

UCSF

UC San Francisco Previously Published Works

Title

Pertussis toxin inhibition of chemotactic factor-induced calcium mobilization and function in human polymorphonuclear leukocytes.

Permalink

<https://escholarship.org/uc/item/3kv6466k>

Journal

Journal of Experimental Medicine, 162(1)

ISSN

0022-1007

Authors

Goldman, DW
Chang, FH
Gifford, LA
[et al.](#)

Publication Date

1985-07-01

DOI

10.1084/jem.162.1.145

Peer reviewed

PERTUSSIS TOXIN INHIBITION OF CHEMOTACTIC
FACTOR-INDUCED CALCIUM MOBILIZATION AND
FUNCTION IN HUMAN POLYMORPHONUCLEAR
LEUKOCYTES

BY D. W. GOLDMAN, F.-H. CHANG, L. A. GIFFORD, E. J. GOETZL, AND
H. R. BOURNE

*From the Howard Hughes Medical Institute, and the Division of Allergy and Immunology,
Departments of Medicine and Pharmacology, University of California Medical Center, San
Francisco, California 94143*

The binding of lipid and peptide chemotactic factors to cell surface receptors on polymorphonuclear leukocytes (PMNL)¹ initiates a series of intracellular biochemical events that lead to increased chemokinetic and chemotactic migration, aggregation, adherence, lysosomal degranulation, and production of superoxide anion (1–3). The rapid stimulation, by chemotactic factors, of phospholipid and protein methylation (4–6), arachidonic acid release (4, 5, 7), phosphatidyl inositol turnover (8–10), internal calcium release and influx of extracellular calcium (11, 12), glucose flux (13), and membrane depolarization (14) suggests that these events are closely coupled to receptor occupancy, yet little is known about the transductional process.

Modulation by guanine nucleotides of the affinity of receptors for *N*-formyl-methionyl peptides in membranes of human PMNL (15), and the activation, by *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), of a GTPase activity in homogenates of PMNL (16) suggested that a guanine nucleotide-binding protein may participate in the transduction of signals from occupied receptors. Hormonally stimulated activation and inhibition of adenylate cyclase are mediated by coupling proteins, termed G_s and G_i, respectively, which have similar subunit structures and amino acid compositions (17); the α subunit of each protein contains a guanine nucleotide-binding site. Cholera toxin-induced ADP-ribosylation of the α subunit of G_s stabilizes G_s in the active state, and results in persistent activation of adenylate cyclase (18–20). A bacterial toxin isolated from *Bordetella pertussis* similarly catalyzes ADP-ribosylation of the α subunit of G_i, and, consequently, attenuates hormonal inhibition of adenylate cyclase activity (18, 19, 21). Although pertussis toxin treatment of rat mast cells does not alter

This work was supported in part by grants HL 31809, AI 19784, and GM 27800 from the National Institutes of Health, Bethesda, MD. Dr. Goldman is an Investigator of the Arthritis Foundation.

¹ *Abbreviations used in this paper:* C5fr, C5 fragments; [Ca²⁺]_i, internal (cytosolic) calcium ion concentration; cAMP, cyclic AMP; DFP, diisopropyl fluorophosphate; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; HBSS, Hank's balanced salt solution; hpf, high power field; LTB₄, leukotriene B₄; OVA, ovalbumin; PMA, 4-β-phorbol-12-myristate-13-acetate; PMNL, polymorphonuclear leukocytes; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

cyclic AMP (cAMP) levels (22), it blocks ligand-stimulated release of histamine (23). That pertussis toxin might be acting on a GTP-binding protein was suggested by the capacity of stable analogs of GTP to promote histamine release (23–25). To determine whether a pertussis toxin-sensitive GTP-binding protein is involved in the coupling of PMNL chemotactic factor receptors to functional activation, we assessed the effects of pertussis toxin on binding of leukotriene B₄ (LTB₄) to human PMNL and on chemotactic factor-induced increases in cytosolic calcium concentration, chemotactic migration, and degranulation.

