Receptor-mediated Phagocytosis in Human Neutrophils Is Associated with Increased Formation of Inositol Phosphates and Diacylglycerol

Elevation in Cytosolic Free Calcium and Formation of Inositol Phosphates Can Be Dissociated from Accumulation of Diacylglycerol

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Abstract

Phagocytosis of C3bi- or IgG-opsonized yeast particles in human neutrophils was found to be associated with an increased formation of inositol phosphates and diacylglycerol. Pertussis toxin only marginally affected phagocytosis of IgG- and C3bi-opsonized particles and the associated formation of second messengers. Forskolin, which induced a threefold rise of cellular cAMP, however, markedly inhibited both C3bi- and IgG-mediated phagocytosis as well as the particle-induced formation of inositol phosphates and diacylglycerol. These observations are in contrast to what was found to occur with chemotactic factors and indicate that chemotactic and phagocytic signaling can be regulated independently in human neutrophils.

Since C3bi-mediated phagocytosis has been shown to occur at vanishingly low cytosolic free calcium levels, calcium-depleted cells were used to study the importance of the inositol cycle for the engulfment of C3bi-opsonized particles. Despite a total lack of receptor-induced formation of inositol phosphates, a significantly increased accumulation of diacylglycerol accompanied the ingestion of C3bi-opsonized particles. These data show that the engulfment of C3bi-opsonized particles can occur independently of both a calcium transient and an increased inositol phosphate production. However, the observed accumulation of diacylglycerol, not derived from phosphoinositides, suggests that this second messenger play a role in the control of the engulfment process.

Introduction

The phagocytic process in human neutrophils is triggered mainly by two different receptor-mediated mechanisms, one that recognizes the C3bi-fragment of the complement system and another that recognizes the Fc domain of immunoglobulin G (1). The transduction mechanisms regulating the phagocytic process have so far only been partly elucidated. What is known is that receptor-mediated phagocytosis occurring via the two major opsonins is associated with a rise in the concentration of cytosolic free Ca^{2+} (2–4). Even more interesting are the observations that in human neutrophils only IgG-mediated ingestion is actually dependent on the rise of cytosolic free Ca^{2+}, whereas the ingestion of C3bi-opsonized particles can occur in the absence of such a rise (3). These data extend the previous observation by Stossel et al. (5) that demonstrated that the ingestion of C3b endotoxin-coated oil droplets by neutrophils could occur in EDTA-containing medium. In addition, it has also been demonstrated that in macrophages (J774 cells) the rise in intracellular Ca^{2+} is not a requirement for the phagocytic uptake of IgG-opsonized particles (2). Consequently, it seems reasonable to propose the existence of at least one additional intracellular messenger participating in the regulation of receptor-mediated phagocytosis.

Receptor-mediated activation of phospholipase C, via a regulatory G protein, and with the subsequent turnover of the inositol cycle, is a well-documented pathway in cellular signal transduction (for review see Berridge [6]). The hydrolysis of phosphatidyl 4,5-bisphosphate [PtdIns(4,5)P_2][1] leads to an accumulation of two major second messengers, inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and 1,2-diacylglycerol. The former induces the mobilization of intracellular calcium whereas the latter is the endogenous activator of protein kinase C. In human neutrophils, both Ca^{2+} and protein kinase C activity appear to be necessary for mediating secretion triggered by chemotactic factors such as FMLP and leukotriene B_4 (LTB_4) (7–9).

The aim of the present study was to investigate initially whether ingestion of IgG- or C3bi-opsonized yeast particles by human neutrophils is associated with an increased formation of inositol phosphates, as recently demonstrated in macrophages (10) and diacylglycerol, but more importantly, whether these second messengers play a role in the control of phagocytosis in these cells.

Methods

Materials. The materials and their sources were as follows: pertussis toxin (List Biological Laboratories Inc., Cambell, CA); forskolin (Calbiochem-Behring Corp., La Jolla, CA); myo-[2-^{3}H]inositol, bis(*O*-aminophenoxyl)ethane-*N,N,N',N'*-tetraacetic acid (quin2), tetraacetomethyl ester of quin2 (quin2-AM), [γ-^{32}P]ATP, Hyperfilm multipurpose, and cAMP assay kit (Amersham International, Amersham, Bucks., UK); Dowex 1 × 8 (200–400 mesh), FMLP, cardiolipin, and diethylenetriaminepentaacetic acid (Sigma Chemical Co., St. Louis, MO); octylglycoside and imidazol (Boehringer Mannheim, Mannheim, Federal Republic of Germany); 1,2-diacylglycerol kinase purified from *Escherichia coli* (Lipidex, Inc., Middleton, WI); silica gel 60 thin-layer chromatography plates (Merck & Co., Darmstadt, Federal

