NM, Lobel P, Kornfeld S: Mannose 6-phosphate receptors and lysosomal enzyme targeting. J Biol Chem 264:12115, 1989

188. Hasilik A, Pohlmann R, Olsen RL, von Figura K: Myeloperoxidase is synthesized as larger phosphorylated precursor. EMBO J 3:2671, 1984

189. Castañón MJ, Spevak W, Adolf GR, Chlebowicz-Sledziewska E, Sledziewski A: Cloning of human lysozyme gene and expression in the yeast *Saccharomyces cerevisiae.* Gene 66:223, 1988

190. Michaelson D, Rayner J, Couto M, Ganz T: Cationic defensins arise from charge-neutralized propeptides: A mechanism for avoiding leukocyte autocytotoxicity. J Leukoc Biol 51:634, 1992

191. Nauseef WM, McCormick SJ, Clark RA: Calreticulin functions as a molecular chaperone in the biosynthesis of myeloperoxidase. J Biol Chem 270:4741, 1995

192. Gorr SU, Darling DS: An N-terminal hydrophobic peak is the sorting signal of regulated secretory proteins. FEBS Lett 361:8, 1995

193. Ganz T, Liu L, Valore EV, Oren A: Posttranslational processing and targeting of transgenic human defensin in murine granulocyte, macrophage, fibroblast, and pituitary adenoma cell lines. Blood 82:641, 1993

194. Gullberg U, Lindmark A, Lindgren G, Persson AM, Nilsson E, Olsson I: Carboxyl-terminal prodomain-deleted human leukocyte elastase and cathepsin G are efficiently targeted to granules and enzymatically activated in the rat basophilic mast cell line RBL. J Biol Chem 270:12912, 1995

195. Garwicz D, Lindmark A, Gullberg U: Human cathepsin G lacking functional glycosylation site is proteolytically processed and targeted for storage in granules after transfection to the rat basophilic/mast cell line RBL or the murine myeloid cell line 32D. J Biol Chem 270:28413, 1995

196. Scepek S, Lindau M: Exocytotic competence and intergranular fusion in cord blood derived eosinophils during differentiation. Blood 89:510, 1997

197. Milgram SL, Mains RE, Eipper BA: Identification of routing determinants in the cytosolic domain of a secretory granule-associated integral membrane protein. J Biol Chem 271:17526, 1996

198. Subramaniam M, Koedam JA, Wagner DD: Divergent fates of P- and E-selectins after their expression and the plasma membrane. Mol Biol Cell 4:791, 1993

199. Disdier M, Morrissey JH, Fugate RD, Bainton DF, McEver RP: Cytoplasmic domain of P-selectin (CD62) contains the signal for sorting into the regulated secretory pathway. Mol Biol Cell 3:309, 1992

200. Borregaard N: Current opinion about neutrophil granule physiology. Curr Opin Hematol 3:11, 1996

201. Le Cabec V, Cowland JB, Calafat J, Borregaard N: Targeting of proteins to granule subsets determined by timing not by sorting: the specific granule protein NGAL is localized to azurophil granules when expressed in HL-60 cells. Proc Natl Acad Sci USA 93:6454, 1996

202. Tsuruta T, Tani K, Shimane M, Ozawa K, Takahashi S, Tsuchimoto D, Takahashi K, Nagata S, Sato N, Asano S: Effects of myeloid cell growth factors on alkaline phosphatase, myeloperoxidase, defensin and granulocyte colony-stimulating factor receptor mRNA expression in haemopoietic cells of normal individuals and myeloid disorders. Br J Haematol 92:9, 1996

203. Segel EK, Ellegaard J, Borregaard N: Development of the phagocytic and cidal capacity during maturation of myeloid cells: Studies on cells from patients with chronic myelogenous leukemia. Br J Haematol 67:3, 1987

204. Rosmarin AG, Weil SC, Rosner GL, Griffin JD, Arnaout MA, Tenen DG: Differential expression of CD11b/CD18 (Mo1) and myeloperoxidase genes during myeloid differentiation. Blood 73:131, 1989

205. Cully J, Harrach B, Hauser H, Harth N, Robenek H, Nagata S, Hasilik A: Synthesis and localization of myeloperoxidase protein in transfected BHK cells. Exp Cell Res 180:440, 1989

206. Liu L, Ganz T: The pro region of human neutrophil defensin contains a motif that is essential for normal subcellular sorting. Blood 85:1095, 1995