Materials and Methods

Materials. Hank's balanced salt solution without phenol red (HBSS) (University of California Cell Culture Facility, San Francisco, CA), diisopropylfluorophosphate (DFP), fMLP, *n*-butyl phthalate, 4- β -phorbol-12-myristate-13-acetate (PMA), phenolphthalein glucuronic acid, human albumin, lyophilized *Micrococcus lysodeikticus* (Sigma Chemical Co., St. Louis, MO), ovalbumin (OVA) (Miles Laboratories, Inc., Elkhart, IN), Ficoll-Paque, 6% (wt/vol) dextran 70 in normal saline (Pharmacia Fine Chemicals, Piscataway, NJ), α -[³²P]-NAD (New England Nuclear, Boston, MA), 5, 6, 8, 9, 11, 12, 14, 15 (*N*)-[³H]-LTB₄ (209 Ci/mole), Quin 2/AM (Amersham Corp., Arlington Heights, IL), A23187 (Calbiochem-Behring, La Jolla, CA), cytochalasin B (Aldrich Chemical Co., Milwaukee, WI), Hydrofluor scintillation fluid (National Diagnostics, Inc., Somerville, NJ) and gel electrophoresis reagents (Bio-Rad, Richmond, CA) were obtained from the suppliers noted. Partially purified chemoattractant fragments of C5 (C5fr) were prepared from yeast-activated human serum, as described (26). Synthetic LTB₄ was kindly supplied by J. Rokach of Merck Frosst Laboratories, Dorval, Canada. Purified pertussis toxin (27) was a gift from R. D. Sekura of the National Institute of Child Health and Human Development, Bethesda, MD.

Preparation of Human PMNL and Treatment with Pertussis Toxin. Human PMNL were prepared as described (28) from sodium citrate-anticoagulated venous blood of normal donors. Erythrocytes were removed by dextran sedimentation, followed by a 20-s hypotonic lysis in 20 vol of ice-cold distilled water. Isotonicity was restored by adding 0.6 M KCl. PMNL of $\geq 96\%$ purity were obtained by centrifugation of mixed leukocytes on Ficoll-Paque cushions (29). The purified PMNL were suspended at 2×10^7 cells/ml in HBSS containing 0.1% (wt/vol) OVA and 10 mM Hepes (pH 7.3) (HBSS-OVA).

2×10^7 PMNL/ml of HBSS-OVA were incubated at 37°C with different concentrations of pertussis toxin for 2 h. Quin 2-containing PMNL were prepared by adding 20 μ M diacetomethoxyester of Quin 2 15 min before the end of incubation. The PMNL were then washed several times and resuspended in HBSS-OVA. Control PMNL were incubated for 2 h at 37°C without any additions.

Measurement of Quin 2 Fluorescence. Chemotactic factor-induced changes in Quin 2 fluorescence, a measure of changes in the cytosolic calcium concentration (30), was determined as previously described (31). 10^7 PMNL were suspended in 2 ml of fresh HBSS-OVA and incubated at 37°C in the dark for 15 min. The fluorescence signal was then recorded with a Perkin-Elmer model 640-40 spectrofluorimeter (Perkin-Elmer, Mountain View, CA) equipped with a magnetic stirrer and a water-jacketed cuvette holder. The monochromators were set at 339 nm for excitation and 492 nm for emission. The maximum and minimum fluorescence signals were determined for each batch of PMNL after the addition of 20 μ l of 1% (wt/vol) Triton X-100, followed by 40 μ l of 0.5 M Tris and 0.5 M EGTA (pH 10). The autofluorescence from an equal number of PMNL without Quin 2 was subtracted from the Quin 2 fluorescence values. The corrected values were used to calculate the intracellular Ca⁺² concentration, as described (30).

Measurement of [³H]LTB₄ binding to PMNL. Replicate suspensions of 10^7 PMNL were incubated with 5×10^{-14} mol [³H]LTB₄ and different concentrations of nonradioactive LTB₄ in a final volume of 0.5 ml for 40 min in an ice-water bath. The PMNL were centrifuged through a phthalate-oil cushion, and the amounts of bound and free LTB₄

were fit to either a one-site or two-site binding model by a method of weighted nonlinear least squares based on the LIGAND program (32) adapted for use on a Hewlett-Packard model 86B personal computer (Hewlett-Packard, Inc., Palo Alto, CA) (33).

Measurement of Chemotactic Migration and Degranulation. Chemotactic migration was performed in modified Boyden chambers (Adaps, Inc., Dedham, MA) assembled with micropore filters of 3 μm pore diameter (Sartorius, Gottingen, Federal Republic of Germany) (28, 33). PMNL were counted in 10 high-power microscope fields (hpf), 5 from each of duplicate filters, at a depth of 80–100 μm from the cell source. The response is expressed as net PMNL/hpf, after subtraction of background migration of PMNL in control chambers lacking a stimulus. The altered chemotactic migration of PMNL pretreated with pertussis toxin is expressed as a percentage of the migration of replicate suspensions of PMNL preincubated in HBSS-OVA alone.

