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Selective Inhibition of IgG-Mediated Phagocytosis in Gelsolin-Deficient Murine Neutrophils

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Phagocytosis and the microbialidal functions of neutrophils require dynamic changes of the actin cytoskeleton. We have investigated the role of gelsolin, a calcium-dependent actin severing and capping protein, in peripheral blood neutrophils from gelsolin-null (Gsn−/−) mice. The phagocytosis of complement opsonized yeast was only minimally affected. In contrast, phagocytosis of IgG-opsonized yeast was reduced close to background level in Gsn−/− neutrophils. Thus, gelsolin is essential for efficient IgG- but not complement-mediated phagocytosis. Furthermore, attachment of IgG-opsonized yeast to Gsn−/− neutrophils was reduced (~50%) but not to the same extent as ingestion (~73%). This was not due to reduced surface expression of the Fcγ-receptor or its lateral mobility. This suggests that attachment and ingestion of IgG-opsonized yeast by murine neutrophils are actin-dependent and gelsolin is important for both steps in phagocytosis. We also investigated granule exocytosis and several steps in phagosome processing, namely the formation of actin around the phagosome, translocation of granules, and activation of the NADPH-oxidase. All these functions were normal in Gsn−/− neutrophils. Thus, the role of gelsolin is specific for IgG-mediated phagocytosis. Our data suggest that gelsolin is part of the molecular machinery that distinguishes complement and IgG-mediated phagocytosis. The latter requires a more dynamic reorganization of the cytoskeleton. The Journal of Immunology, 2000, 165: 2451–2457.

Polyomorphonuclear leukocytes (neutrophils) constantly rearrange their actin cytoskeleton to perform chemotaxis and phagocytosis. Such rearrangements depend on actin severing and capping proteins of which gelsolin is the best-characterized example (1). Gelsolin is an 82-kDa cytoplasmic protein widely expressed in mammalian cells. Upon activation by Ca2+, gelsolin severs actin filaments, binds, and caps barbed ends of actin filaments. Gelsolin-actin complexes dissociate in the presence of polyphosphoinositides (2, 3). Gelsolin-null (Gsn−/−) mice have normal embryonic development and longevity (4). Gsn−/− mice show a prolonged bleeding time due to decreased platelet shape changes. Migration of neutrophils into peritoneal exudates is delayed, and fibroblasts have excessive stress fibers and decreased motility. The GTP-binding protein rac is overexpressed in Gsn−/− tissues and regulates gelsolin in fibroblasts (5).

Neutrophil functions, such as adhesion, chemotaxis, phagocytosis and finally microbial killing by secretion of oxidative metabolites and granular enzymes into the phagosomal space, depend on rapid and dynamic cytoskeletal rearrangements. Gelsolin constitutes about 1% of the total neutrophil protein and is a good candidate to mediate Ca2+-dependent actin rearrangements (6). Phagocytosis in neutrophils is well studied, but its molecular mechanisms are still poorly understood. The two main pathways are complement-receptor (CR)-mediated and Fcγ-receptor (FcγR)-mediated phagocytosis (reviewed in Ref. 7). The important receptors are CR1, CR3, and CR4 for CR-mediated phagocytosis and FcγRII and FcγRIII for FcγR-mediated phagocytosis in non-stimulated neutrophils.

Binding and clustering of CRs by complement-opsonized particles mediates serine phosphorylation of the receptor and the formation of punctuate structures containing F-actin, paxillin, α-actinin, and vinculin assembled to the membrane (8). This leads to a sifting of the opsonized particle into the membrane without protruding pseudopods (9). FcγR-mediated phagocytosis has been shown to have another morphology with protruding pseudopodia encircling the prey, thus demanding another kind of actin remodeling (9). FcγR-receptor stimulation mediates signaling via immune receptor tyrosine-based activation motifs (ITAM) with the initiation of a signaling cascade involving phosphorylation of syk, src-kinases activation of PI3 kinases, rac and phospholipase C, that stimulate protein kinase C-activation and calcium release from intracellular stores (7–9).