207. Shapiro SD, Fliszar CJ, Broekelmann TJ, Mecham RP, Senior RM, Welgus HG: Activation of the 92 kDa gelatinase by stromelysin and 4-aminophenylmercuric acetate. J Biol Chem 270:6351, 1995

208. Segal AW: The NADPH oxidase of phagocytic cells is an electron pump that alkalinizes the phagocytic vacuole. Protoplasma 184:86, 1995

209. Khannagupta A, Kolibaba K, Zibello TA, Berliner N: NB4 cells show bilineage potential and an aberrant pattern of neutrophil secondary granule protein gene expression. Blood 84:294, 1994

210. Johnston JJ, Rintels P, Chung J, Sather J, Benz EJ Jr, Berliner N: Lactoferrin gene promoter: Structual integrity and nonexpression in HL60 cells. Blood 79:2998, 1992

211. Hirata RK, Chen S-T, Weil SC: Expression of granule protein mRNA in acute promyelocytic leukemia. Hematol Pathol 7:225, 1993

212. Newburger PE, Speier C, Borregaard N, Walsh CE, Whitin JC, Simons ER: Development of the superoxide-generating system during differentiation of the HL-60 human promyelocytic leukemia cell line. J Biol Chem 259:3771, 1984

213. Le Cabec V, Calafat J, Borregaard N: Sorting of the specific granule protein, NGAL during granulocytic maturation of HL-60 cells. Blood 89:2117, 1997

214. Brion C, Miller SG, Moore H-PH: Regulated and constitutive secretion. Differential effects of protein synthesis arrest on transport of glycosaminoglycan chains to the two secretory pathways. J Biol Chem 267:1477, 1992

215. Poo M, Dan Y, Song H, Morimoto T, Popov S: Calcium-dependent vesicular exocytosis: From constitutive to regulated secretion. Cold Spring Harb Symp Quant Biol 60:349, 1995

216. Borregaard N, Boxer LA, Smolen JE, Tauber AI: Anomalous neutrophil granule distribution in a patient with lactoferrin deficiency: Pertinence to the respiratory burst. Am J Hematol 18:255, 1985

217. Hamanaka SC, Gilbert CS, White DA, Parmley RT: Ultrastructural morphology, cytochemistry, and morphometry of eosinophil granules in Chédiak-Higashi syndrome. Am J Pathol 143:618, 1993

218. Usha HN, Pradhu PD, Sridevi M, Baindur K, Balakrishnan CM: Chédiak-Higashi Syndrome. Indian Pediatr 31:1115, 1994

219. Barbosa MDFS, Nguyen QA, Tchernev VT, Ashley JA, Detter JC, Blades SM, Brandt SJ, Chotai D, Hodgman C, Solari RCE,

Lovett M, Kingsmore SF: Identification of the homologous beige and Chediak-Higashi syndrome genes. Nature 382:262, 1996

220. Brown EJ, Schreiber SL: A signaling pathway to translational control. Cell 23:517, 1996

221. Nielsen FC, Østergaard L, Nielsen J, Christiansen J: Growth-dependent translation of IGF-II mRNA by a rapamycin-sensitive pathway. Nature 377:358, 1995

222. Quinn MT, Parkos CA, Walker L, Orkin SH, Dinauer MC, Jesaitis AJ: Association of a Ras-related protein with cytochrome b of human neutrophils. Nature 342:198, 1989

223. Hu Z-B, Ma W, Uphoff CC, Metge K, Gignac SM, Drexler HG: Myeloperoxidase: Expression and modulation in a large panel of human leukemia-lymphoma cell lines. Blood 82:1599, 1993

224. Berliner N, Hsing A, Graubert T, Sigurdsson F, Zain M, Bruno E, Hoffman R: Granulocyte colony-stimulating factor induction of normal human bone marrow progenitors results in neutrophil-specific gene expression. Blood 85:799, 1995

225. Date Y, Nakazoto M, Shiomi K, Toshimori H, Kangawa K, Matsuo H, Matsukura S: Localization of human neutrophil peptide (HNP) and its messenger RNA in neutrophil series. Ann Hematol 69:73, 1994

226. Mars WM, Stellrecht CM, Stass S, Frazier ML, Saunders GF: Localization of an abundant myeloid mRNA to individual leukocytes in mixed cell populations. Leukemia 1:167, 1987