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1. *Abbreviations used in this paper:* InsP, inositol monophosphate; $InsP_2$, inositol bisphosphate; $InsP_3$, inositol trisphosphate; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; quin2, bis(o-aminophenoxyl)ethane-*N,N,N',N'*-tetraacetic acid; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; quin2-AM, tetraacetomethyl ester of quin2.

Republic of Germany); dextran and Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden).

Preparation of human neutrophils. Blood from healthy volunteers was collected in heparin-containing tubes, and the neutrophils were isolated according to the method described by Böyum (11). In short, after elimination of erythrocytes by dextran sedimentation followed by a brief hypotonic lysis, the cell suspension was centrifuged on a Ficoll-Paque gradient to separate the polymorphonuclear leukocytes from lymphocytes, monocytes, and platelets. The neutrophils were then washed twice before being suspended in a calcium containing medium: 138 mM NaCl, 6 mM KCl, 1 mM $MgSO_4$, 1.1 mM $CaCl_2$, 0.1 mM EGTA, 1 mM $NaHPO_4$, 5 mM $NaHCO_3$, 5.5 mM glucose, and 20 mM Hepes (pH 7.4). In some experiments calcium and EGTA were excluded from the medium. Throughout this paper these media are referred as the "calcium-containing medium" and "calcium-free medium," respectively. Cellular counting of neutrophils was performed either in a Bürker chamber or with a model ZF counter with a 100-channel pulse height analyzer from Coulter-Electronics, Ltd., Dunstable, UK.

Depletion of intracellular calcium. Depletion of intracellular calcium, to inhibit agonist-induced activation of phospholipase C, was carried out by incubating the neutrophils (5×10^7 cells/ml) in calcium-free medium with 50 μM quin2-AM and 1 mM EGTA for 1 h at 37°C in a humidified atmosphere of 5% CO_2 (12). The subsequent exposure to C3bi-opsonized particles was performed in calcium-free medium supplemented with 1 mM EGTA.

Determination of cAMP content. Neutrophils (10^7 cells/ml) were incubated with forskolin (100 μM) for various periods of time at 37°C. The reactions were terminated by adding ice-cold ethanol (final concentration 65%). The different samples were then allowed to settle for 1 h in a freezer, after which the different supernatants containing cAMP were collected. The precipitated materials were washed with fresh 65% ice-cold ethanol to ensure that the extraction of cAMP was as complete as possible. The supernatants were then centrifuged at 2,000 *g* for 15 min at 4°C, and the precipitates were discarded. The remaining supernatants were then evaporated under nitrogen, and the cAMP content was assayed with a commercial kit (Amersham International).

Determination of phagocytosis. Phagocytosis of FITC-labeled yeast particles was monitored by a fluorescence-quenching method (3, 13), in which the extracellular particles are quenched by adding trypan blue. The IgG-coated particles were opsonized by incubating yeast with purified anti-yeast IgG (24 μg/ml) in the presence of 20% heat-inactivated (56°C, 30 min) human serum; C3bi-coated particles were opsonized by incubating the yeast with 20% fresh human serum. All particles were incubated at 37°C for 30 min and washed before use. Yeast particles were presented to neutrophils in a ratio of 5:1. Phagocytosis is calculated as the number of yeast particles ingested per 100 cells.

Determination of inositol phosphate formation. Phosphoinositides and inositol phosphates were labeled by incubating cells (5×10^7/ml) with myo-[2-^{3}H]inositol (50 μCi/ml) in the previously described calcium-containing medium (except for a reduction of the Ca^{2+} concentration to 0.5 mM) or in calcium-free medium for 2 h in an atmosphere of 5.0% CO_2 at 37°C. For the determination of particle-induced changes in the formation of inositol phosphates, the labeled cells were washed, resuspended, and put on ice. The cells were then mixed with a solution of either IgG- or C3bi-opsonized yeast particles (4°C). The mixtures of cells and yeast particles were then put in a water bath (37°C) for various periods of time (see illustrations). The phagocytic process was terminated by adding ice-cold trichloroacetic acid (final concentration 15%, vol/vol). For extraction of the inositol phosphates, we used a previously described procedure (7, 9, 14), i.e., samples were first put on ice for 15 min and then centrifuged. The supernatants thus obtained were washed three times with a fivefold excess of diethyl ether. The washed extracts were adjusted to pH 7.5 with Tris (final concentration 0.2 M), and the inositol phosphates were separated, by stepwise elution, from small Dowex anion-exchange columns. After addition of 67% Aquasol, the radioactivity of the different fractions was determined by liquid-scintillation counting. In the present study no attempts were made to distinguish between the two isomers of $InsP_3$ (7, 15).

