Isolation and Characterization of Gelatinase Granules From Human Neutrophils

By Lars Kjeldsen, Henrik Sengeløv, Karsten Lollike, Morten H. Nielsen, and Niels Borregaard

We recently confirmed the existence of gelatinase granules as a subpopulation of peroxidase-negative granules by double-labeling immunogold electron microscopy on intact cells and by subcellular fractionation. Further characterization of gelatinase granules has been hampered by poor separation of specific and gelatinase granules on both two-layer Percoll gradients and sucrose gradients. We have developed a three-layer Percoll density gradient that allows separation of the different granules and vesicles from human neutrophils; in particular, it allows separation of specific and gelatinase granules. This allows us to characterize these two granule populations with regard to their content of membrane proteins, which become incorporated into the plasma membrane during exocytosis. We found that gelatinase granules, defined as peroxidase-negative granules containing gelatinase but lacking lactoferrin, contain 50% of total cell gelatinase, with the remaining residing in specific granules. Furthermore, we found that 20% to 25% of both the adhesion protein Mac-1 and the NADPH-oxidase component cytochrome b558 is localized in gelatinase granules. Although no qualitative difference was observed between specific granules and gelatinase granules with respect to cytochrome b558 and Mac-1, stimulation of the neutrophil with FMLP resulted in a selective mobilization of the least dense peroxidase-negative granules, ie, gelatinase granules, which, in concert with secretory vesicles, furnishes the plasma membrane with Mac-1 and cytochrome b558. This shows that gelatinase granules are functionally important relative to specific granules in mediating early inflammatory responses. © 1994 by The American Society of Hematology.

From the Granulocyte Research Laboratory, Department of Hematology, State University Hospital, Rigshospitalet, and the Department of Pathological Anatomy, University of Copenhagen, Denmark.

Submitted September 21, 1993; accepted November 4, 1993.


Address reprint requests to Lars Kjeldsen, MD, Granulocyte Research Laboratory, Department of Hematology L-4041, State University Hospital, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology.

GELATINASE GRANULES IN HUMAN NEUTROPHILS

Figure 1: Density profile of three-layer Percoll density gradient. Density gradients were prepared as described in Materials and Methods by applying the postnuclear supernatant on top of a 3 X 9 mL Percoll gradient (1.12/1.09/1.05 g/mL) followed by centrifugation at 37,600 g for 30 minutes. The location of the visible bands is indicated.

Figure 2: Photo of three-layer Percoll density gradient before and after centrifugation of postnuclear supernatant. The resulting bands, the \( \alpha \)-band, the \( \beta_1 \)-band, the \( \beta_2 \)-band, and the \( \gamma \)-band, are readily visible.

Materials and Methods

Isolation of Neutrophils. Neutrophils were isolated from blood donated by healthy volunteers. Blood was anticoagulated in 25 mmol/L sodium citrate, 126 mmol/L NaCl, and 3 mmol/L KCl. Red blood cells were allowed to sediment for 45 minutes by the addition of equal amounts of 2% (wt/vol) Dextran (Pharmacia, Uppsala, Sweden) in saline. The leukocyte-rich supernatant was centrifuged on Lymphoprep (Nygaard, Oslo, Norway) at 400 g for 15 minutes. Remaining erythrocytes were lysed by hypotonic shock in ice-cold water for 30 seconds and toxicity was restored by the addition of 1.8% NaCl. Cells were washed once in saline and resuspended in the desired buffer. All steps except dextran sedimentation were performed at 4°C.

Stimulation of Neutrophils. For stimulation, the cells were resuspended in Krebs Ringer phosphate (KRP; 130 mmol/L NaCl, 5 mmol/L KCl, 1.27 mmol/L MgSO\(_4\), 0.95 mmol/L CaCl\(_2\), 5 mmol/L glucose, 10 mmol/L Na\(_2\)HPO\(_4\)/Na\(_2\)HPO\(_4\), pH 7.4) at 3 X 10⁸ cells/mL and preincubated for 5 minutes at 37°C. After the addition of the stimulus (10 nmol/L FMLP or 2 ng/mL phorbol myristate acetate [PMA]) cells were incubated for 15 minutes. The incubation was stopped by centrifugation at 200 g for 6 minutes. The supernatant, termed S₀, was aspirated and the cells were resuspended in saline for subsequent subcellular fractionation. Release of granule markers was calculated as content in S₀ as a percentage of the content in S₀ + S₁ + P₁ (see below).