227. Nagaoka I, Yomogida S, Nakamura S, Iwabuchi K, Yamashita T: Evaluation of the expression of the cationic peptide gene in various types of leukocytes. FEBS Lett 302:279, 1992

228. Herwig S, Su Q, Zhang W, Ma YS, Tempst P: Distinct temporal patterns of defensin mRNA regulation during drug-induced differentiation of human myeloid leukemia cells. Blood 87:350, 1996

229. Rado TA, Bollekens J, St Laurent G, Parker L, Benz EJ: Lactoferrin biosynthesis during granulocytopoiesis. Blood 64:1103, 1984

230. Rado TA, Wei X, Benz EJ Jr: Isolation of lactoferrin cDNA from a human myeloid library and expression of mRNA during normal and leukemic myelopoiesis. Blood 70:989, 1987

231. Graubert T, Johnston J, Berliner N: Cloning and expression of the cDNA encoding mouse neutrophil gelatinase—Demonstration of coordinate secondary granule protein gene expression during terminal neutrophil maturation. Blood 82:3192, 1993

232. Austin GE, Zhao W-G, Zhang W, Austin ED, Findley HW, Murtagh JJ: Identification and characterization of the human myeloperoxidase promoter. Leukemia 9:848, 1995

233. Takahashi H, Nukiwa T, Yoshimura K, Quick CD, States DJ, Holmes MD, Whang-Peng J, Knutsen T, Crystal RG: Structure of the human neutrophil elastase gene. J Biol Chem 263:14739, 1988

234. Srikanath S, Rado TA: A 30-base pair element is responsible for the myeloid-specific activity of the human neutrophil elastase promoter. J Biol Chem 269:32626, 1995

235. Hohn P, Popescu NC, Hanson RD, Salvesen G, Ley TJ: Genomic organization and chromosomal localization of the human cathepsin G gene. J Biol Chem 264:13412, 1989

236. Grisolano JL, Sclar GM, Ley TJ: Early myeloid cell-specific expression of the human cathepsin G gene in transgenic mice. Proc Natl Acad Sci USA 91:8989, 1994

237. Sturrock AB, Franklin KF, Rao G, Marshall BC, Rebentisch MB, Lemons RS, Hoidal JR: Structure, chromosomal assignment, and expression of the gene for proteinase-3. The Wegener's granulomatosis autoantigen. J Biol Chem 267:21193, 1992

238. Clarke S, Greaves DR, Chung LP, Tree P, Gordon S: The human lysozyme promoter directs reporter gene expression to activated myelomonocytic cells in transgenic mice. Proc Natl Acad Sci USA 93:1434, 1996

239. Linzmeier R, Michaelson D, Liu L, Ganz T: The structure of neutrophil defensin genes. FEBS Lett 321:267, 1993

240. Skalnik DG, Strauss EC, Orkin SH: CCAAT displacement protein as a repressor of the myelomonocytic-specific gp91-phox gene promoter. J Biol Chem 266:16736, 1991

241. Newburger PE, Skalnik DG, Hopkins PJ, Eklund EA, Curnutte JT: Mutations in the promoter region of the gene for gp91-phox in x-linked chronic granulomatous disease with decreased expression of cytochrome b(558). J Clin Invest 94:1205, 1994

242. Luo W, Skalnik DG: CCAAT displacement protein competes with multiple transcriptional activators for binding to four sites in the proximal gp91(phox) promoter. J Biol Chem 271:18203, 1996

243. Luo W, Skalnik DG: Interferon regulatory factor-2 directs transcription from the gp91(phox) promoter. J Biol Chem 271:23445, 1996

244. Dinauer MC, Pierce EA, Bruns GAP, Curnutte JT, Orkin SH: Human neutrophil cytochrome b light chain (p22-phox). Gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. J Clin Invest 86:1729, 1990

245. Back A, East K, Hickstein D: Leukocyte integrin CD11b promoter directs expression in lymphocytes and granulocytes in transgenic mice. Blood 85:1017, 1995

246. Dziennis S, Van Etten RA, Pahl HL, Morris DL, Rothstein TL, Blosch CM, Perlmutter RM, Tenen DG: The CD11b promoter directs high-level expression of reporter genes in macrophages in transgenic mice. Blood 85:319, 1995