Determination of 1,2-diacylglycerol formation. For determination of particle-induced changes in cellular diacylglycerol contents, neutrophils were washed and resuspended in the calcium-containing medium or calcium-free medium and put on ice. The cells were then mixed with a solution of either IgG- or C3bi-opsonized yeast particles (4°C). The mixtures of cells and yeast particles were then put in a water bath (37°C) for various periods of time (see illustrations). The phagocytic process was terminated by adding an ice-cold solution of chloroform/methanol (1:2, vol/vol), and cellular lipids were extracted according to the method developed by Bligh and Dyer (16). The assay used was essentially as previously described by others (17, 18). Each sample, which had been evaporated under nitrogen, was solubilized in 20 μl of a mixture containing 5 mM cardiolipin, 225 mM octylglycoside, and 1 mM diethylenetriaminepentaacetic acid, and then sonicated for 15 s and left for an additional 10 min at room temperature. The samples containing the solubilized lipids were then mixed with 50 μl of a reaction solution containing 100 mM imidazol-HCl (pH 6.6), 100 mM NaCl, 25 mM $MgCl_2$, and 2 mM EGTA; 2 μl of a solution containing 100 mM dithiothreitol and 1 mM diethylenetriaminepentaacetic acid; 13 μl of distilled water; and 5 μl of a 1,2-diacylglycerol kinase solution (final activity 0.02 U). The samples were then incubated for 5 min at 26°C, after which the enzymatic reactions were initiated by the addition of 10 μl of a 10 mM [γ-^{32}P]ATP solution (sp act 70,000 cpm/nmol). After 15 min the reactions were stopped by adding 2 ml of chloroform/methanol (1:2, vol/vol) and the lipids were extracted as above. The samples were evaporated under nitrogen and resuspended in 30 μl of chloroform before application on a silica gel 60 thin-layer chromatography plate. The plates were developed with chloroform/methanol/acetic acid (65:15:5, vol/vol/vol), air-dried, and subjected to autoradiography. The spots corresponding to phosphatidic acid were scraped into scintillation vials, and, after adding 5 ml of Aquasol to each vial, the radioactivity was determined in a scintillation counter (LKB Produkter, Bromma, Sweden).

Results

Phagocytosis of C3bi- and IgG-opsonized particles and formation of $InsP_3$ and diacylglycerol. Ingestion of either C3bi- or IgG-opsonized particles (Fig. 1, *A* and *D*), is associated with an increased accumulation of $InsP_3$ (Fig. 1, *B* and *E*). This increase precedes the formation of the less phosphorylated inositols, i.e., inositol monophosphate (InsP) and inositol bisphosphate ($InsP_2$) (data not shown). It could also be demonstrated that the ingestion of either C3bi- or IgG-opsonized yeast particles is associated with an increased accumulation of diacylglycerol, the natural activator of protein kinase C (Fig. 1, *C* and *F*). This increase in cellular diacylglycerol content is much higher and more sustained than that observed after exposure to FMLP (17, 19).

Effects of pertussis toxin on C3bi- and IgG-mediated phagocytosis and the associated formation of $InsP_3$. Pertussis toxin (200 ng/ml) had only minor or no effects on phagocytosis of both C3bi- and IgG-opsonized particles and the associated formation of $InsP_3$ (Table I). The toxin also lacked effect on particle-induced formation of diacylglycerol (data not shown). In comparison with the effects of pertussis toxin on the generation of intracellular signals as well as on several functional responses triggered by chemotactic factors (7, 9, 20), its effects on phagocytosis and the associated formation of second messengers, shown in the present study, were minute. Not even if the dose of pertussis toxin was raised to 1.2 μg/ml (2 h at 37°C) were the effects anything but minor (data not shown).