Subcellular fractionation. After incubation of neutrophils with diisopropylfluorophosphate (DFP; 5 mmol/L; Aldrich Chemical Co., Milwaukee, WI) for 5 minutes and centrifugation at 200 g for 6 minutes, neutrophils were resuspended at 3 to 5 X 10⁷/mL in disruption buffer (100 mmol/L KCl, 3 mmol/L NaCl, 1 mmol/L ATPNa₂, 3.5 mmol/L MgCl₂, 10 mmol/L PIPES, pH 7.2) containing 0.5 mmol/L phenylmethylsulfonyl fluoride (PMSF). Cells were disrupted by nitrogen cavitation (pressurized for 5 minutes) as described. Nuclei and intact cells were pelleted by centrifugation at 400 g for 15 minutes (P₁). Ten milliliters of the postnuclear supernatant (S₁) was applied on top of a 3 X 9 mL three-layer Percoll gradient (1.05/1.09/1.12 g/mL) containing 0.5 mmol/L PMSF and centrifuged at 37,000 g for 30 minutes. This resulted in a gradient with 4 visible bands, from the bottom designated the \( \alpha \)-band, the \( \beta_1 \)-band, the \( \beta_2 \)-band, and the \( \gamma \)-band. The cytosol was present above the \( \gamma \)-band on top of the Percoll. The gradient was collected in fractions of 1 mL each by aspiration from the bottom of the tube. All fractions were assayed for markers as described below.

Marker assays. Myeloperoxidase (azurophil granules), lactoferrin (specific granules), gelatinase (gelatinase granules), albumin (secretory vesicles), and HLA (plasma membranes) were all measured by enzyme-linked immunosorbent assay (ELISA) as described. Specific granules were also identified by vitamin B₁₂-binding protein as described by Gottlieb et al and Kane and Peters. Secretory vesicles were also identified by 'atent alkaline phosphatase, ie, alkaline phosphatase only measurable in the presence of detergent (0.2% Triton X-100). Furthermore, fractions were assayed for \( \beta_2 \)-microglobulin and for the \( \alpha \) subunit CD11b of Mac-1, both assessed by ELISA as described. General procedures for the ELISAs were as described for NGAL ELISA below, except that samples for the CD11b ELISA were solubilized by 25 mmol/L N-octyl glucoside and 0.2% cytochrome c. The content of cytochrome b₅₅₈ was quantitated by dithionite-reduced-minus-oxidized difference spectra using an
NGAL was measured by ELISA.16 Antibodies against NGAL were obtained as described in Kjeldsen et al.17 NGAL-ELISA was performed using 96-well flat-bottom immunoplates (Nunc, Roskilde, Denmark). The plates were coated overnight with anti-NGAL antibodies, diluted 1:2,000, followed by the addition of biotinylated anti-NGAL antibody and avidin-peroxidase (Dakopatts, Glostrup, Denmark). The plates were coated overnight with anti-NGAL antibodies, diluted 1:2,000, followed by the addition of biotinylated anti-NGAL antibody and avidin-peroxidase (Dakopatts, Glostrup, Denmark). The plates were incubated for 1 hour unless otherwise stated. Color was developed during 30 minutes of incubation in 0.1 mol/L sodium phosphate, 0.1 mol/L citric acid buffer, pH 5.0, containing 0.04% o-phenylenediamine and 0.03% H2O2 (100 μl/well), and stopped by the addition of 100 μL 1 mol/L H2SO4. The plates were washed three times in washing buffer (0.5 mol/L NaCl, 3 mmol/L KCl, 8 mmol/L Na2HPO4/KH2PO4, pH 7.2, 1% Triton X-100) and fixed for 5 hours at room temperature in 1% osmium tetroxide. Absorbance was read at 492 nm in a Multiscan Plus ELISA-reader (Labsystems, Helsinki, Finland).

Subcellular fractions were centrifuged on an Airfuge (Beckmann, Palo Alto, CA) to sediment the Percoll. The biologic material from each fraction was aspirated and resuspended in cavitation buffer and disrupted by nitrogen cavitation followed by subcellular fractionation on a three-layer Percoll density gradient as described in Materials and methods. The gradient was fractionated by aspiration from the bottom of the tube into 37 fractions of 1 ml each. The lower the fraction number, the denser the fraction. The content of various markers was measured in each fraction as described. (A) The distributions of myeloperoxidase (MPO), latent alkaline phosphatase (LAP), and HLA. (B) The distributions of lactoferrin and gelatinase. (C) The distributions of vitamin B12-binding protein (vit. B12 BP), NGAL, and β2-microglobulin (β2mic). (D) The distributions of cytochrome b568 and CD11b. The results are average distributions in 3 to 7 subcellular fractionation experiments (the actual number for each marker is shown in Table 1).