247. Pahl HL, Rosmarin AG, Tenen DG: Characterization of the myeloid-specific CD11b promoter. Blood 79:865, 1992

248. Böttinger EP, Shelley CS, Farokhzad OC, Arnaout MA: The human beta 2 integrin CD18 promoter consists of two inverted ets cis-elements. Mol Cell Biol 14:2604, 1994

249. Rosmarin AG, Caprio D, Levy R, Simkevich C: CD18 (beta(2) leukocyte integrin) promoter requires PU.1 transcription factor for myeloid activity. Proc Natl Acad Sci USA 92:801, 1995

250. Srivastava M: Genomic structure and expression of the human gene encoding cytochrome b(561), an integral protein of the chromaffin granule membrane. J Biol Chem 270:22714, 1995

251. Johnston J, Yang-Feng T, Berliner N: Genomic structure and mapping of the chromosomal gene for transcobalamin I (TCN1): Comparison to human intrinsic factor. Genomics 12:459, 1992

252. Huhtale P, Tuuttila A, Chow LT, Lohi J, Keski-Oja J, Tryggvarson K: Complete structure of the human gene for 92-kDa type IV collagenase. Divergent regulation of expression for the 92- and 72-kilodalton enzyme genes in HT-1080 cells. J Biol Chem 266:16485, 1991

253. Gum R, Lengyel E, Juarez J, Chen JH, Sato H, Seiki M, Boyd D: Stimulation of 92-kDa gelatinase B promoter activity by ras is mitogen-activated protein kinase kinase 1-independent and requires multiple transcription factor binding sites including closely spaced PEA3/ets and AP-1 sequences. J Biol Chem 271:10672, 1996

254. Weiss MJ, Ray K, Henthorn PS, Lamb B, Kadesch T, Harris H: Structure of the human liver/bone/kidney alkaline phosphatase gene. J Biol Chem 263:12002, 1988

255. Roos D, Deboer M, Kuribayashi F, Meischl C, Weening RS, Segal AW, Ahlin A, Nemet K, Hossle JP, Bernatowska-Matuszkiewicz E, Middleton-Price H: Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. Blood 87:1663, 1996

256. Austin GE, Zhao WG, Zhang W, Austin ED, Findley HW, Murtagh JJ: Identification and characterization of the human myeloperoxidase promoter. Leukemia 9:848, 1995

257. Zhao WG, Regmi A, Austin ED, Braun JE, Racine M, Austin GE: Cis-elements in the promoter region of the human myeloperoxidase (MPO) gene. Leukemia 10:1089, 1996

258. Yoshimura K, Chu CS, Crystal RG: Enhancer function of a 53-bp repetitive element in the 5′ flanking region of the human

neutrophil elastase gene. Biochem Biophys Res Commun 204:38, 1994

259. Hickstein D, Baker DM, Gollahon KA, Back AL: Identification of the promoter of the myelomonocytic leukocyte integrin CD11b. Proc Natl Acad Sci USA 89:2105, 1992

260. Kinashi T, Springer TA: Steeel factor and c-kit regulate cell-matrix adhesion. Blood 83:1033, 1994

261. Eklund EA, Skalnik DG: Characterization of a gp91-phox promoter element that is required for interferon gamma-induced transcription. J Biol Chem 270:8267, 1995

262. Sposi NM, Zon LI, Care A, Valtieri M, Testa U, Gabbianelli M, Mariani G, Bottero L, Mather C, Orkin S, Peschle C: Cell cycle-dependent initiation and lineage-dependent abrogation of GATA-1 expression in pure differentiating hematopoietic progenitors. Proc Natl Acad Sci USA 89:6353, 1992

263. Labbaye C, Valtieri M, Barberi T, Meccia E, Masella B, Pelosi E, Condorelli GL, Testa U, Peschle C: Differential expression and runctional of GATA-2, NF-E2, and GATA-1 in normal adult hematopoiesis. J Clin Invest 95:2346, 1995

264. Levanon D, Negreanu V, Bernstain Y, Bar-am I, Avivi L, Groner Y: AML1, AML2, and AML3, the human members of the runt domain gene-family: cDNA structure, expression, and chromosomal localization. Genomics 23:425, 1994

265. Tanaka T, Tanaka K, Ogawa S, Kurokawa M, Mitani K, Nishida J, Shibata Y, Yazaki Y, Hirai H: An acute myeloid gene, AML1, regulates hematopoietic myeloid cell differentiation and transcriptional activation antagonistically by two spliced forms. EMBO J 14:341, 1995