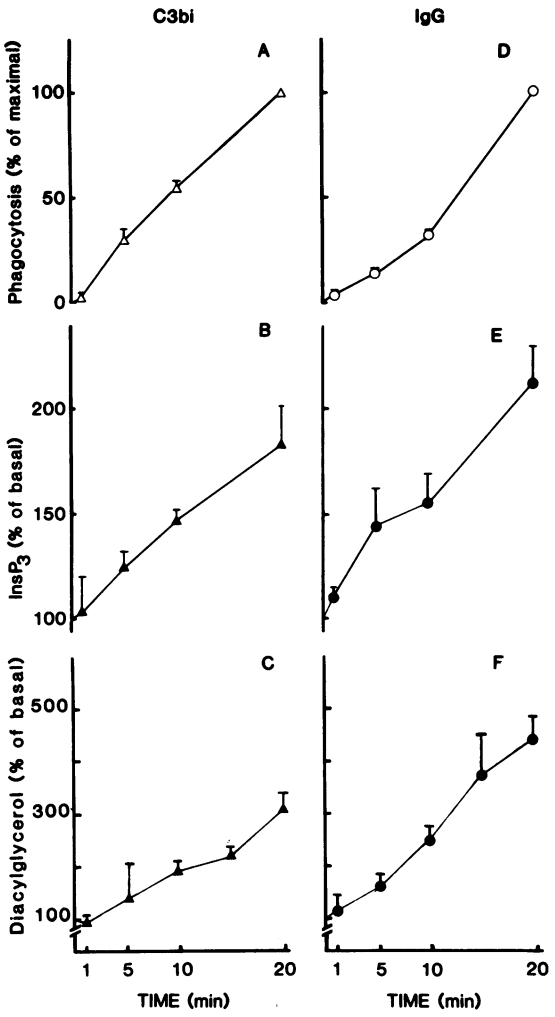

Figure 1. Human neutrophils were incubated with opsonized yeast particles at 37°C for various periods of time before the number of phagocytosed particles were counted or the contents of inositol phosphates or diacylglycerol were determined. (*A–C*) Results obtained using C3bi-opsonized yeast particles; (*D–F*) results obtained with IgG-opsonized yeast particles. The values given are mean±SEM of four to six separate experiments. The 100% level for C3bi- and IgG-phagocytosis was 230±20 and 256±16 ingested yeast particles per 100 cells, respectively. The basal level for $InsP_3$, i.e., 100%, was 233±18 dpm. The amount of diacylglycerol in unstimulated cells was 241±20 pmol/10^7 cells. Statistically significant changes in reference to time zero values, i.e., 100%, were evaluated using Student's *t* test for paired samples.

Effects of forskolin on C3bi- and IgG-mediated phagocytosis and the associated formation of $InsP_3$ and diacylglycerol. Purified neutrophils were preincubated with forskolin (100 μM), an agent which, by activating adenylate cyclase, raises the cellular level of cAMP (21). In our experiments forskolin induced a threefold increase (312% of controls, mean of three experiments) in cellular cAMP level after 10 min. In the presence of forskolin, this elevated level remained for an additional 40 min. The forskolin-induced rise in cAMP correlated with the decreased phagocytosis of both C3bi- and IgG-opsonized particles (65±4% for IgG and 60±5% for C3bi, Fig. 2, *dashed lines*). The inhibition of receptor-mediated phagocytosis was associated with a corresponding reduction in the formation of

Table I. Effects of Pertussis Toxin on Phagocytic Capacity and $InsP_3$ Formation in Human Neutrophils

	Phagocytosis	$InsP_3$
	% of control	
C3bi yeast	75±11* (*n* = 10)	83±4 (*n* = 5)
IgG yeast	67±13* (*n* = 10)	74±5* (*n* = 5)

Purified human neutrophils were preincubated with pertussis toxin (200 ng/ml) for 2 h at 37°C under a humidified atmosphere of 5.0% CO_2. In these cells this batch of pertussis toxin blocked completely any FMLP-induced response. In experiments determining the formation of $InsP_3$ the medium was supplemented with 50 μCi of myo-[^{3}H]inositol/ml. After the cells were washed, they were incubated with opsonized yeast particles for 20 min at 37°C, and the number of phagocytosed particles were then counted and/or $InsP_3$ content was determined. The pertussis toxin-induced changes in phagocytic capacity and $InsP_3$ formation are given as percent of control. The values given are mean±SEM for four to seven experiments. The 100% level for C3bi and IgG phagocytosis were 231±45 and 256±60 ingested yeast particles per 100 cells, respectively. The basal level for $InsP_3$, i.e., 100%, was 365±86 dpm. Statistical analyses were done using Student's *t* test for paired samples comparing pertussis toxin-treated cells with nontreated cells (i.e., cells preincubated with buffer for the same period of time) for the number of experiments given within parentheses. * $P < 0.05$.