Electron microscopy. Subcellular fractions were centrifuged on an Airfuge (Beckmann, Palo Alto, CA) to sediment the Percoll. The biologic material from each fraction was aspirated and resuspended in cavitation buffer and disrupted by nitrogen cavitation followed by subcellular fractionation on a three-layer Percoll density gradient as described in Materials and methods. The gradient was fractionated by aspiration from the bottom of the tube into 37 fractions of 1 ml each. The lower the fraction number, the denser the fraction. The content of various markers was measured in each fraction as described. (A) The distributions of myeloperoxidase (MPO), latent alkaline phosphatase (LAP), and HLA. (B) The distributions of lactoferrin and gelatinase. (C) The distributions of vitamin B12-binding protein (vit. B12 BP), NGAL, and β2-microglobulin (β2mic). (D) The distributions of cytochrome b568 and CD11b. The results are average distributions in 3 to 7 subcellular fractionation experiments (the actual number for each marker is shown in Table 1).
### Table 1. Distribution of Biochemical Markers After Subcellular Fractionation of Unperturbed Neutrophils on Three-Layer Percoll Density Gradients

<table>
<thead>
<tr>
<th>Marker</th>
<th>α-band</th>
<th>β₁-band</th>
<th>β₂-band</th>
<th>γ-band</th>
<th>Cytosol</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloperoxidase</td>
<td>73.4 (4.2)</td>
<td>19.5 (3.3)</td>
<td>4.5 (0.8)</td>
<td>2.2 (1.0)</td>
<td>0.4 (0.3)</td>
<td>99.7 (8.5)</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>8.2 (3.0)</td>
<td>83.3 (4.6)</td>
<td>6.6 (1.3)</td>
<td>1.8 (1.0)</td>
<td>0.1 (0.1)</td>
<td>102.1 (10.6)</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>2.1 (1.4)</td>
<td>47.1 (4.7)</td>
<td>40.0 (3.1)</td>
<td>10.3 (2.0)</td>
<td>0.5 (0.5)</td>
<td>91.6 (14.8)</td>
</tr>
<tr>
<td>Latent alkaline phosphatase</td>
<td>0.4 (0.7)</td>
<td>1.4 (1.6)</td>
<td>21.1 (4.2)</td>
<td>74.4 (3.7)</td>
<td>2.8 (2.2)</td>
<td>97.2 (13.5)</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.4 (1.5)</td>
<td>8.1 (2.0)</td>
<td>28.7 (3.3)</td>
<td>59.8 (4.3)</td>
<td>2.1 (1.5)</td>
<td>95.6 (8.6)</td>
</tr>
<tr>
<td>HLA</td>
<td>2.6 (2.6)</td>
<td>5.2 (2.2)</td>
<td>10.5 (4.5)</td>
<td>71.6 (6.8)</td>
<td>10.1 (4.5)</td>
<td>82.9 (11.1)</td>
</tr>
<tr>
<td>Vitamin B₆-binding protein</td>
<td>8.0 (1.9)</td>
<td>78.3 (4.2)</td>
<td>9.5 (1.3)</td>
<td>2.8 (2.0)</td>
<td>1.4 (1.1)</td>
<td>93.7 (8.9)</td>
</tr>
<tr>
<td>NGAL</td>
<td>9.5 (2.1)</td>
<td>79.6 (1.7)</td>
<td>8.0 (0.7)</td>
<td>2.1 (0.4)</td>
<td>0.9 (0.5)</td>
<td>105.4 (7.5)</td>
</tr>
<tr>
<td>β₂-Microglobulin</td>
<td>3.5 (0.7)</td>
<td>44.7 (1.0)</td>
<td>17.9 (0.8)</td>
<td>26.5 (1.9)</td>
<td>7.5 (0.7)</td>
<td>90.9 (3.3)</td>
</tr>
<tr>
<td>Cytochrome b₅₆₅</td>
<td>0</td>
<td>61.1 (6.2)</td>
<td>21.9 (2.7)</td>
<td>17.0 (5.6)</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Mac-1</td>
<td>2.0 (1.2)</td>
<td>55.5 (6.4)</td>
<td>24.7 (4.0)</td>
<td>17.0 (3.3)</td>
<td>0.8 (1.1)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Neutrophils at 3 to 5 × 10⁷ cells/mL were disrupted by nitrogen cavitation followed by centrifugation to pellet nuclei and unbroken cells. The supernatant, S₁, was applied on three-layer Percoll density gradients and centrifuged as described in Materials and Methods. This resulted in a gradient with four visible bands, from the bottom designated the α-band (corresponding to fractions no. 1 through 7), the β₁-band (corresponding to fractions no. 8 through 15), the β₂-band (corresponding to fractions no. 16 through 19), and finally the γ-band (corresponding to fractions no. 20 through 26). The remaining fractions represent the cytosol. The content of the various markers in the regions defined above is expressed as the content recovered in the region as a percentage of the total content recovered in the gradient. The recovery of each marker is calculated as total content recovered on the gradient in percentage of content in S₁. Values are in percentages and are the mean of seven experiments (except for Mac-1, NGAL, and for β₂-microglobulin, which represent 6, 4, and 2 experiments, respectively) with the SD given in parentheses.