266. Frank R, Zhang J, Hiebert S, Meyers S, Nimer S: AML1 but not the AML1/ETO fusion protein can transactivate the GM-CSF promoter. Blood 84:229a, 1994 (abstr, suppl 1)

267. Nuchprayoon I, Meyers S, Scott LM, Suzow J, Hiebert S, Friedman AD: PEB2/CBF, the murine homolog of the human myeloid AML1 and PEB2β/CBFβ proto-oncoproteins, regulates the murine myeloperoxidase and neutrophil elastase genes in immature myeloid cells. Mol Cell Biol 14:5558, 1994

268. Suzow J, Friedman AD: The murine myeloperoxidase promoter contains several functional elements, one of which binds a cell type-restricted transcription factor, myloid neclear factor 1 (MyNF1). Mol Cell Biol 13:2141, 1993

269. Kastan MB, Stone KD, Civin CI: Nuclear oncoprotein expression as a function of lineage, differentiation stage, and proliferative status of normal human hematopoietic cells. Blood 74:1517, 1989

270. Duprey SP, Boettiger D: Developmental regulation of c-myb in normal myeloid progenitor cells. Proc Natl Acad Sci USA 82:6937, 1985

271. Westin EH, Gallo RC, Arya SK, Eva A, Souza LM, Baluda MA, Aaronson SA, Song-Staal F: Differential expression of the amv gene in human hematopoietic cells. Proc Natl Acad Sci USA 79:2194, 1982

272. Clarke MF, Kokowska-Latallo JF, Westin E, Smith M, Prochownik EV: Constitutive expression of a c-myb cDNA blocks Friend murine erythroleukemia cell differentiation. Mol Cell Biol 8:884, 1988

273. Burk O, Mink S, Ringwald M, Klempnauer KH: Synergistic activation of the chicken mim-1 gene by v-myb and C/EBP transcription factors. EMBO J 12:2027, 1993

274. Ness SA, Kowenz-Leutz E, Casini T, Graf T, Leutz A: Myb and NF-M: Combinatorial activators of myeloid genes in heterologous cell types. Genes Dev 7:749, 1993

275. Hromas R, Orazi A, Neiman RS, Maki R, Van Beveran C, Moore J, Klemsz M: Hematopoietic lineage- and stage-restricted expression of the ETS oncogene family member PU.1. Blood 82:2998, 1993

276. Chen HM, Zhang P, Voso MT, Hohaus S, Gonzalez DA, Glass CK, Zhang DE, Tenen DG: Neutrophils and monocytes express high levels of PU.1 (spi-1) but not spi-B. Blood 85:2918, 1995

277. Voso MT, Burn TC, Wulf G, Lim B, Leone G, Tenen DG: Inhibition of hematopoiesis by copetitive binding of transcrption factor PU.1. Proc Natl Acad Sci USA 91:7932, 1994

278. Chen HM, Ray Gallet D, Zhang P, Hetherington CJ, Gonzalez DA, Zhang DE, Moreau-Gachelin F, Tenen DG: PU.1 (spi-1) autoregulates its expression in myeloid cells. Oncogene 11:1549, 1995

279. Hohaus S, Petrovick MS, Voso MT, Sun T, Zhang DE, Tenen DG: PU.1 (Spi-1) and C/EBP alpha regulate expression of the granulocyte-macrophage colony-stimulating factor receptor alpha gene. Mol Cell Biol 15:5830, 1995

280. Smith LT, Gonzalez D, Tenen DG: The myeloid specific G-CSF receptor promoter conatins a functional site for the myeloid transcription factor PU.1 (Spi-1). Blood 84:372a, 1994 (abstr, suppl 1)

281. Pahl HL, Scheibe RJ, Zhang DE, Chen HM, Galson DL, Maki RA, Tenen DG: The proto-oncogene PU.1 regulates expression of the myeloid-specific CD11b promoter. J Biol Chem 268:5014, 1993

282. Rosmarin AG, Caprio DG, Kirsch DG, Handa H, Simkevich CP: GABP and PU.1 compete for binding, yet cooperate to increase CD18 (beta(2) leukocyte integrin) transcription. J Biol Chem 270:23627, 1995

283. Eichbaum QG, Iyer R, Raveh DP, Mathieu C, Ezekowitz RA: Restriction of interferon gamma responsiveness and basal expression of the myeloid human Fc gamma R1b gene is mediated by a functional PU.1 site and a transcription initiator consensus. J Exp Med 179:1985, 1994