An actin ring that slowly dissolves after ingestion is completed encircles the phagosome (10). The actin ring may be a barrier, preventing granules from fusion with the phagosome. Gelsolin translocates to the actin ring and might be important for its removal (P.S. and O.S., unpublished observations). Intracellular Ca2+ stores and granules also translocate to the phagosome and phagolysosome fusion takes place (11). In neutrophils, this is a calcium-dependent process (12).

Extracellular secretion of granule proteins is another neutrophil activity, important for killing, but also causing tissue damage. The cortical actin network may form a physical barrier that prevents...
granules from reaching the plasma/phagosomal membrane. The role of gelsolin could be to break up this barrier. It has indeed been shown that actin depolymerization in itself is sufficient to induce secretion in some cell types and that gelsolin can stimulate secretion (13–15).

We have investigated neutrophils from Gsn−/− mice to study the role of gelsolin in neutrophil functions. Our results show the feasibility of using transgenic mice to study the specific role of a protein in peripheral blood neutrophils. Gelsolin is the prototype of a protein sequestering and capping proteins. It is thought to play a central role in the actin dynamics of phagocytes. We show here that gelsolin plays an important role in FcγR-mediated phagocytosis, but not in several other phagocyte functions.

Materials and Methods

Materials

Rabbit-anti-yeast Abs were produced by Dakopatts (Glostrup, Denmark). Percoll was purchased from Pharmacia (Uppsala, Sweden) and HBSS from Life Technologies (Grand Island, NY). FITC, FITC-Phalloloid, lyso-phosphatidyl choline, cytochalasin B, and polyclonal rabbit anti-lactoferrin Ab were obtained from Sigma (St. Louis, MO). Polyclonal rabbit anti-myeloperoxidase (MPO) Ab was provided by Dr. Inge Olson in Lund, Sweden and rabbit anti-gelsolin Ab by Dr. Christine Chapponnier, CMU, Geneva, Switzerland. Goat IgG, FITC-labeled, and nonlabeled goat anti-rat F(ab')2, and dichlorotriazinylaminofluorescein-labeled goat anti rabbit F(ab')2 were purchased from Jackson ImmunoResearch (West Grove, PA).

Rat monoclonal anti mouse CD16/32 (clone 2.4G2) was bought from PharMingen (San Diego, CA) and anti-mouse CD18 (clone C71/16) from AMS, Biotechnology (Täby, Sweden).

Preparation of mouse neutrophils

Mouse blood (200–400 μl per animal) was obtained from either wild-type or Gsn−/− mice (C57BL/6) (4) in EDTA-tubes by tail bleeding. NaCl (0.9%) was added to a final volume of 4 ml. After centrifugation at 450 × g for 10 min, cells were resuspended in 1 ml HBSS supplemented with 0.2% EDTA. The cells were overlaid a 3-layer Percoll gradient 75%, 67%, and 52% Percoll, respectively, diluted in HBSS (100% Percoll = 9 parts Percoll and 1 part 100% HBSS) and centrifuged at 1500 × g for 30 min. The neutrophils were harvested from the 67%/75% interface after carefully removing the cells from the upper phases. After one wash, remaining red cells in the neutrophil fraction were eliminated by hypotonic lysis (0.5 mM water for 35 s and 0.5 ml 1.8% NaCl). After a final wash, 50,000–500,000 cells, >90% neutrophils, identified by staining the nuclei with Türk's reagent, were obtained per mouse. These cells were kept in Medium 199 or external solution (E.S.: 140 mM NaCl, 5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 10 mM HEPES, pH 7.2, 8.9 mM glucose) and used within 6 h.

Phagocytosis assay

Phagocytosis was assayed in suspension and not with adherent cells to minimize the contribution active cell movement, which is known to be influenced by gelsolin (4). Furthermore, additional receptors may be activated upon adherence to surfaces and influence the phagocytic uptake. Heat-killed bakers yeast (Saccharomyces cerevisiae) (106/ml) were labeled with FITC (0.25 mg/ml) in 0.2 M carbonate buffer (pH 9.5) for 60 min at 37°C. The preparations were washed in PBS and mounted.