Abbreviation: ND, not determined.

and left overnight in a 50% mixture (vol/vol) of epoxypropane and Epon before a final embedding in Epon. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Philips 201 electron microscope (Philips, Eindhoven, Holland).

Morphometrical analysis of peroxidase-negative granules was performed by measuring the diameters of granules displaying double membrane configuration. Only granules from the β₁-band and β₂-band were evaluated.

### RESULTS

Subcellular fractionation on two-layer Percoll density gradients (1.05/1.12 g/mL) results in generation of three vis-

![Fig 4. Ultrastructure of material from α-, β₁-, β₂-, and γ-band isolated on Percoll density gradients. Four hundred microliters of each fraction was centrifuged in an airfuge to remove Percoll. The biologic material from fractions 2 and 3 (α-band), 10 and 11 (β₁-band), 16 and 17 (β₂-band), and 23 and 24 (γ-band), respectively, was pooled and processed for electron microscopy as described in Materials and Methods. The micrographs show (A) α-band, (B) β₁-band, (C) β₂-band, and (D) γ-band. Bars: 0.5 μm.](image-url)
GELATINASE GRANULES IN HUMAN NEUTROPHILS

was collected and resuspended in boiling for. Four hundred microliters centrifuged in an airfuge to pellet the Percoll. The biologic material and latent alkaline phosphatase/HLA (lane 34) were enriched in gelatinase granules are marked (*) on either side in electrophoresis on a layer of Percoll with a density of 1.09 g/mL leads to a less-visible bands, as previously described. The α-band contains azurophil granules, the β-band specific and gelatinase granules, and the γ-band light membrane structures, including secretory vesicles and plasma membranes. The density profile within the β-band is rather steep. Introducing a third layer of Percoll with a density of 1.09 g/mL leads to a less-steep density profile (Fig 1), resulting in generation of 4 visible bands, from the bottom designated the α-band, the β1-band, the β2-band, and the γ-band, with the cytosol on top of the Percoll (Fig 2). This gradient was fractionated by aspiration from the bottom of the tube into 37 fractions of 1 mL each. Figure 3A and B shows the distribution profiles of markers for the different compartments of the neutrophil, and Table 1 gives the distribution of these markers in the different regions of the gradient. The gradient is largely unchanged compared with the two-layer Percoll gradient regarding the content of the α- and the γ-band (Fig 3A and Table 1). The α-band contains the majority of the azurophil granule marker myeloperoxidase, whereas the γ-band contains secretory vesicles identified by latent alkaline phosphatase and plasma membrane identified by HLA. The characteristic “shoulder” in the profile of latent alkaline phosphatase extends into the β2-band (21% of latent alkaline phosphatase activity is contained within the β2-band; Table 1). The β1-band contains the majority of the specific granule marker lactoferrin (83%), whereas the majority of gelatinase is located in the β1-band and in the γ-band, which both contain very little lactoferrin (Fig 3B and Table 1). We have identified a subpopulation of peroxidase-negative granules that on double-labeling immunogold electron microscopy are positive for gelatinase but lack lactoferrin; we have defined these as gelatinase granules. The remaining peroxidase-negative granules all contain lactoferrin; although 80% contain gelatinase as well, these are defined as specific granules. Thus, a total separation of gelatinase from lactoferrin is impossible because a significant portion of gelatinase resides in lactoferrin-containing granules. Although there is a gradual transition from specific to gelatinase granules, we here arbitrarily define gelatinase granules as all granules containing in fraction no. 16 and higher, albeit 6% of lactoferrin is contained within these fractions (Table 1). According to this definition, gelatinase granules contain 50.3% of total cell gelatinase (40.0% in the β2-band, 10.3% in the γ-band).