284. Perez C, Coeffier E, Moreau-Gachelin F, Wietzerbin J, Benech PD: Involvement of the transcription factor PU.1/Spi-1 in myeloid cell-restricted expression of an interferon-inducible gene encoding the human high-affinity Fc gamma receptor. Mol Cell Biol 14:5023, 1994

285. Scott LM, Civin CI, Rorth P, Friedman AD: A novel temporal expression pattern of three C/EBP family members in differentiationg myelomonocytic cells. Blood 80:1725, 1992

286. Zhang DE, Hohaus S, Voso MT, Chen HM, Smith LT, Hetherington CJ, Tenen DG: Function of PU.1 (spi-1), C/EBP, and AML1 in early myelopoiesis: Regulation of multiple myelod CSF receptor promoters. Curr Top Microbiol Immunol 211:137, 1996

287. Khanna-Gupta A, Lawson N, Zibello T, Berliner N: Overexpression of CCAAT displacement protein (CDP/cut) in 32Dcl3 cells silences multiple secondary granule proteins. Blood 88:630a, 1996 (abstr, suppl 1)

288. Osada S, Yamamoto H, Nishihara T, Imagawa M: DNA binding specificity of the CCAAT/enhancer-binding protein transcription factor family. J Biol Chem 271:3891, 1996

289. Cham BP, Gerrard JM, Bainton DF: Granulophysin is located in the membrane of azurophilic granules in human neutrophils and mobilizes to the plasma membrane following cell stimulation. Am J Pathol 144:1369, 1994

290. Saito N, Pulford KAF, Breton-Gorius J, Massé J-M, Mason DY, Cramer EM: Ultrastructural localization of the CD68 macrophage-associated antigen in human blood neutrophils and monocytes. Am J Pathol 139:1053, 1991

291. Nanda A, Brumell JH, Nordstrom T, Kjeldsen L, Sengeløv H, Borregaard N, Rotstein OD, Grinstein S: Activation of proton pumping in human neutrophils occurs by exocytosis of vesicles bearing vacuolar-type H+-ATPases. J Biol Chem 271:15963, 1996

292. Olsson I: The intracellular transport of glucosaminoglycans (mucopolysaccharides) in human leukocytes. Exp Cell Res 54:318, 1969

293. Mason DY, Cramer EM, Massé J-M, Crystal R, Bassot J-M, Breton-Gorius J: Alpha$_1$-antitrypsin is present within the primary granules of human polymorphonuclear leukocytes. Am J Pathol 139:623, 1991

294. Gabay JE, Scott RW, Campanelli D, Griffith J, Wilde C, Marra MN, Seeger M, Nathan CF: Antibiotic proteins of human polymorphonuclear leukocytes. Proc Natl Acad Sci USA 86:5610, 1989

295. Flodgaard H, Østergaard E, Bayne S, Svendsen A, Thomsen J, Engels M, Wollmer A: Covalent structure of two novel neutrophile leucocyte-derived proteins of porcine and human origin. Neutrophile elastase homologues with strong monocyte and fibroblast chemotactic activities. Eur J Biochem 197:535, 1991

296. Pohl J, Pereira HA, Martin NM, Spitznagel JK: Amino acid sequence of CAP37, a human neutrophil granule-derived antibacterial and monocyte-specific chemotactic glycoprotein structurally similar to neutrophil elastase. FEBS Lett 272:200, 1990

297. Weiss J, Olsson I: Cellular and subcellular localization of the bactericidal/permability-increasing protein of neutrophils. Blood 69:652, 1987

298. Spitznagel JK, Dalldorf FG, Leffell MS, Folds JD, Welsh IRH, Cooney MH, Martin LE: Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. Lab Invest 30:774, 1974

299. Ganz T, Selsted M, Szklarek D, Harwig SSL, Daher K, Bainton DF, Lehrer RI: Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 76:1427, 1985

300. Ohlsson K, Olsson I: The neutral proteases of human granulocytes. Isolation and partial characterization of granulocyte elastase. Eur J Biochem 42:519, 1974

301. Cramer E, Pryzwansky KB, Villeval J-C, Testa U, Breton-Gorius J: Ultrastructural localization of lactoferrin and myeloperoxidase in human neutrophils by immunogold. Blood 65:423, 1985