$InsP_3$ and diacylglycerol (Fig. 2). In contrast, similar treatment with forskolin had no effect on the FMLP-induced formation of $InsP_3$ (data not shown).

Effects of calcium-depletion on C3bi-mediated phagocytosis and the associated formation of diacylglycerol and inositol phosphates. Neutrophils were loaded with 50 μM quin2 in a calcium-depleted medium ([Ca^{2+}] ~ 10^{-9} M) to depress the cytosolic free Ca^{2+} level. In six separate experiments this treatment lowered the cytosolic free Ca^{2+} level to 40 nM or less; this

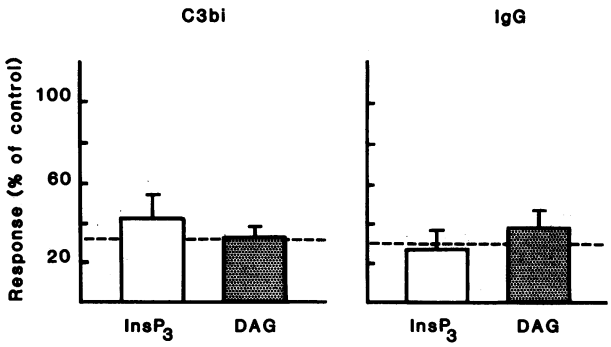

Figure 2. Purified human neutrophils were preincubated with forskolin (100 μM) for 30 min before stimulation with opsonized yeast particles (20 min). Control cells were treated similarly but without forskolin. After these incubations the number of phagocytosed particles were counted or cellular contents of $InsP_3$ or diacylglycerol (DAG) were determined. (*Dashed line*) Effect of forskolin on phagocytosis. The values given are mean±SEM of five to eight separate experiments. The 100% level for C3bi and IgG phagocytosis was 233±31 and 208±27 ingested yeast particles per 100 cells, respectively. The basal level for $InsP_3$, i.e., 100%, was 236±28 dpm. The amount of diacylglycerol in unstimulated cells was 174±30 pmol/10^7 cells. Statistically significant changes produced by forskolin were tested using Student's *t* test for paired samples.

level is far below the K_d of known Ca^{2+}-binding proteins (22). Addition of ionomycin (500 nM) or C3bi-opsonized particles to these cells did not affect their cytosolic free Ca^{2+} level, as long as these cells were maintained in a Ca^{2+}-depleted medium. This excludes the possibility of any rise in the cytosolic free calcium level occurring during these experimental conditions (data not shown). Compared with control cells, the calcium-depleted cells retained the major part of their phagocytic capacity for C3bi-particles (Fig. 3 *A*). In experiments performed in Ca^{2+}-depleted medium with cells not loaded with quin2, it could be shown that 81% of this reduction (mean of three separate experiments) is due to the lack of extracellular calcium, which is known to result in a decreased association between yeast particles and cells. In calcium-depleted cells, as in control cells, the phagocytosis of C3bi-opsonized particles was associated with a time-dependent accumulation of diacylglycerol (Fig. 3 *B*). The kinetics of diacylglycerol formation in calcium-depleted cells is largely unaffected during the initial 5 min of the phagocytic period, but after this time accumulation becomes slower. However, despite a substantial uptake of C3bi-particles and formation of diacylglycerol, no formation of $InsP_3$ could be detected (Fig. 3, *inset*). Neither was the $InsP_2$ and InsP fractions affected, which would have been the case if an early and transient hydrolysis of PtdIns(4,5)P_2 had occurred. This agrees well with previously published data demonstrating that the activity of phospholipase C in intact cells is not regulated by Ca^{2+} but is instead dependent on a certain minimum concentration of this cation (23–25).