The stimulation of release of β -glucuronidase and lysozyme from PMNL was determined in HBSS-OVA containing 5 $\mu\text{g}/\text{ml}$ of cytochalasin B. Each supernatant was assayed colorimetrically for β -glucuronidase, as described (26), and for lysozyme by measuring the rate of digestion of *Micrococcus lysodeikticus*, and the resultant decrease in turbidity of the suspension (34). The amount of enzyme release is expressed as a percentage of the total amount of activity present in replicate suspensions of PMNL that had been disrupted by sonication on ice. The percentage of enzyme released in the absence of a stimulus was subtracted to determine the net percentage release. The altered release of enzymes by PMNL pretreated with pertussis toxin is presented as a percentage of the release by PMNL preincubated in HBSS-OVA alone. In separate experiments, the simultaneous addition to PMNL of 100 ng/ml of pertussis toxin and a stimulus had no effect on the amount of β -glucuronidase and lysozyme released by the stimulus alone, and the presence of up to 100 ng/ml of pertussis toxin with the respective substrates failed to alter the expression of activity of either enzyme.

Pertussis Toxin Catalyzed ADP-ribosylation of PMNL Homogenates. PMNL were treated with 10^{-4} M DFP in HBSS without calcium and magnesium at 37°C for 30 min (35). The PMNL were washed several times, resuspended at 10^8 cells/ml in ice cold 0.1 M sucrose containing 2 mM EDTA and 5 mM Tris-HCl (pH 7.2), and disrupted in a Dounce homogenizer with 120 strokes at 4°C. The homogenate was centrifuged at 800 g for 10 min at 4°C and the supernatants were stored at -70°C .

Pertussis toxin-catalyzed ADP-ribosylation of aliquots from the 800 g supernatants was performed in 0.1 M sucrose containing 10 μM α - ^{32}P]NAD, 2.5 mM MgCl_2 , 1 mM ATP, 10 mM thymidine, 1 mM dithiothreitol, and 5 mM Tris-HCl (pH 7.2), as previously described (19, 36). The reaction was terminated by the addition of 5 vol of Laemmli sample buffer (37). Protein was precipitated with 20% (wt/vol) of trichloroacetic acid, and the pellet was washed with anhydrous diethyl ether. The protein pellet was resuspended in Laemmli sample buffer, and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (37). To quantitate incorporation of ^{32}P into the 41 kilodalton (kD) protein, the band was cut from dried gels and counted in a scintillation counter.

Results

Effects of Pertussis Toxin on Cytosolic Calcium. Quin 2-containing PMNL were used to monitor changes in the cytosolic concentration of intracellular calcium ($[\text{Ca}^{+2}]_{\text{in}}$). Concentrations of LTB_4 and fMLP >1 and 10 nM, respectively, elicited a maximal increase of 1.8–2.5-fold in $[\text{Ca}^{+2}]_{\text{in}}$, from a resting value of 69–145 nM to a peak value of 150–351 nM (range, $n = 12$) within 0.6–1.5 min (Fig. 1). Pretreatment of PMNL with 100 ng/ml of pertussis toxin for 2 h inhibited the LTB_4 - and fMLP-induced increases in $[\text{Ca}^{+2}]_{\text{in}}$ by $98 \pm 2\%$ (mean \pm SD; $n = 4$) without altering the baseline value for $[\text{Ca}^{+2}]_{\text{in}}$ (Fig. 1). The inhibition of LTB_4 -induced changes in $[\text{Ca}^{+2}]_{\text{in}}$ by pertussis toxin was concentration-dependent with half-maximal inhibition occurring at a concentration of 30 ng/ml pertussis toxin.