FACS analysis

Mouse neutrophils (20,000 cells/tube) were pelleted and resuspended in 0.1 ml cold 4% paraformaldehyde. After 30 min, the cells were washed twice and incubated for 1 h with 0.1 ml either rat monoclonal anti-mouse CD16/32 (clone 2.4G2) or anti-mouse CD18 (clone C71/16) diluted 1:100 in 0.1% BSA/PBS. After one wash, 0.1 ml of an FITC-labeled secondary Ab, goat anti-rat F(ab')2, fragments diluted 1:200 in 0.1% BSA/PBS supplemented with 5% goat IgG, was added. Cells were washed and resuspended in 0.2 ml PBS.

Cell labeling and fluorescence recovery after photobleaching (FRAP)

Neutrophils (106/ml) were incubated at 4°C for 1 h with 100 μl Krebs-Ringer’s phosphate buffer supplemented with 10 mM glucose, 1.2 mM Mg2+, and 1 mM Ca2+ (KRG) with FITC-labeled monoclonal rat-anti mouse CD16/32 (5 μg/ml) in KRG. Cells were plated on cool 3-well glass slides for 10 min, rinsed, and mounted by adding 50 μl ice-cold KRG and placing a coverslip over the well. The chambers were sealed with hot wax-vaseline mixture (1:1). The equipment for FRAP measurements has been described in detail elsewhere (19). Fluorescently labeled receptors in a small circular area (the bleach spot) on the cell surface were illuminated and photobleached (×100–1000 intensity, 500 ms) with an Argon laser (type 2020-03; Spectra Physics, Mountain View, CA) at 488 nm through a 587 nm filter. A Zeiss Universal microscope (Zeiss, Germany). The 63× oil-immersion planachromatic objective used gave an estimated bleach spot radius of 0.89 μm at 1/e2 intensity. Fluorescence in the bleach spot was measured before bleaching and for 120 s after bleaching at increasing time intervals. The diffusion coefficient (D, × 10−5 cm2/s) and the mobile fraction (R, %) was calculated according to Axelrod et al. (20) and Jacobson et al. (21). On small mouse neutrophils, up to 10% of the total cell surface might be bleached and thereby reduce R. However, bleaching would be identical in neutrophils from both Gsn+ and wild-type mice and would not affect the comparison. Measurements were taken within 60 min after labeling and equilibration of the cells to microscope stage temperature. The preparation was kept at 15–17°C on a temperature-regulated microscope stage to minimize internalization of the receptors.

Capacitance measurements of secretion

For patch-clamp recordings, the neutrophils were plated on glass coverslips, in some cases coated with 1% BSA. Capacitance recordings with the time domain technique were performed as described for human neutrophils (22). Mouse neutrophils have an initial capacitance of 1.6 ± 0.1 pF on albumin and 2.3 ± 0.13 on glass in the presence of cytochalasin B (control cells), which probably reflects some spontaneous degranulation on glass. Gsn− neutrophils display similar initial capacitance values (1.7 ± 0.15 on albumin and 2.4 ± 0.14 on glass with 5 μM cytochalasin B). The capacitance values for mouse neutrophils on glass are around 25% smaller than human neutrophils (23). The cells were stimulated with either 20 μM GTPyS or 100 μM Ca2+ in the pipette to elicit a significant secretory response (22, 23).

Visualization of F-actin with FITC-labeled phalloidin during phagocytosis

Neutrophils were adhered on glass slides for 5 min at 37°C. Complement-opsonized yeast was added. After 3, 10, and 15 min, cells were fixed in cold 4% paraformaldehyde for 30 min. After thorough washes, cells were incubated for 20 min in lyso-phosphatidylcholine (100 μg/ml) and FITC-phalloidin (0.6 μM). The preparations were washed in PBS and mounted. A Zeiss Axiosvert S100 microscope equipped with epifluorescence and differential interference contrast and a 100 × objective was used. Images were acquired with a cooled charge-coupled device camera (Vivicam, VisiRon System, Puchheim, Germany) and the MetaFluor software (Universal Imaging, West Chester, PA).

