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Role of Guanine Nucleotide Regulatory Protein in Polyphosphoinositide Degradation and Activation of Phagocytic Leukocytes by Chemoattractants

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Leukocyte activation by chemoattractants provides an important model to study the biochemical mechanisms of stimulus-response coupling in these cells. Well-defined chemotactic factors induce readily quantifiable responses in phagocytic leukocytes. These include directed migration and the production and release of toxic substances including oxygen radicals and lysosomal enzymes. The development of radiolabeled synthetic oligopeptides with potent chemotactic activity allowed the demonstration of chemoattractant receptors on polymorphonuclear leukocytes (PMNs) as well as macrophages. In membrane preparations from these cells, these receptors exist in high- and low-affinity states which are regulated by guanosine di- and triphosphates. This suggested that chemoattractant receptors interact with guanine nucleotide regulatory proteins (N or G proteins). Although chemoattractants elicit a rapid but transient increase in intracellular cAMP levels, they neither stimulate nor inhibit membrane-bound adenylate cyclase, suggesting a novel role for N proteins in certain receptor-transduction mechanisms. Stimulation of phagocytes by chemoattractants is also associated with a rapid increase in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) which appears to result from the production of inositol 1,4,5-triphosphate (IP_3) as a consequence of the diesteric cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2). Treatment of phagocytes with pertussis toxin (PT), which ADP-ribosylates and thereby inactivates certain N proteins, abolishes the cells' responsiveness to chemoattractants. More direct evidence for a role of a PT-sensitive N protein in leukocyte activation was provided by the demonstration that chemoattractants stimulate the hydrolysis of PIP_2 in PMN membranes only in the presence of GTP. This receptor-mediated hydrolysis of PIP_2 is not observed in plasma membranes prepared from PT-treated PMNs. Therefore, these studies suggest that occupancy of chemoattractant recep-

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tors activates a PT-sensitive N protein. The activated N protein shifts the Ca^{2+} requirement for phospholipase C activity from supraphysiological levels to ambient cytosolic Ca^{2+} concentrations. Cleavage of PIP_2 results in the formation of the second messenger molecules, IP_3 and 1,2-diacylglycerol, which can initiate cellular activation. These messengers also seem to activate responses which feed back to attenuate receptor stimulation of phospholipase.

Key words: receptor, phospholipase C, transduction mechanism

The function of phagocytic leukocytes in host defense is to destroy harmful substances which have been identified by the immune system. To fulfill this task, phagocytes such as polymorphonuclear leukocytes (PMNs) and macrophages migrate along gradients of chemoattractants produced during humoral or cellular immune responses. Endogenous humoral chemotactic factors include C5a, which is a proteolytic cleavage product arising from complement activation [reviewed in 1,2], and thrombin, which arises from the clotting cascade [3]. Potent cell-derived chemoattractants are the arachidonate metabolite leukotriene B_4 (LTB_4) and the phospholipid mediator of anaphylaxis, platelet-activating factor (PAF, 1-o-alkyl-2-acetyl-phosphatidylcholine), which may be produced by PMNs as an amplification signal [4–6]. Lymphocytes also release chemotactic factors (termed lymphocyte derived chemotactic factor, LDCF) upon stimulation by antigens [1]. Certain products of bacterial protein synthesis are potent chemoattractants and N-formylated synthetic oligopeptides (eg, N-formyl-methionyl-leucyl-phenylalanine, fMet-Leu-Phe), which are similar to these products, are powerful tools with which to study leukocyte activation. Chemoattractants, at higher concentrations than are required for stimulation of directed migration, stimulate phagocytes to release toxic substances such as oxygen radicals or hydrolytic enzymes [7,8]. The ease of obtaining relatively large numbers of purified phagocytes as well as their easily quantifiable responses to defined stimuli make them excellent candidates with which to study mechanisms of cellular regulation.

