Phagocytosis in Human Platelets: Localization of Acid Phosphatase-Positive Phagosomes Following Latex Uptake

By Jon C. Lewis, Jorge E. Maldonado, and Kenneth G. Mann

During the phagocytic process, the sequence of events occurring in polymorphonuclear leukocytes includes attachment of foreign particles to the phagocyte surface, engulfment of the particle by the phagocyte, formation of a phagocytic vacuole, and finally the release of hydrolytic enzymes into the phagocytic vacuole. Through in vitro studies, the ability of platelets to isolate various substances from the external medium has been demonstrated. This finding has resulted in attempts to equate the phagocytosis of platelets to that described for polymorphonuclear leukocytes. Ultrastructural studies have demonstrated uptake of Thorotrast, viruses, polystyrene particles, and bacteria by platelets, but none of these investigations has clearly demonstrated phagocytic vacuoles within platelets. Identification of the platelet as a phagocytic cell, therefore, has been inconclusive. We report the use of polystyrene latex particles to study phagocytosis in human platelets. Our results clearly illustrate uptake of extracellular material by platelets and the time-dependent isolation of that material in acid phosphatase-positive vacuoles.

MATERIALS AND METHODS

Blood samples of healthy volunteers who had given informed consent were taken in plastic syringes and immediately transferred to plastic centrifuge tubes containing heparin (10 U/ml), EDTA (0.3%, final concentration), or sodium citrate (0.4%, final concentration) as an anticoagulant. Platelet-rich plasma (PRP) was prepared, and cells, fixed at room temperature, were processed for electron microscopy by procedures routinely used in our laboratory. In some experi-

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ments, aspirin (15 grains) was taken orally by volunteers during a 24-hr period before having blood drawn.

For experiments involving latex uptake, PRP was warmed in a 37°C water bath for 5-10 min. After temperature equilibration, 50 μl of an untreated 0.5% latex suspension in distilled water (0.087 μ average diameter, styrene butadiene, Dow Diagnostics, Midland, Mich.) were added to each 1 ml of PRP. The tubes were gently agitated to mix the contents and then were returned to the 37°C bath for 1, 3, 5, 10, 15, 20, 30, 45, 60, and 90 min before processing for acid phosphatase activity. The quantitation of latex particle density within platelet organelles was done by the procedure described by Kodama and Kodama. For these calculations, the cellular area in sq μ (determined from electron micrographs) was multiplied by the geometric thickness of 0.075 μ (average section thickness) to determine the approximate volume in cu μ of the organelle being considered.

Acid phosphatase cytochemistry was carried out by a modification of the technique described by McKeever and Balentine. Platelets, fixed at 1-3°C in cacodylate-buffered glutaraldehyde for 4 hr, were washed and then incubated in the medium described by McKeever and Balentine for 15 hr. After incubation, the cells were rinsed with 2% acetic acid, followed by rinsing with 0.1 M cacodylate containing 5% sucrose, and then were postfixed with 1% OsO₄, stained with uranyl acetate, and embedded.

The periodate-alkaline-bismuth reaction was carried out by the procedure of Ainsworth et al., as modified by Burton and Hinkley. Thin sections, picked up on uncoated copper specimen screens, were oxidized in periodate for 10-20 min before being stained with chelated bismuth for 45 min. Sections treated in this manner were viewed without further processing.

RESULTS

When platelets were fixed immediately after addition of latex to the PRP, they appeared morphologically identical to control platelets (Fig. 1). Cells varied in shape from discoid to round and had numerous alpha-granules, a few

![Fig. 1. Control platelets. Open-channel system, mitochondria, and alpha-granules. One large (0.4-μ diameter) electron-lucent, debris-containing vacuole (small arrow) and a smaller electron-dense organelle (large arrow) are shown. x 21,700.](image-url)
Table 1. Sequence of Events in Latex-Particle Phagocytosis