Immunofluorescence

Mouse neutrophils were adhered for 5 min to 3-well microscope slides. Complement-opsonized yeast particles were added. After 3, 10, and 15 min at room temperature, the cells were washed three times in 0.1% BSA/PBS. A secondary anti-rabbit Ab was added 1:100 in 0.1% BSA/PBS supplemented with 5% IgG from host species. After 30 min, the cells were washed carefully, mounted, and analyzed with fluorescent microscopy.
Nitro blue tetrazolium (NBT) test

Neutrophils were incubated in E.S. at 37°C with opsonized yeast particles in the presence of 1.7 mg/ml NBT (yellow) for 20 min at 37°C. In the presence of oxidative metabolites, NBT is reduced to formazan, which forms a blue precipitate. Cells were observed under the microscope for blue precipitates around the yeast particles.

Statistical analysis

The average number of ingested and bound yeast particles was determined from the pool of all cells observed under the same conditions (n = 150–450 from three to nine independent experiments). Mean and SE of mean were calculated and the probability that two pools were identical was determined by Student’s t test on two independent populations (performed with Microsoft Excel). Differences between pools were considered statistically significant at p < 0.05.

Results

Gelsolin is necessary for FcyR-mediated phagocytosis, but not for CR-mediated phagocytosis

To analyze the role of gelsolin in the regulation of phagocytosis, we studied the uptake of yeast through the main phagocytic receptors of neutrophils, namely FcγRs and CRs. Fig. 1A shows the uptake of complement-opsonized yeast by wild-type and Gsn− neutrophils. The overall uptake of yeast (40 min) was the same under these conditions. However, there were kinetic differences: Gsn− neutrophils took up significantly less than wild-type controls during the first 5–20 min (p < 0.001). The situation was different for IgG-opsonized yeast (Fig. 1B): its uptake was markedly inhibited in Gsn− cells at all time points (p < 0.001). We also determined the number of yeast particles attached to the outside of cell. Similar to phagocytosis, the association of complement opsonized particles to Gsn− neutrophils was reduced up to 20 min (Fig. 1C).

After 40 min of incubation, the differences were no longer significant. For IgG-opsonized particles, a marked decrease in the number of cell associated particles was found in Gsn− cells at all time points (Fig. 1D, p < 0.03).

Particle uptake requires pseudopod formation upon particle attachment, pseudopod extension, and closure of the phagosome. There is recent evidence that these steps are regulated differentially. For example, in macrophages, the tyrosine kinase syk and phosphoinositide 3-kinase appears to be required for closure of the phagosome, rather than initial pseudopod formation (24, 25). A decreased number of internalized particles, but an increased number of cell-associated particles characterize failure of phagosome closure. In contrast, the decreased number of cell-associated particles in Gsn− neutrophils suggest that gelsolin is needed for phagocytic steps preceding pseudopod extension and phagosome closure.

Phagocytosis and binding of IgG-opsonized particles are active processes

We compared complement and IgG-mediated phagocytosis with conditions that are known to inhibit phagocytosis (Fig. 2). Inhibition of actin polymerization by cytochalasin B reduced particle ingestion and attachment. IgG-mediated phagocytosis (Fig. 2A)
was more sensitive to cytochalasin B and more affected by the absence of gelsolin than complement-mediated phagocytosis (Fig. 2B). Furthermore, particle ingestion was more sensitive than particle binding. At 4°C, almost no particles were attached to mouse neutrophils and none were internalized. Nonopsonized particles were poorly bound and internalized. In fact, IgG-mediated phagocytosis by Gsn− cells was not significantly different from phagocytosis of nonopsonized particles by control cells (p = 0.14). Thus, attachment and internalization of IgG-opsonized particles to mouse neutrophils are active, actin-dependent processes, and gelsolin is important for both.

Previous studies had shown that FcγR-, but not CR-mediated phagocytosis is a Ca2+-dependent process (26). Our results suggest that gelsolin is the sensor for Ca2+-dependent actin rearrangements occurring during FcγR-mediated phagocytosis. However, alternative explanations might involve effects of gelsolin on cell surface expression of FcγRs or on lateral receptor mobility.