Discussion

In analogy with what is known about receptors for chemotactic factors, the present investigation shows that activation of C3bi and IgG receptors on human neutrophils also results in an increased formation of $InsP_3$ and diacylglycerol, the two major second messengers in cellular transduction signaling. However, there are important differences between the chemotactic factor-induced and the phagocytosis-associated (C3bi or IgG) formation of these second messengers. First, the amount of diacylglycerol formed in association with C3bi- and IgG-mediated phagocytosis is much higher (present study) than the amount produced upon FMLP-induced stimulation of human neutrophils (own results and references 17 and 19). Secondly, C3bi- and IgG-mediated formation of second messengers was only slightly affected by pertussis toxin at a concentration that totally abolished signaling and functions elicited by chemotactic factors. Even at a sixfold higher toxin concentration, phagocytosis and the associated generation of second messengers were largely unaffected. This agrees well with a previous study (although that study dealt with a different kind of particle and much higher concentrations of pertussis toxin), which failed to demonstrate that pertussis toxin had any effect at all on receptor-mediated phagocytosis (26). In contrast, both chemotactic and secretory functions elicited by chemoattractants such as FMLP, C5a, and LTB_4 are entirely suppressed by pertussis toxin. It is presently clear that there is a large variety of regulatory G proteins coupled to phospholipase C, some of which are sensitive to ADP-ribosylation by pertussis toxin and some that are not (27–29). However, even when dealing with the 41-kD pertussis toxin-sensitive G protein in neutrophils, it is not possible to obtain a complete ribosylation of this protein with the toxin. It has been shown by different investigators, using the same method as in the present study, that incubations with 100–2,000 ng/ml of pertussis toxin result in 63–89% ADP-ribosylation of the 41-kD protein (20, 30,,31). There are three alternative explanations for the observed differences in sensitivity to pertussis toxin between chemotactic factors and opsonins: phagocytosis requires (*a*) no G protein or (*b*) perhaps more likely, much less active G protein than chemotaxis; or (*c*) the phagocytic process engages a G protein insensitive to pertussis toxin and thus distinct from the protein participating in transduction signaling induced by chemotactic factors. Thirdly, the present study indicates a difference in sensitivity to high levels of cAMP between the transduction mechanism of particulate stimuli (C3bi and IgG) and that of chemotactic stimuli (own results and reference 32). Although we cannot exclude other mechanisms for the action of the forskolin-induced rise in cAMP, various studies have indicated that phos-

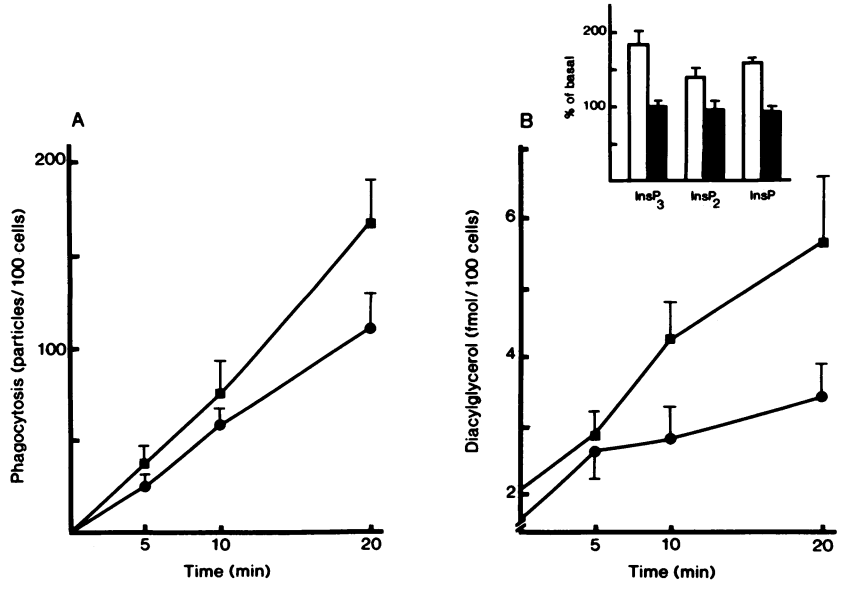

Figure 3. Human neutrophils were incubated with 50 μM quin2-AM in calcium-free medium supplemented with 1 mM EGTA ($[Ca^{2+}] \sim 10^{-9}$) at 37°C and in an atmosphere of 5.0% CO_2 for 60 min. Thereafter C3bi-opsonized particles were added. (*A*) Time-dependent ingestion of C3bi particles for (■) normal, nondepleted cells and (●) calcium-depleted cells. (*B*) C3bi-induced accumulation of diacylglycerol in (■) normal, nondepleted cells (■) and (●) calcium-depleted cells. The basal levels of diacylglycerol were 2.1±0.3 fmol per 100 cells in nondepleted cells and 1.6±0.3 fmol per 100 cells in calcium-depleted cells; these values were stable for at least 20 min. (*Inset*) Relative effects of C3bi-opsonized particles on the accumulation of inositol phosphates after 20 min in nondepleted cells (*open bars*) and calcium-depleted cells (*solid bars*). The values given are means±SEM of four to nine experiments.