<table>
<thead>
<tr>
<th>Time of Exposure (min)</th>
<th>Latex Location</th>
<th>Latex Density (particles/cu μ)</th>
<th>Acid Phosphatase Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Extracellular</td>
<td>15</td>
<td>Discrete organelles</td>
</tr>
<tr>
<td>15</td>
<td>Open-channel system</td>
<td>500</td>
<td>Discrete organelles</td>
</tr>
<tr>
<td>45</td>
<td>Dense vacuoles</td>
<td>1000</td>
<td>Discrete organelles</td>
</tr>
<tr>
<td>60</td>
<td>Dense vacuoles</td>
<td>1000</td>
<td>Dense vacuoles</td>
</tr>
</tbody>
</table>

mitochondria randomly distributed throughout the cytoplasm, and sometimes a distinct band of circumferentially located microtubules. Elements of the open-channel system and the dense tubular system also were clearly delineated. Occasionally, cells contained membrane-limited vacuoles similar to secondary lysosomes observed in platelets from other species.\(^{14,15}\) These structures generally had diameters smaller than 0.3 μ and varied in electron density (Fig. 1). Minimal internalization of latex was observed at time zero. Latex particles, associated with less than 1% of the cells, generally were bound to extracellular membranes.

Many particles were observed in the open-channel system of cells maintained in latex suspension for between 10 and 30 min (Table 1). This accumulation was accompanied by a slight tendency toward irregularity in cell shape and a dilation of the open-channel system. A few degranulated platelets were found during this period; however, as previously reported by White,\(^5\) the number and distribution of alpha-granules, mitochondria, and microtubules generally did not seem to be affected by the latex. In all cells exposed to latex for less than 45 min, acid phosphatase activity was typically localized to electron-opaque, membrane-limited organelles with diameters in the range of 0.1-0.2 μ (Fig. 2).

Significant ultrastructural alterations were found in cells incubated with latex for 45-60 min. Prominent dilations of the open-channel system filled with latex particles were found in 50% of the platelets. In addition, these platelets contained numerous electron-opaque, membrane-limited, latex-containing vacuoles (Fig. 3). In contrast to cisternae of the open-channel system, no free space was observed in the electron-opaque vacuoles, and latex particles within the vacuoles were packed tightly together, resulting in an approximate particle density of 1000 particles/cu μ. This value was a 67-fold increase in concentration, when compared to the original extracellular suspension, and was twice the concentration of particles found in the most densely packed profiles of the open-channel system (Table 1). Typically, one or two electron-opaque, latex-containing vacuoles, having diameters in the range 0.1-0.5 μ, were observed in each platelet section. When latex exposure exceeded 60 min, an extensive amount of acid phosphatase activity was associated with latex particles in the electron-opaque vacuoles (Fig. 4). Particles in these structures were surrounded and often obscured by heavy deposition of lead phosphate (Fig. 4). Enzyme product was not observed in cisternae of the open-channel system.

Periodate-Alkaline-Bismuth Staining

Ultrastructural differences between plasma membranes and lysosomal membranes have been demonstrated in several cell types.\(^6\) Because the external
Fig. 2. Platelets exposed to latex for 15 min and subsequently reacted for acid phosphatase. Particles are found in cisternae of open-channel system (arrow), while reaction product is localized to cytoplasmic structures. x 29,000.

Fig. 3. (A) Electron-opaque, latex-containing vacuoles (small arrows) are observed in platelets exposed to latex for 60 min. Latex is also seen in cisternae of open-channel system (large arrow). Citrate anticoagulant. x 30,000. (B) Electron-opaque, latex-containing vacuole. x 50,000.
membranes of platelets and membranes of the open-channel system are rich in glycoproteins, an on-grid stain that was specific for these compounds was used to determine if membranes of electron-dense, latex-containing vacuoles differed from other platelet membranes. The results of the periodate alkaline bismuth stain are seen in Figs. 5 and 6. The outer surface coat of platelets exhibited an intensely electron-opaque staining after oxidation and reaction with the bismuth reagent (Fig. 5). Distribution of the stain on the exterior surface was granular, while the underlying plasma membrane stained in a less pronounced fashion (Fig. 5B). Profiles of the open-channel system appeared to stain identically to the outer surface coat, but membranes of the electron-opaque, latex-containing vacuoles lacked the granular, electron-opaque surface coat (Fig. 6).

Fig. 4. Phosphatase-positive, latex-containing vacuoles, 60-min exposure to latex. × 37,500.