Surface expression of phagocytic receptors is not reduced in Gsn− neutrophils

We compared the cell surface expression of Fc receptors and CRs on wild-type and Gsn− neutrophils in suspension by FACS-analysis with Abs against defined FcγRs and CR3. The FACS data showed no detectable difference in either CR3 (CD18) or FcγRII/III (CD16/32) surface expression on wild-type and Gsn− neutrophils, respectively (Fig. 3). Thus, the inhibition of FcγR-mediated phagocytosis in Gsn− neutrophils was not due to decreased cell surface density of receptors.

Lateral mobility of Fcγreceptors II/III is not altered in Gsn− neutrophils

Phagocytic receptors must move in the plane of the membrane to mediate phagocytosis (27). To investigate whether the difference in FcγR-mediated phagocytosis is due to altered receptor mobility in the neutrophil membrane, FRAP was used. We found no differences (p = 0.94) between the FcγR diffusion coefficients of Gsn− (3.54 × 10−10 ± 0.30 cm2/s, n = 39) and wild-type (3.57 × 10−10 ± 0.27 cm2/s, n = 41) neutrophils. The calculated diffusion coefficients of mouse neutrophil Fcγ-receptors are similar to what has been shown for CR1/CD35 and the fMLP-receptor in human neutrophils (2.7–4.5 and 5 × 10−10 cm2/s, respectively) (28, 29).

Note that the only available Ab against mouse FcγRs (2.4G2) binds both mFcγRII and mFcγRIII. Thus, our data represent the average mobility of FcγRIIs and FcγRIIs on mouse neutrophils.

FRAP analysis also reveals the mobile fraction, R, of receptors that can move freely in the plane of the membrane without confinement by cytoskeletal structures or other interactions (30). Like the diffusion coefficients, the mobile fraction of receptors was unaltered in Gsn− neutrophils (25.4 ± 2.6%, n = 41 for wild-type vs 27.8 ± 1.9%, n = 39 for Gsn−, p = 0.45). Therefore, the cytoskeletal elements that may regulate FcγR movement in the plane of the plasma membrane are functional in the absence of gelsolin.

We conclude that the gelsolin-sensitive step in FcγR-mediated phagocytosis is downstream of FcγR movement in the plasma membrane. However, we cannot exclude that adherence of the cells, which is needed for this technique, influences receptor mobility.

Secretion induced by Ca2+ and GTPγS is not dependent on gelsolin

Gelsolin might be important not only for Ca2+-dependent phagocytosis, but also for Ca2+-dependent exocytosis. Indeed, it has been suggested from morphological studies that granules are prevented from fusing with the plasma membrane by the submembraneous actin network and that Ca2+/gelsolin-dependent actin severing might therefore induce exocytosis (31). Several pieces of experimental evidence also argue in favor of this model, including the enhanced neutrophil secretion in the presence of cytochalasin B (32), and a direct effect of gelsolin on Ca2+-dependent secretion in permeabilized mast cells (15). As gelsolin is regulated by the GTP-binding protein rac (5), and rac activates secretion in mast cells (33), the activation of secretion by GTPγS (23) might also involve a gelsolin-dependent pathway.

To test these hypotheses, we investigated secretion in Gsn− neutrophils using patch-clamp capacitance measurements. Perfusion of 100 μM Ca2+ and/or 20 μM GTPγS into single cells via the patch-pipette induced secretion in wild-type mouse neutrophils. The Ca2+ concentrations were chosen based on our previous studies in human neutrophils (22), where 100 μM Ca2+ was needed to elicit significant release of primary and secondary granules.

Neither amplitude nor kinetics of Ca2+ or GTPγS-induced capacitance increases were different in Gsn− vs wild-type neutrophils (Fig. 4, A and B). Normal capacitance increases occurred in Gsn− neutrophils both in the presence and the absence of cytochalasin B, suggesting that gelsolin does not enhance secretion like cytochalasin B and is not involved in this enhancement. Thus, we conclude that gelsolin is not required for Ca2+- and GTPγS-induced secretion.