The ultrastructure of the different bands was investigated by electron microscopy. Figure 4A through D shows electron micrographs of material from the α-, β1-, β2-, and γ-band, respectively. The ultrastructure of the α-band and the γ-band is in agreement with previous publications. The ultrastructures of the β1- and β2-bands, respectively, are very similar, because both contain electron-dense rounded granules, although the β2-fraction appears more heterogeneous, possibly because of contamination with light membrane structures. From morphometric analysis of 84 granules from the β1-band and 79 granules from the β2-band, it appears that the β2 granules are slightly but significantly smaller than the β1 granules (average diameter of β2 granules 187 nm compared with 236 nm of β1 granules, P < .00001, two sample t-test [unpaired]). This is in agreement with previous findings using double-labeling immunogold electron microscopy on whole cells, which showed gelatinase gran-
It has been demonstrated by several investigators that both Mac-1 and cytochrome b$_{558}$ translocate from internal stores to the plasma membrane on activation of neutrophils.$^{8,11,31,32}$ The contribution of the different granule/vesicle subsets to the upregulation of cytochrome b$_{558}$ and Mac-1 in the plasma membrane on stimulation can now be investigated by subcellular fractionation of neutrophils.
stimulated with various secretagogues. Figure 6 and Table 2 show the distribution of myeloperoxidase, lactoferrin, gelatinase, albumin (a matrix marker for secretory vesicles), cytochrome b558, Mac-1, and HLA in control cells and in cells stimulated with 10 nM FMLP or 2 μg/mL PMA before subcellular fractionation. It is evident that stimulation of neutrophils with FMLP hardly mobilizes any specific granules (release of 2.2% of lactoferrin), whereas 26% of gelatinase is exocytosed. It is observed that gelatinase is selectively depleted from the lightest peroxidase-negative granules, i.e., gelatinase granules residing in the β2-band (reduced from 40% to 18.7%) and in the γ-band (reduced from 10.3% to 4.3%). Likewise, the content of both cytochrome b558 and Mac-1 in the β2-band is reduced from 22% and 25% to 7.1% and 9.2%, respectively, with a parallel increase in the γ-band content of these two proteins. Furthermore, as previously shown, a translocation of cytochrome b558 and Mac-1 occurs within the γ-band from secretory vesicles to the plasma membrane, because secretory vesicles are almost fully mobilized on stimulation with FMLP (as visualized by disappearance of albumin after FMLP stimulation; Fig 6). This translocation to the plasma membrane of approximately 15% of total cell cytochrome b558 and Mac-1 is overlooked if one only focuses on the total content of these proteins in the γ-band. On stimulation with PMA, one observes a further upregulation of cytochrome b558 and Mac-1 in the plasma membrane, with the major contribution coming from specific granules (Fig 6 and Table 2). In contrast to gelatinase granules, specific granules are not fully mobilized on stimulation with PMA, because 34% of lactoferrin remains in the β2-band.

**DISCUSSION**

We describe here a novel method for subcellular fractionation of human neutrophils on three-layer Percoll density gradients. In contrast to previous fractionation protocols, this protocol offers separation of all established secretory organelles of the neutrophil, in particular, separation of gelatinase granules from specific (lactoferrin containing) granules. The present results are very much in line with our data obtained by double-labeling immunogold electron microscopy and with the findings of Graves et al. and Jones et al. and confirm the recent assumptions of the existence of heterogeneity among peroxidase-negative granules. These granules constitute a continuum from granules rich in lactoferrin and vitamin B12-binding protein but devoid of gelatinase and scarcely mobilized by inflammatory mediators to granules rich in gelatinase but devoid of lactoferrin or vitamin B12-binding protein and responsive to stimulation of the cells by inflammatory mediators. These latter granules, the gelatinase granules (equivalent to "specific granules containing gelatinase but lacking vitamin B12-binding protein," as designated by Graves et al.), should therefore be considered a subpopulation of peroxidase-negative granules. They contain approximately 50% of total cell gelatinase, with the remaining 50% being localized together with lactoferrin in specific granules. It should be stressed that we reserve the term specific granules for peroxidase-negative granules containing lactoferrin and vitamin B12-binding protein.