REGULATION OF CHEMOATTRACTANT-RECEPTOR BINDING BY N PROTEINS

With the availability of chemically defined radiolabeled ligands it has been possible to demonstrate that chemoattractants bind to specific receptors on the plasma membrane of PMNs and macrophages [2]. Competitive binding studies with a series of closely related N-formylated synthetic oligopeptides demonstrated a close correlation between their binding affinities and biological potencies [9]. Covalent cross-linking techniques have revealed that the receptor for these synthetic oligopeptides has an apparent molecular weight of 60–70,000 Da, which is reduced to 32,000 Da upon deglycosylation [10]. Intact human PMNs and monocytes typically express approximately 50–80,000 binding sites with a single affinity of K_D ~20nM [2]. When plasma membranes are purified from these cells, the chemoattractant receptors exist in two affinity states with K_Ds of approximately 0.8 and 20 nM, with ca 25% of the binding sites in their high-affinity state [11]. Upon addition of guanosine diphosphate or triphosphates, the proportion of high-affinity receptors on PMN or macro-

phage membranes is drastically reduced [12,13]. Guanosine di- and triphosphate-mediated conversion of high-affinity sites to low-affinity sites is typical of receptors which either stimulate or inhibit adenylate cyclase via stimulatory or inhibitory N proteins [reviewed in 14]. We therefore examined whether the chemoattractant receptor was similarly linked to adenylate cyclase.

EVIDENCE THAT THE CHEMOATTRACTANT RECEPTOR IS NOT LINKED TO ADENYLATE CYCLASE

Several studies with intact human PMNs demonstrated that chemoattractants induce a transient elevation in intracellular cAMP levels which peaked at 15–20 sec [15–17]. Interestingly, the Ca^{2+} ionophore A23187 elicited a similar transient increase while the adenylate-cyclase-stimulating hormones prostaglandin E_1 (PGE_1) and isoproterenol produced a more prolonged increase in cAMP levels [17]. Chemoattractants and the Ca^{2+} ionophore were found to enhance intracellular cAMP levels in human PMNs by a unique mechanism that was distinctly different from that utilized by PGE_1 or isoproterenol [17]. Chelation of extracellular Ca^{2+} by EGTA or inhibition of intracellular Ca^{2+} redistribution by TMB-8 (8-(N,N-diethylamine)-octyl-3,4,5-trimethoxybenzoate) reduced cAMP elevations in response to chemoattractants or the Ca^{2+} ionophore by up to 100% without affecting cAMP increases in response to PGE_1 or isoproterenol. In contrast, α_2-adrenergic treatment of intact PMNs inhibited cAMP elevation to PGE_1 as expected but did not decrease chemoattractant- or A23187-elicited cAMP elevations [17]. More direct studies in purified PMN plasma membranes [17] confirmed the differences between chemoattractants and PGE_1 or isoproterenol in the mechanism of cAMP elevations observed in intact cells (Table I). Adenylate cyclase activity in PMN plasma membranes could be stimulated either with PGE_1 or isoproterenol in a GTP-dependent manner, demonstrating the presence of functional receptors, N_s, and catalytic unit of adenylate cyclase. Furthermore, the inhibitory N_i also was functional in these membranes since α_2-agonist treatment caused a significant reduction in adenylate cyclase activation in response to PGE_1. However, the chemotactic peptide over a wide dose range was neither stimulatory nor inhibitory when added with or without GTP to those membrane preparations. These data indicated that chemoattractant receptors are not coupled to adenylate

TABLE I. Modulation of Adenylate Cyclase in PMN Membranes by α_2-Adrenergic Agonist but not fMet-Leu-Phe*

Stimulus	Dose (μM)	Pretreatment (pmol cAMP/min/mg protein)[a]		
		Buffer	α_2 Agonist	fMet-Leu-Phe
Prostaglandin E_1	10.0	49 ± 2	31 ± 5[b]	49 ± 6
	1.0	39 ± 6	28 ± 3[b]	40 ± 4
Isoproterenol	100.0	42 ± 2	5 ± 2[b]	38 ± 2
fMet-Leu-Phe	10.0	0 ± 1		
	1.0	0 ± 1		

*PMN membranes were pretreated for 3 min with buffer, α_2 agonist (epinephrine (30 μM) plus propanolol (10 μM) or fMet-Leu-Phe (1 μM) before addition of a stimulus for 10 min (adapted from [17]).

[a]Values represent the mean net stimulated cAMP production after subtraction of unstimulated values.