Phagosomal actin reorganization, granule translocation, and NBT reduction occur normally in Gsn− neutrophils

As shown above, CR-mediated phagocytosis is slowed down, but not substantially inhibited in the absence of gelsolin. However, gelsolin might not only be important for formation of the phagosome (as is the case for FcγR-mediated phagocytosis), but also for later events in phagocytosis, such as remodeling of the actin cytoskeleton after particle ingestion, phagolysosome fusion, and NADPH oxidase activation. We first investigated whether gelsolin was translocated to the phagosome. As shown in Fig. 5, A and B, in wild-type mouse neutrophils, gelsolin was enriched around the phagosome. Thus, the periphagosomal localization of gelsolin is
Another possible function of gelsolin might be in the regulation of the NADPH-oxidase activation during phagocytosis. Indeed, the small GTP-binding protein rac plays a role in both NADPH oxidase activation and regulation of gelsolin function. To study NADPH oxidase activation in the phagosome, we used the NBT test. In both, Gsn− and wild-type neutrophils, an intense periphagosomal NBT staining was observed in all phagocytosing cells (Fig. 5, Q and R). Thus, gelsolin is not required for the activation of the NADPH-oxidase in the phagosome.

**Discussion**

We have investigated the role of gelsolin in actin-dependent neutrophil functions. Our data clearly show that gelsolin plays a specific role in IgG-mediated phagocytosis. Phagocytosis of IgG-opsonized particles was strongly inhibited in Gsn− cells, whereas phagocytosis of complement-opsonized particles was merely delayed and several steps in phagosome maturation were unaffected.

Gelsolin exerts a dual role on actin filaments (reviewed in Ref. 34). First, upon activation by Ca2+, gelsolin severs filaments and generates capped barbed ends. Second, binding of polyphosphoinositides dissociates gelsolin from these filaments providing free ends for polymerization. Both functions may be localized in subcellular domains. Localized accumulation of polyphosphoinositides could provide a mechanism to direct actin polymerization to specific areas near the plasma membrane like the pseudopods during phagocytosis. The selective inhibition of IgG-mediated phagocytosis in Gsn− cells correlates well with earlier reports, showing calcium-dependence of IgG-mediated phagocytosis in neutrophils and calcium-independence of CR-mediated phagocytosis (26). In macrophages, phagocytosis via FcR is calcium-independent (35). Thus, gelsolin might be the calcium sensor in IgG-mediated phagocytosis by neutrophils. The morphology of FcyR-mediated phagocytosis differs from CR-mediated phagocytosis. The pseudopods protrude from the cell body when an IgG-opsonized particle is ingested, whereas a complement-opsonized particle appears to sink down into the membrane (9). Different cytoskeletal structures are associated with complement- and IgG-mediated phagocytosis in macrophages (8), and different GTP-binding proteins seem to be involved (36). The formation of pseudopods in FcyR-mediated phagocytosis might require a more intense actin reorganization that depends on gelsolin.

Several intracellular mediators of phagocytosis have been identified recently and the following hypothetical signaling cascade of IgG-mediated phagocytosis emerges: The activation of rac in FcγR-mediated phagocytosis probably occurs via activation of src and syk tyrosine kinases, leading to activation of PI3-kinase (24, 37), which in turn activates the GTP-binding protein rac (38). Gelsolin has been identified as an effector of rac (5), providing a possible link to cytoskeletal rearrangements in phagocytosis, as we show here. The precise role of the proposed syk-PI3-kinase-rac-gelsolin cascade remains to be proven. However, syk, PI3-kinase, and rac are needed to complete phagocytosis (24, 25, 36, 39, 40). Rac appears to be specific for IgG-mediated phagocytosis and rho for complement-mediated phagocytosis (36), although a role for rho in FcyRI-mediated phagocytosis has also been reported (41). Lamellodipodia-like structures engulf IgG-opsonized particles in macrophages (8). In fibroblasts, lamellodipodia formation is mediated by rac via gelsolin (5) and rac promotes the dissociation of gelsolin from actin filaments in neutrophils (42). Together, these data lead to the hypothesis that FcyRs activate rac, which interacts with gelsolin, leading to ingestion of the IgG-coated particle. Complement-opsonized particles may activate multiple signaling pathways. Gelsolin appears to be involved but is not essential, because in its absence, CR-mediated phagocytosis is merely delayed.