302. Csernok E, Lüdemann J, Gross WL, Bainton DF: Ultrastructural localization of proteinase 3, the target antigen of anti-cytoplasmic antibodies circulating in Wegener's granulomatosis. Am J Pathol 137:1113, 1990

303. Cross AS, Wright DG: Mobilization of sialidase from intracellular stores to the surface of human neutrophils and its role in stimulated adhesion responses of these cells. J Clin Invest 88:2067, 1991

304. László L, Doherty FJ, Watson A, Self T, Landon M, Lowe J, Mayer RJ: Immunogold localization of ubiquitin-protein conjugates in primary (azurophilic) granules of polymorphonuclear neutrophils. FEBS Lett 279:175, 1991

305. Buescher ES, Livesey SA, Linner JG, Skubitz KM, McIlheran SM: Functional, physical and ultrastructural localization of CD15 antigens to the human polymorphonuclear leukocyte secondary granule. Anat Rec 228:306, 1990

306. Ducker TP, Skubitz KM: Subcellular localization of CD66, CD67, and NCA in human neutrophils. J Leukoc Biol 52:11, 1992

307. Fletcher MP, Gallin JI: Human neutrophils contain an intracellular pool of putative receptors for the chemoattractant N-formylmethionyl-leucyl-phenylalanine. Blood 62:792, 1983

308. Jesaitis AJ, Naemura JR, Painter RG, Sklar LA, Cochrane CG: Intracellular localization of N-formyl chemotactic receptor and Mg^{2+} dependent ATPase in human granulocytes. Biochim Biophys Acta 719:556, 1982

309. Singer II, Scott S, Kawka DW, Kazazis DM: Adhesomes: Specific granules containing receptors for laminin, C3bi/fibrinogen, fibronectin, and vitronectin in human polymorphonuclear leukocytes and monocytes. J Cell Biol 109:3169, 1989

310. Rotrosen D, Gallin JI, Spiegel AM, Malech HL: Subcellular localization of Giα in human neutrophils. J Biol Chem 263:10958, 1988

311. Volpp BD, Nauseef WM, Clark RA: Subcellular distribution and membrane association of human neutrophil substrates for ADP-ribosylation by pertussis toxin and cholera toxin. J Immunol 142:3206, 1989

312. Goldschmeding R, van Dalen CM, Faber N, Calafat J, Huizinga TWJ, van der Schoot CE, Clement LT, von dem Borne AEGK: Further characterization of the NB 1 antigen as a variably expressed 56-62 kD GPI-linked glycoprotein of plasma membranes and specific granules of neutrophils. Br J Haematol 81:336, 1992

313. Mollinedo F, Burgaleta C, Velasco G, Arroyo AG, Acevedo A, Barasoain I: Enhancement of human neutrophil functions by a monoclonal antibody directed against a 19 kDa antigen. J Immunol 149:323, 1992

314. Lacal PM, Barasoaín I, Sánchez A, García-Sancho J, Flores I, Mollinedo F: A monoclonal antibody that detects a specific human neutrophil antigen involved in phorbol myristate acetate- and formylmethionyl-leucyl-phenylalanine-triggered respiratory burst. J Immunol 148:161, 1992

315. Maridonneau-Parini I, de Gunzburg J: Association of rap1 and rap2 proteins with the specific granules of human neutrophils. Translocation to the plasma membrane during cell activation. J Biol Chem 267:6396, 1992

316. Quinn MT, Mullen ML, Jesaitis AJ, Linner JG: Subcellular distribution of the Rap1A protein in human neutrophils: Colocalization and cotranslocation with cytochrome b_{559}. Blood 79:1563, 1992

317. Suchard SJ, Burton MJ, Stoehr SJ: Thrombospondin receptor expression in human neutrophils coincides with the release of a subpopulation of specific granules. Biochem J 284:513, 1992

318. Porteu F, Nathan CF: Mobilizable intracellular pool of p55 (type I) tumor necrosis factor receptors in human neutrophils. J Leukoc Biol 52:122, 1992

319. Plesner T, Ploug M, Ellis V, Ronne E, Hoyer-Hansen G, Wittrup M, Pedersen TL, Tscherning T, Dano K, Hansen NE: The receptor for urokinase-type plasminogen activator and urokinase is translocated from two distinct intracellular compartments to the plasma membrane on stimulation of human neutrophils. Blood 83:808, 1994