[b]$P < .01$ compared with buffer pretreatment.

cyclase and utilize neither N_s nor N_i in the customary manner of hormones that regulate this enzyme. Since our observations in intact cells had shown that cAMP elevations by chemoattractants were mediated by a Ca^{2+}-dependent mechanism and since cellular activation by these stimuli were also associated with rapid increases in cytosolic Ca^{2+}, it was hypothesized that chemoattractant receptors utilized N proteins in a transduction mechanism linked to the generation of a Ca^{2+} signal [1,2,18].

EVIDENCE THAT AN N PROTEIN MEDIATES POLYPHOSPHOINOSITIDE DEGRADATION BY CHEMOATTRACTANT RECEPTORS

Recent findings in numerous cell types, including PMNs, monocytes, and differentiated HL-60 cells, have demonstrated a close correlation between the rapid rises in cytosolic [Ca^{2+}] and degradation of polyphosphoinositides observed after hormone stimulation [19–23]. Substantial evidence exists [reviewed in 24] indicating that receptor occupancy by Ca^{2+}-mobilizing hormones can lead to the activation of a polyphosphoinositide-specific phospholipase C which degrades PIP_2 into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). These two products can serve as intracellular messengers since introduction of IP_3 into permeabilized cells induces the release of stored Ca^{2+} from the endoplasmic reticulum [25], thereby promoting activation of Ca^{2+}/calmodulin-dependent protein kinases, while DAG can directly activate protein kinase C [reviewed in 26].

To determine whether N proteins play a role in chemoattractant-induced phospholipase C activation, we developed an assay system for this enzyme in purified PMN plasma membranes [27]. Incubation of purified plasma membranes with [γ-^{32}P]ATP in the presence of $MgCl_2$ results in rapid incorporation of radioactivity into phosphatidic acid, phosphatidylinositol 4-phosphate (PIP), and PIP_2. Receptor-mediated degradation of PIP_2 in these ^{32}P-labeled membranes could be monitored under defined conditions where the concentrations of regulatory agents such as guanine nucleotides or Ca^{2+} could be varied. In agreement with other reports [28], millimolar [Ca^{2+}] promoted PIP_2 hydrolysis in the absence of agonist [27], but at physiologic Ca^{2+} levels neither chemoattractant nor GTP alone led to PIP_2 hydrolysis (Table II). However, fMet-Leu-Phe stimulated PIP_2 hydrolysis in PMN membranes at low (2 μM) concentrations of Ca^{2+} if GTP was also present. Neither guanosine diphosphate nor other nucleotides were effective. Therefore, stimulation of phospholipase C by the chemoattractant receptor apparently is mediated by the activation of the N protein with GTP. This conclusion was substantiated by demonstrating that nonhydrolyzable analogs of GTP such as GppNHp or GTPγS stimulate PIP_2 hydrolysis in the absence of the chemoattractant. Interestingly, the Ca^{2+} requirements for phospholipase C activation were reduced by approximately two orders of magnitude by the addition of GTP. Coaddition of fMet-Leu-Phe further reduced the Ca^{2+} requirement to submicromolar levels [29]. Thus, the function of the GTP-activated N protein appears to be to reduce the requirement of the phospholipase C for Ca^{2+} so that the enzyme becomes active at ambient [Ca^{2+}] [29].

The specific requirement for guanosine triphosphates is typical for all members of the family of N proteins, which include N_s and N_i of the adenylate cyclase system, transducin of the light-activated cGMP phosphodiesterase in outer segments of retina, as well as N_o, a ubiquitous protein of unknown function [reviewed in 30]. These four N proteins are heterotrimers which have distinct α subunits but share common β and

TABLE II. Receptor-Stimulated PIP_2 Hydrolysis in Isolated PMN Plasma Membranes*

Stimulus	% PIP_2 remaining[a] $CaCl_2$ added	
	2 μM	1,000 μM
None	96 ± 5	54 ± 7
10 μM GTP	94 ± 6	52 ± 8
0.1 μM fMet-Leu-Phe	93 ± 2	45 ± 8
0.1 μM fMet-Leu-Phe + 10 μM GTP	72 ± 2[b]	45 ± 8

*Plasma membrane preparations were phosphorylated with [γ^{32}P]ATP and incubated for an additional 60 sec at the indicated Ca^2 concentration with the indicated stimuli (adapted from reference [27]).
[a]Values represent mean percentage ± SEM of radioactivity remaining in PIP_2 compared with samples incubated with buffer containing 0.5 mM EGTA.
[b]$P < .01$ compared to buffer at the same $CaCl_2$ concentration.