**FIGURE 4.** Patch-clamp capacitance recordings reveal gelsolin-independent granule secretion. Representative traces of capacitance changes from neutrophils stimulated with either 20 μM GTP·S (A) or 100 μM Ca2+ (B) via the patch pipette. The traces show Gsn− (thick line) compared with control (thin line) cells. Initial capacitance is subtracted for clarity and changes were smoothed by adjacent averaging. A, Capacitance traces from a Gsn− neutrophil and a control neutrophil plated on glass with 5 μg/ml cytochalasin B in the medium stimulated with GTP·S (20 μM). B, Capacitance traces from a Gsn− neutrophil and a control neutrophil plated on BSA-coated glass stimulated with 100 μM Ca2+. C, Quantitative analysis of secretion induced by GTP·S and/or Ca2+ on glass or albumin ± cytochalasin B in Gsn− (a) and control (a) neutrophils. Each bar represents the average maximal capacitance of three to seven cells ± SEM. The neutrophils were plated and stimulated under the following conditions: 1 + 2, plating on glass in the presence of cytochalasin B; 1, stimulation with 20 μM GTP·S; 2, stimulation with 100 μM Ca2+ and 20 μM GTP·S; 3 + 4, plating on albumin in the absence of cytochalasin B; 3, stimulation with 100 μM Ca2+; 4, stimulation with 100 μM Ca2+ and 20 μM GTP·S. Differences between control and Gsn− cells are not statistically significant (0.36 < p < 1).
Which step of the phagocytic process depends on gelsolin? We found that attachment of IgG-opsonized particles is the first step in phagocytosis to be reduced, but clearly not abolished in Gsn− neutrophils. This is also the first step that depends on the formation of new actin filaments (Fig. 2 and Ref. 43). Therefore, we hypothesize that gelsolin is specifically involved in the cytoskeletal changes associated with early pseudopod formation during IgG-mediated phagocytosis in neutrophils.

Once the cell has ingested the particle, the processing of the phagosome is generally unaffected in Gsn− cells. Gelsolin is dispensable for formation of actin filaments around the phagosome, translocation of granules, intraphagosomal superoxide production, and granule fusion with the plasma membrane excluding gelsolin as the Ca2+ sensor in neutrophils. If rac is important for NADPH-oxidase function (44), it does not require gelsolin downstream to activate the oxidase. Other actin severing and capping proteins may compensate for the lack of gelsolin in these processes. We have dissected phagocytosis and phagosomal processing and adjusted well-established methods to probe each function. However, it remains possible, that other assay conditions (e.g., cells in suspension as for phagocytosis) reveal a role of gelsolin in one of these functions.

The choice of the phagocytic receptor influences the fate of the phagocytosed particle. Two examples illustrate this point. The protozoan parasite Toxoplasma gondii actively invades mammalian cells (45). The parasitophorous vacuole does not fuse with lysosomes and the parasite avoids killing. Rerouting the parasite to IgG-mediated phagocytosis is sufficient to render the vacuole fusogenic (46). CRs mediate phagocytosis of Mycobacterium tuberculosis, but the pathogen effectively avoids killing by unknown mechanisms. However, coating M. tuberculosis with Ab redirects the bacterium to fusion-competent phagosomes (reviewed in Ref. 47). Thus IgG-mediated phagocytosis enhances killing of the phagocytosed pathogens. Understanding the molecular differences between IgG- and complement-mediated phagocytosis will identify potential targets for pharmaceutical intervention. We have identified gelsolin as part of the IgG-specific phagocytic machinery. Therefore, it will be interesting to see whether gelsolin knockout mice are susceptible to infections that involve IgG-mediated phagocytosis.
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