Fig. 5. (A) Sections stained with alkaline-bismuth. Intensely stained surface coat (SC) appears granular, while staining of underlying plasma membrane is less pronounced. Open-channel system (OCS) stains similarly to exterior surfaces. × 20,700. (B) High magnification of surface coat of platelet. × 130,500.
Effects of Anticoagulant and Aspirin

Phagocytosis by platelets was independent of both the choice of anticoagulant and the effects of aspirin. When either EDTA or sodium citrate was used as an anticoagulant in place of heparin, no discernible differences were observed either in platelet ultrastructure or in the ability of platelets to phagocytize latex particles. Similarly, oral ingestion of aspirin had no apparent effect either on the uptake of latex or on the formation of acid phosphatase-positive, latex-containing vacuoles.

DISCUSSION

Uptake of various particles by platelets in vitro is a widely recognized phenomenon, and ultrastructural evidence is accumulating to suggest that removal of foreign material from the blood is a major physiologic role for these cells. This hypothesis has been supported through biochemical studies of Kuramoto et al. in which significant increases in production of lactate and CO2 are observed when platelets are challenged with latex. Similar results have been reported by Cooper et al.; however, these later authors were hesitant to attribute the metabolic response to phagocytosis of latex because particles were observed inside the platelets within 2 min after contact, but variation in production of lactate was not significant until platelets had been challenged with latex for more than 10 min. Both Kuramoto et al. and Cooper et al. reported marked increases in metabolism when platelets were exposed to latex for 30–60 min. This time corresponds to the period during which electron-opaque, latex-containing vacuoles were noted in our experiments.

Precise identification of subcellular structures through electron microscopy is frequently difficult, but our preliminary data support the hypothesis that the electron-opaque, latex-containing vacuoles are phagosomes. This hypothesis is substantiated by two lines of evidence: (1) localization of acid phosphatase activity and (2) unique membrane-staining characteristics obtained with alkaline-bismuth. Several workers have demonstrated acid phosphatase activity within human platelets. In these reports, moderate enzyme activity was found in the alpha-granule peripheral matrix and the alpha-granule nucleoid, whereas intense reaction product was noted in large pleomorphic platelet granules, which may correspond to non-alpha-granule lysosomes. None of these workers reported enzyme activity in cisternae of the open-channel system, an
observation confirmed by us. Our observation of acid phosphatase activity in electron-dense, latex-containing vacuoles, therefore, is significant and suggests that these structures are secondary lysosomes. Information obtained through the use of periodate–alkaline–bismuth staining, a reaction reported to be specific for mucosubstances and polysaccharides containing 1,2-glycols, further supports this suggestion. As documented in our results, a distinct ultrastructural difference is found between external plasma membranes that contain a granular surface coat and membranes of the electron-opaque vacuoles. This distinction can be accounted for if the membranes are of different origins or if membranes of the latex-containing vacuoles have undergone dramatic structural alterations. In platelets, these alterations could include enzymatic degradation of the glycoprotein coat on membrane surfaces during the process of secondary lysosome formation.

Our results and the results of others suggest that two distinct sequential phenomena occur when platelets are challenged with latex particles: (1) adhesion of particles to platelet surfaces and sequestration within cisternae of the open-channel system through a process accommodated within baseline metabolic levels, and (2) formation of phagocytic vacuoles through an active process related to increased levels of glucose metabolism. DeJesus et al. initially suggested the occurrence of two stages during the phagocytosis of latex by platelets. These authors, when studying phagocytosis-stimulated nitroblue tetrazolium (NBT) reduction, noted that particle-induced reduction was inhibited about 80% by 3 mM EDTA. This observation correlated with a study earlier reported by Mustard and Packham, who found that EDTA was inhibitory to particle engulfment but did not interfere with adhesion of particles to platelet surfaces. Although our results support the concept of a two-stage process in latex phagocytosis by platelets, we did not observe inhibition of either open-channel system uptake (adhesion) or formation of electron-opaque vacuoles (engulfment) when calcium chelators were used as anticoagulants. The fact that we used PRP in our experiments, whereas both Mustard and Packham and DeJesus et al. used washed platelets, may explain this discrepancy in our results. Further work is needed to understand fully the phagocytic process in platelets, but it is apparent that under certain circumstances the human platelet can act as a true phagocyte.

REFERENCES


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