γ subunits. Certain bacterial toxins provide useful tools to identify N proteins since they specifically ADP-ribosylate and thereby modify the activity of the α subunits. For example, choleratoxin (CT) ADP ribosylates the 43,000-Da α_s and the 39,000-Da α_t subunits of N_s and transducin, respectively. Similarly, Bordetella pertussis toxin (PT) mediates ADP-ribosylation of the 41,000-Da α_i, the 39,000 α_o, and also the α_t subunit of N_i, N_o, and transducin, respectively. When plasma membranes from PMNs or monocytes were ADP-ribosylated with CT, radioactive proteins with molecular weights of approximately 43 kDa were detected, while PT resulted in the appearance of an intensely labeled band near the 40–41-kDa area [31–34]. Interestingly, cytosolic fractions from PMNs and monocytes contain significant amounts of PT-sensitive substrates of ca 41 kDa, suggesting that this N protein may differ from N_i, which is membrane bound [34; unpublished observation].

Choleratoxin has previously been shown to raise cAMP levels in PMNs and to inhibit chemotaxis [35]. PMNs were therefore treated with PT to determine whether a PT-sensitive N protein was involved in the chemoattractant receptor transduction mechanism. Exposure of PMNs to PT (1.0 μg/ml, 90 min) inhibited their chemotactic responses to fMet-Leu-Phe (Table III), the complement product C5a [21], LTB_4, and PAF [36–38]. The inhibitory effects of PT were specifically directed toward cellular activation by chemoattractants, since O_2^- production of PMNs in response to the Ca^{2+} ionophore, phorbol myristate acetate (PMA), or the lectins Con A and wheat germ agglutinin were largely unaffected (Table III). Similarly, PT blocked O_2^- production, enzyme release, and arachidonic acid release in guinea pig PMNs only in response to fMet-Leu-Phe but not A23187 [32,33].

To obtain information on the mechanism of PT-mediated inhibition of cellular activation by chemoattractants, Ca^{2+} mobilization was monitored in PMNs with the two fluorescent Ca^{2+} indicators, Quin 2 and chlortetracycline (Fig. 1). Enhanced Quin 2 fluorescence correlates with increased cytosolic [Ca^{2+}] while decreases in chlortetracycline fluorescence are thought to monitor losses of membrane-associated Ca^{2+} [39,40]. Cells which had been pretreated with PT failed to mobilize Ca^{2+} in response to fMet-Leu-Phe in both of these assay systems [21,37,41]. As observed in

TABLE III. Effects of PT on Biological Responses of PMN*

Stimulus		O_2^- production[a]		Lysozyme secretion[b]		Chemotaxis[c]	
		Buffer	PT	Buffer	PT	Buffer	PT
fMet-Leu-Phe	100 nM	163 ± 44	51 ± 14[d]	41 ± 7	31 ± 7[d]		
	10 nM	32 ± 6	6 ± 4[d]	28 ± 8	20 ± 8	54 ± 2	20 ± 11[d]
	1 nM	16 ± 11	0 ± 0			43 ± 2	20 ± 12[d]
PAF[e]	5 μM	115 ± 23	23 ± 11[d]	19 ± 6	19 ± 9		
	1 μM	80 ± 19	11 ± 6[d]	18 ± 8	2 ± 1 [d]		
	100 nM	35 ± 26	2 ± 2[d]	4 ± 2	1 ± 1		
Con A	50 μg/ml	281 ± 59	221 ± 47[d]	40 ± 7	35 ± 8		
	10 μg/ml	26 ± 13	18 ± 11	21 ± 4	24 ± 6		
A23187	5 μM	198 ± 6	106 ± 43	67 ± 17	67 ± 16		
PMA	10 ng/ml	268 ± 62	273 ± 87	44 ± 7	49 ± 7		
	2 ng/ml	146 ± 29	126 ± 52	22 ± 5	15 ± 4		

*Cells were incubated with buffer or 1.0 μg PT/ml for 90 min at 37°C, before assaying for O_2^- production, lysozyme secretion, or chemotaxis (adapted from reference [21]).
[a]Net O_2^- production expressed as difference ± SEM between absorbance of stimulated and unstimulated PMNs.
[b]Net lysozyme secretion as % of maximum release obtained with Triton X-100.
[c]Net distance (nm) of migration into the filter after subtraction of unstimulated migration.
[d]$P < .05$ by paired t-test.
[e]Platelet activating factor.

the biological functions, PT treatment failed to affect Quin 2 or chlortetracyline fluorescence changes induced by lectins or the Ca^{2+} ionophore.