The separation of gelatinase granules from specific granules enables us to estimate the relative distribution within peroxidase-negative granule subpopulations of both Mac-1 and cytochrome b558, whose subcellular localizations have been a matter of debate during recent years. It was concluded, based on colocalization with lactoferrin in double-labeling immunogold electron microscopy, that specific granules were the intracellular reservoir of leukocyte adhesion receptors (including Mac-1) and cytochrome b558. On the other hand, Mollinedo et al. claimed both Mac-1 and cytochrome b558 to be located mainly within gelatinase granules. We find the two proteins to be located in both specific and gelatinase granules, but find only 20% to 25% of Mac-1 and cytochrome b558 residing in gelatinase granules, whereas the majority (55% and 61%) is located in specific granules. The localization of Mac-1 in both specific and gelatinase granules is in line with the results obtained by Jones et al. and Graves et al., although they found less Mac-1 in specific granules (32% and 26%, respectively) and more in gelatinase granules (35% and 44%, respectively). Furthermore, the fact that the majority of Mac-1 within the γ-band of unperturbed cells is not located in the plasma membrane but rather in secretory vesicles was not considered in those studies. The quantitative differences in distribution of Mac-1 within peroxidase-negative granules may be caused by the use of different solubilization procedures. Only after including n-octyl glucoside and CTAB in the solubilization protocol in the CD11b ELISA were we able to measure all Mac-1 present in specific granules.

The heterogeneity within peroxidase-negative granules may reflect differential but overlapping synthesis and packaging of different granule proteins during granulopoiesis. Ultrastructural studies on neutrophil precursors have indicated that lactoferrin is synthesized and packaged at the early myelocytic stage preceding the packaging of gelatinase. Although hypothetical, it is possible that the synthesis of gelatinase continues after lactoferrin synthesis has ceased. The content of different peroxidase-negative granules could therefore simply reflect the actual proteins that are synthesized at the time of granule formation. Furthermore, the possibility exists that a constant synthesis of both Mac-1 and cytochrome b558 occurs during the time of specific granule formation and later during gelatinase granule formation. This would result in an equal density of cytochrome b558 and Mac-1 in the membrane of gelatinase granules and specific granules, respectively, but given the lower number and smaller size/surface of gelatinase granules, the total content of these two proteins will be much less in these granules than in specific granules.

The biologic significance of storing Mac-1 and cytochrome b558 in three different secretory organelles is very intriguing. We have recently shown that a strict hierarchy exists regarding both calcium sensitivity and kinetics of mobilization of the different organelles within the neutrophil. Secretory vesicles are mobilized faster and at lower cytosolic Ca2+ levels than gelatinase granules, which again are mobilized faster and at lower cytosolic Ca2+ levels than specific granules. This could imply that secretory vesicles are rapidly exocytosed, as the neutrophil reaches inflamed endothel...
rium, leading to upregulation of surface Mac-1 and subsequent firm adhesion of the cell. Later, in the inflammatory response, a further upregulation of Mac-1 from gelatinses granules ensures a persistent firm attachment of the neutrophil,\(^{39}\) with a concomitant secretion of gelatinses, allowing the cell to traverse the basement membrane of the endothelium. The parallel partial translocation of cytochrome b\(_{558}\) and subsequent activation of the NADPH-oxidase could be of importance in activation of latent gelatinses before diapedesis,\(^{40}\) and could mediate expression of ICAM-1 on the surface of the endothelial cell.\(^{37}\) Considering the harmful effects to the host of reactive oxygen species, it seems reasonable to store the majority of cytochrome \(b_{558}\) in specific granules, because specific granules are unlikely to be mobilized by weak stimuli involved in neutrophil adhesion and diapedesis.

The use of the three-layer Percoll density gradient presented here makes it possible to further characterize the structure and content of gelatinses granules with the potential of elucidating the mechanisms underlying the graded eosinoy of peroxidase-negative granule subpopulations.

ACKNOWLEDGMENT

The expert technical assistance of Charlotte Horn and Fia L. Olsen is greatly appreciated. Antibodies against the \(\alpha\) subunit of Mac-1 were generously provided by Dr. Timothy A. Springer (Center for Blood Research, Harvard Medical School, Boston, MA).

REFERENCES


Isolation and characterization of gelatinase granules from human neutrophils

L Kjeldsen, H Sengelov, K Lollike, MH Nielsen and N Borregaard