320. Bjerrum OW, Bjerrum OJ, Borregaard N: β2-microglobulin in neutrophils: An intragranular protein. J Immunol 138:3913, 1987

321. Murphy G, Bretz U, Baggiolini M, Reynolds JJ: The latent collagenase and gelatinase of human polymorphonuclear neutrophil leucocytes. Biochem J 192:517, 1980

322. Hibbs MS, Bainton DF: Human neutrophil gelatinase is a component of specific granules. J Clin Invest 84:1395, 1989

323. Ringel EW, Soter NA, Austen KF: Localization of histaminase to the specific granule of the human neutrophil. Immunology 52:649, 1984

324. Matzner Y, Vlodavsky I, Bar-Ner M, Ishai-Michaeli R, Tauber AI: Subcellular localization of heparanase in human neutrophils. J Leukoc Biol 51:519, 1992

325. Heiple JM, Ossowski L: Human neutrophil plasminogen activator is localized in specific granules and is translocated to the cell surface by exocytosis. J Exp Med 164:826, 1986

326. Kane SP, Peters TJ: Analytical subcellular fractionation of human granulocytes with reference to the localization of vitamin B12-binding proteins: Analytical subcellular fractionation of human granulocytes with reference to the localization of vitamin B_{12}-binding protein. Clin Sci Mol Med 49:171, 1975

327. Petrequin PR, Todd RF III, Devall LJ, Boxer LA, Curnutte JT III: Association between gelatinase release and increased plasma membrane expression of the Mo1 glycoprotein. Blood 69:605, 1987

328. Jones DH, Anderson DC, Burr BL, Rudloff HE, Smith CW, Krater SS, Schmalstieg FC: Quantitation of intracellular Mac-1 (CD11b/CD18) pools in human neutrophils. J Leukoc Biol 44:535, 1988

329. Jones DH, Schmalstieg FC, Dempsey K, Krater SS, Nannen DD, Smith CW, Anderson DC: Subcellular distribution and mobilization of MAC-1 (CD11b/CD18) in neonatal neutrophils. Blood 75:488, 1990

330. Jones DH, Schmalstieg FC, Hawkins HK, Burr BL, Rudloff HE, Krater S, Smith CW, Anderson DC: Characterization of a new mobilizable Mac-1 (CD11b/CD18) pool that co-localizes with gelatinase in human neutrophils, in Springer TA, Anderson DC, Rosenthal AS, Rothlein RR (eds): Structure and Function of Molecules Involved in Leukocyte Adhesion. New York, NY, Springer-Verlag, 1989, p 107

331. Balsinde J, Diez E, Mollinedo F: Arachidonic acid release from diacylglycerol in human neutrophils. Translocation of diacylglycerol-deacylating enzyme activities from an intracellular pool to plasma membrane upon cell activation. J Biol Chem 266:15638, 1991

332. Mollinedo F, Gómez-Cambronero J, Cano E, Sánchez-Crespo M: Intracellular localization of platelet-activating factor synthesis in human neutrophils. Biochem Biophys Res Commun 154:1232, 1988

333. Bjerrum OW, Borregaard N: Dual granule localization of the dormant NADPH oxidase and cytochrome b_{559} in human neutrophils. Eur J Haematol 43:67, 1989

334. Detmers PA, Zhou D, Powell D, Lichenstein H, Kelley M, Pironkova R: Endotoxin receptors (CD14) are found with CD16 (Fc gamma RIII) in an intracellular compartment of neturophils that contains alkaline phosphatase. J Immunol 155:2085, 1995

335. Werfel T, Sonntag G, Weber M, Götze O: Rapid increases in the membrane expression of neutral endopeptidase (CD10), aminopeptidase N (CD13), tyrosine phosphatase (CD45), and Fcγ-RIII (CD16) upon stimulation of human peripheral leukocytes with human C5a. J Immunol 147:3909, 1991

336. Jack RM, Lowenstein BA, Nicholson-Weller A: Regulation of C1q receptor expression on human polymorphonuclear leukocytes. J Immunol 153:262, 1994

337. Tedder TF, Fearon DT, Gartland GL, Cooper MD: Expression of C3b receptors on human B cells and myelomonocytic cells but not natural killer cells. J Immunol 130:1668, 1983

1997 89: 3503-3521

Granules of the Human Neutrophilic Polymorphonuclear Leukocyte

Niels Borregaard and Jack B. Cowland

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.