Since PT releases the inhibitory regulation of adenylate cyclase by N_i, it was important to examine whether inhibition of cellular activation by PT was mediated by increases in intracellular cAMP levels. PT treatment of PMNs did not affect basal cAMP levels. Furthermore, PT treatment actually reduced cAMP elevations in response to fMet-Leu-Phe by 80% (Table IV). In contrast, PT did not significantly affect cAMP elevations induced by PGE_1. These findings support the hypothesis that fMet-Leu-Phe utilizes a Ca^{2+}-dependent mechanism to raise cAMP levels since Ca^{2+} mobilization in response to fMet-Leu-Phe was also abolished by PT treatment [21].

After having established that PT preferentially blocks leukocyte activation by chemoattractants, it remained to be shown that this toxin prevented the hydrolysis of PIP_2 by chemoattractants in PMNs and therefore the formation of second messengers. The effects of PT pretreatment on PIP_2 degradation in PMNs stimulated with fMet-Leu-Phe or Con A were measured (Table V). Polyphosphoinositide labeling of PMNs with $^{32}PO_4$ was not affected by PT pretreatment. Stimulation with fMet-Leu-Phe or Con A resulted in a significant, rapid loss of PIP_2 compared to unstimulated PMNs, presumably due to its phosphodiesteric cleavage to IP_3 and DAG [29]. This decrease in PIP_2 in response to fMet-Leu-Phe was clearly abolished in PT-treated PMNs [21]. Similarly, studies in HL-60 cells demonstrated that fMet-Leu-Phe could no longer stimulate IP_3 release after cells had been treated with PT [42]. Additionally, if plasma membranes were prepared from PT-treated PMNs, PIP_2 hydrolysis in response to the chemoattractant was completely inhibited [27]. This lack of PIP_2 degradation stimulated by chemoattractants presumably results from the inactivation of the N protein by PT since PIP_2 could still be hydrolyzed by high levels of Ca^{2+} in these membranes [27].