pholipase C, as well as other enzymes involved in the resynthesis of phosphoinositides, may be possible targets for cAMP (32, 33).

The abilities of human neutrophils to move and to engulf foreign organisms by phagocytosis are vital for their role in host defense. For optimal efficiency, it seems reasonable to postulate that human neutrophils are able to perform these two processes simultaneously and independently. This is initially accomplished by specific receptors for chemotaxis and phagocytosis. In addition, the present observations indicate that the transduction mechanisms coupled to these separate receptors can, on at least two different levels, be regulated independently of one another.

The phagocytic process is dependent on a well-functioning microfilamentous system, with actin as a major constituent (34). We have, as have others, previously excluded the possibility of a rise in cytosolic free Ca^{2+} or activation of protein kinase C playing a major role in ligand-induced actin polymerization in human neutrophils (35, 36). However, these findings do not contradict the suggestion that some metabolite in the inositol cycle is directly involved in the regulation of cellular actin (37, 38). In the present study, by using Ca^{2+}-depleted cells, it was demonstrated that the engulfment of C3bi-particles can occur independently of both calcium and increased production of inositol phosphates. This is in agreement with a recent report demonstrating that FMLP-induced polymerization of actin can occur in Ca^{2+}-depleted cells independently of increased hydrolysis of PtdIns(4,5)P_2 in human neutrophils (12). It should be pointed out that a decreased hydrolysis of PtdIns(4,5)P_2 could result in a net increase in the amount of this phospholipid, in particular if there is a simultaneous activation of the phosphatidylinositol-4-phosphate kinase. Consequently, our data do not exclude the possibility that a net accumulation of PtdIns(4,5)P_2 is the signal leading to polymerization of actin.

The observations that the engulfment process is associated with, but not dependent on, both an increased turnover of phosphoinositides and a subsequent rise of cytosolic free Ca^{2+}, might appear contradictory. However, it must be kept in mind that during phagocytosis the uptake of particles is directly followed by other cellular events, such as fusion with primary and secondary granules as well as activation of the respiratory burst, processes that might well be dependent on the inositol cycle and/or a rise in cytosolic free calcium. This idea is supported by experiments showing that in human neutrophils the fusion between different granules and the plasma membrane is indeed Ca^{2+}-dependent (39). In fact, we have recently been able to show that the fusion between phagosomes and granules, following ingestion, is a calcium-dependent process in human neutrophils (Stendahl et al., unpublished data).

Despite inhibition of phospholipase C activity, the ingestion of C3bi-opsonized particles was still associated with a significantly increased accumulation of diacylglycerol, the endogenous activator of protein kinase C. As could be expected, the accumulation of diacylglycerol was reduced as a consequence of the inhibition of C3bi-induced breakdown of PtdIns(4,5)P_2. The data clearly indicate the existence of at least one additional source for the generation of diacylglycerol. We do not know the identity of the precursor(s) participating in diacylglycerol formation during C3bi-mediated phagocytosis, however, it has been shown in human neutrophils, as well as in other cell types, that diacylglycerol accumulation can result from agonist-induced breakdown of phosphatidylcholine (40–43). Because of the observed generation of diacylglycerol, concomitant with phagocytosis under conditions in which Ca^{2+} does not play a role, protein kinase C activity appears to be a potential signal for the engulfment process in human neutrophils. In fact, the inhibition of both IgG- and C3b-mediated phagocytosis by forskolin can be reversed by addition of a direct activator of protein kinase C (phorbol 12-myristate 13-acetate or 1-oleoyl 2-acetyl glycerol) (44). However, it is possible that diacylglycerol not generated from phosphoinositides can have as yet uncharacterized signaling effects, other than activation of protein kinase C, in receptor-mediated phagocytosis.

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