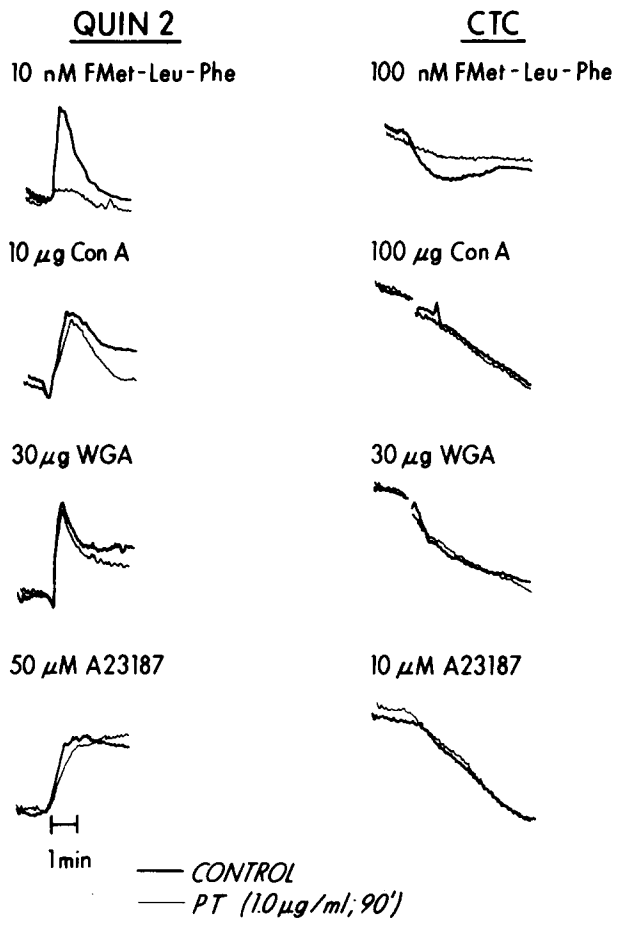

Fig. 1. Effects of pertussis toxin (PT) on Ca^{2+} mobilization. Polymorphonuclear leukocytes (PMNs) were incubated with buffer (dark lines) or with 1.0 μg PT/ml (light lines) for 90 min at 37°C and loaded with the fluorescent Ca^{2+} probes during the last 40 min. Quin 2 or chlorotetracycline (CTC) fluorescence was then measured after addition of stimuli as indicated.

MECHANISM FOR TERMINATION OF LEUKOCYTE ACTIVATION

Leukocyte responses such as PIP_2 degradation, Ca^{2+} mobilization, O_2^- production, and light scatter phenomena induced by a single dose of chemoattractant are transient (ca 2 min) [36,39,43,44]. Therefore, some mechanism which regulates the shutoff of these responses must exist. Termination could occur by feedback inhibition directed at any step of the activation pathway, including receptor-ligand interactions or subsequent events. Since chemoattractants promote DAG formation and increase cAMP levels via a Ca^{2+}-dependent process, it is possible that Ca^{2+}/phospholipid and/or cAMP-dependent protein kinases modify certain components in the transduction pathway, thereby inactivating them.

Numerous studies have demonstrated that sustained elevations of intracellular cAMP levels inhibit phagocyte activation by chemoattractants [15,35,45]. In contrast, cAMP-elevating drugs do not inhibit O_2^- production or enzyme secretion elicited by

TABLE IV. Effects of PT Pretreatment on Stimulated cAMP Production in Intact Human PMNs*

Stimulus	Pretreatment conditions (pmol cAMP/10^7 PMN)[a]	
	Buffer	PT
None	7.5 ± 2.2	8.4 ± 1.8
fMet-Leu-Phe 1.0 μM	18.0 ± 3.3[b]	10.3 ± 0.2
PGE_1 10.0 μM	11.5 ± 1.4[c]	11.0 ± 1.0[c]

*Cells were treated without or with 1.0 μg of PT/ml for 90 min at 37°C before cAMP measurements were taken 20 sec after addition of the indicated stimulus (adapted from reference [21]).
[a]Values represent mean ± SEM pmol cAMP/10^7 PMNs measured 20 sec after addition of stimulus.
[b]$P < .01$ for fMet-Leu-Phe vs none.
[c]$P < .05$ for PGE_1 vs none.

TABLE V. Effect of PT Treatment on Stimulated PIP_2 Degradation in Intact Human PMNs*

Stimulus	Pretreatment conditions	
	Buffer	PT
None	100[a]	100
fMet-Leu-Phe 100 nM	82 ± 3	100 ± 4[b]
Con A 100 μg/ml	88 ± 3	81 ± 9

*Cells were treated without or with 1.0 μg PT/ml for 90 min at 37°C before PIP_2 measurements were taken 15 sec after addition of the indicated stimulus (adapted from reference [27]).
[a]Values represent mean percentage ± SEM of radioactivity remaining in PIP_2 in stimulated compared to unstimulated cells.
[b]$P < .05$ compared with buffer pretreatment for the same stimulus.

PMA [46] and cause only 20–30% inhibition of these functions in response to A23187 [47]. However, lectin-stimulated PMN activation is also prevented by cAMP-elevating drugs [47]. Therefore cAMP, presumably through the activation of cAMP-dependent protein kinases, must interfere with a step in the transduction mechanism which is shared by chemoattractants and lectins but not by Ca^{2+} ionophores or PMA. The most likely target which fits these criteria is phospholipase C. Studies in human PMNs indicate that cAMP-elevating agents abrogate PIP_2 degradation in response to both fMet-Leu-Phe and Con A (manuscript in preparation). Similar findings have been reported for platelets, where prostacyclin reduces the amount of inositol-phosphates formed after challenge with thrombin [48]. However, the exact mechanism whereby cAMP inhibits leukocyte activation appears to be complex, since multiple steps in the polyphosphoinositide chain as well as phospholipase A_2 activation were affected in guinea pig PMNs treated with cAMP-elevating drugs [49].

Recent reports indicate that protein kinase C plays a role in deactivation of leukocyte responses to chemoattractants: preincubation of rabbit PMNs with high

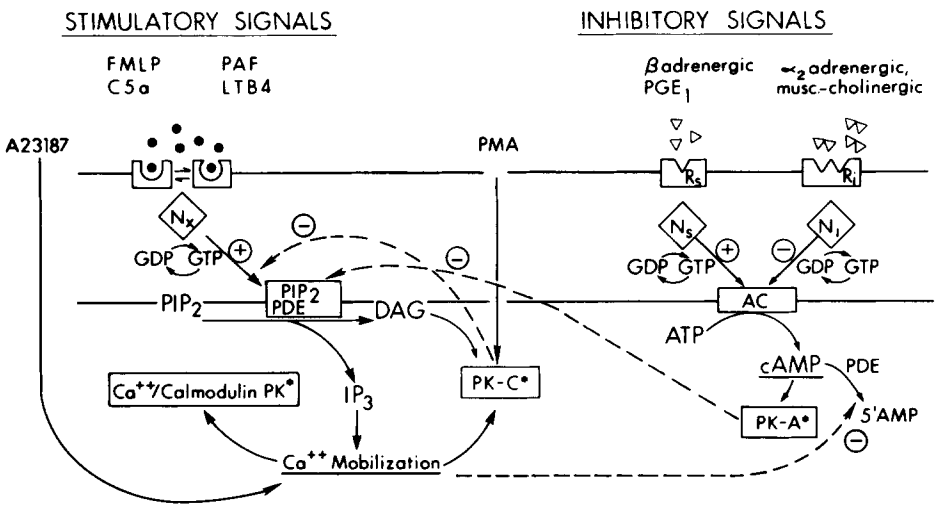

Fig. 2. A model for the regulation of leukocyte responses. Chemoattractant receptors exist in high- and low-affinity states that are allosterically regulated by a guanine nucleotide binding protein (N). The well-characterized model for regulation of adenylate cyclase (AC) is depicted on the right. The dotted lines indicate inhibitory pathways whereas the solid lines show stimulatory pathways. DAG, diacylglycerol; PK-A, cAMP-dependent protein kinase; PK-C, Ca^{2+}/phospholipid-dependent protein kinase [adapted from 18].

levels (100 ng/ml) of PMA prevented subsequent Ca^{2+} mobilization and enzyme release induced by fMet-Leu-Phe but not A23187 [50]. Additionally, PMA has been shown to inhibit Ca^{2+} mobilization and IP_3 formation in response to thrombin, α_1, or muscarinic receptor occupancy [51,52]. These inhibitory effects of PMA appear to be mediated through activation of protein kinase C since newly described inhibitors of this kinase release the block of IP_3 formation by PMA [53]. Studies with human PMNs indicate that PMA also reduces IP_3 formation in response to fMet-Leu-Phe. The locus of the PMA effect appears to involve the coupling of the activated N protein to phospholipase C [54].

MODEL OF REGULATION OF LEUKOCYTE RESPONSES

The observations summarized above are consistent with the schematic representation of leukocyte activation depicted in Figure 2. Occupancy of chemoattractant receptors leads to the replacement of GDP by GTP on an N protein. This N protein is sensitive to PT and has a molecular weight of ca 41 kDa, but unlike N_i, it does not inhibit adenylate cyclase and can easily be removed from the plasma membrane by sonication. The activated N protein stimulates phospholipase C to hydrolyze PIP_2, forming the Ca^{2+} mobilizer IP_3 and protein kinase C activator DAG. Agents that inhibit the function of this N protein, such as PT, diminish cellular activation by chemoattractants but not other stimuli such as the Ca^{2+} ionophore A23187 or PMA, which bypass this process. The products of PIP_2 degradation generate both positive and negative regulatory signals probably mediated by protein kinases. This model of leukocyte regulation appears to be applicable to other hormone receptor systems which initiate PIP_2 hydrolysis. Indeed, recent reports indicate that muscarinic cholinergic, bradykinin, and α_1 receptors utilize a similar pathway [24,55,56].

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