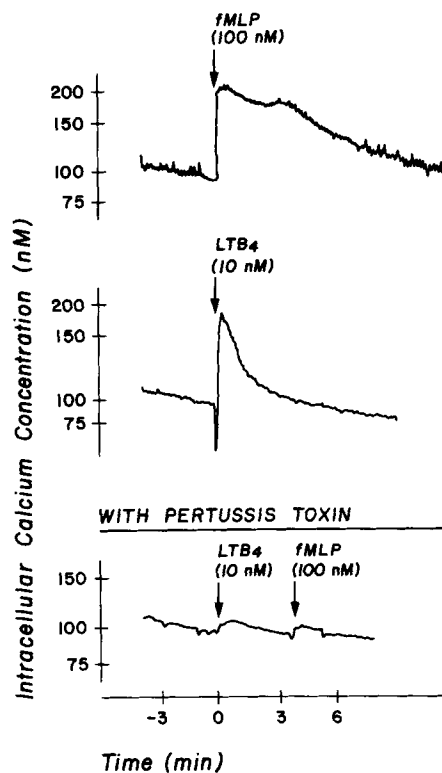


FIGURE 1. Pertussis toxin inhibition of the chemotactic factor-induced increase in $[Ca^{2+}]_{in}$ in human PMNL. The fluorescent signal from Quin 2-containing control PMNL was recorded for 3 min before and 10 min after the addition of either 10 nM LTB_4 or 100 nM fMLP. The fluorescent signal from Quin 2-containing PMNL that had been treated with 100 ng/ml pertussis toxin was recorded for 3 min before and 3 min after the addition of LTB_4 , then 100 nM fMLP was added, and the fluorescent signal was recorded for an additional 3 min.

In initial studies designed to assess the time course of pertussis toxin inhibition of the effects of LTB_4 on $[Ca^{2+}]_{in}$, PMNL were pretreated with pertussis toxin for 30 and 90 min before washing and adding 10^{-7} M LTB_4 . Pertussis toxin at 100, 300, and 1,000 ng/ml inhibited LTB_4 -induced increases in $[Ca^{2+}]_{in}$ by means of 5%, 6%, and 4% at 30 min, and 58%, 82%, and 80% at 90 min. A lag time of 30–60 min has also been observed for the effects of pertussis toxin on rat mast cells (22), pancreatic islet cells (38), and 3T3 cells (39), and is presumed to be required for binding of pertussis toxin and transport of the active subunit to the intracellular site of action (40). Pertussis toxin suppressed the increase in $[Ca^{2+}]_{in}$ at concentrations of LTB_4 up to 3×10^{-7} M, indicating that the toxin inhibits the increase in $[Ca^{2+}]_{in}$ stimulated by both the high-affinity and low-affinity subsets of LTB_4 receptors.

Effect of Pertussis Toxin on Chemotaxis and Enzyme Release. The effects of pertussis toxin on chemotactic migration and lysosomal enzyme release were assessed to examine the possible role of increases in $[Ca^{2+}]_{in}$ in the activation of PMNL. Chemotaxis elicited by LTB_4 , C5fr, and fMLP, and the release of β -glucuronidase elicited by LTB_4 and fMLP in the presence of cytochalasin B were

inhibited by pertussis toxin with a concentration-dependence similar to that for inhibition of chemotactic factor-induced changes in $[Ca^{+2}]_{in}$ (Figs. 2 and 3). Net release of $12.4 \pm 0.2\%$ and $27.5 \pm 4.8\%$ (mean \pm SD; $n = 3$) of lysozyme from control PMNL was elicited by 10 nM LTB₄ and 1 μ M fMLP, respectively. The release of lysozyme by the two chemotactic factors was decreased by means of 88.7% and 87.6%, respectively, after pretreatment with 100 ng/ml of pertussis toxin. The inhibition by pertussis toxin was specific for chemotactic factor-induced release of lysosomal enzyme. Neither the release of β -glucuronidase evoked by the calcium ionophore A23187 or by PMA, which stimulates a secretory response without raising $[Ca^{+2}]_{in}$ (41), was significantly affected by pertussis toxin (Fig. 3). Lysozyme secretion elicited from PMNL treated with 100 ng/ml of pertussis toxin was 91.1% and 78.6% of the release elicited from control PMNL in response to PMA and A23187, respectively.

Effect of Pertussis Toxin on LTB₄ Receptors. Incubation of PMNL with 100 ng/ml pertussis toxin significantly reduced the number of high-affinity receptors for LTB₄ by a mean of 61% on PMNL, and consistently induced a threefold increase in their affinity for LTB₄, without affecting the number or agonist affinity of the low-affinity LTB₄ receptors (Table I). This suggests that pertussis toxin acts at a step in the signal transduction process that is closely linked to the LTB₄ receptor.

ADP-ribosylation of PMNL Homogenates. Preactivated pertussis toxin specifically catalyzes the transfer of [³²P]-labeled ADP-ribose from [³²P]NAD to a 41 kD protein in PMNL homogenates (Fig. 4). The protein comigrates on SDS-PAGE with the ADP-ribosylated α subunit of G_i from human erythrocyte

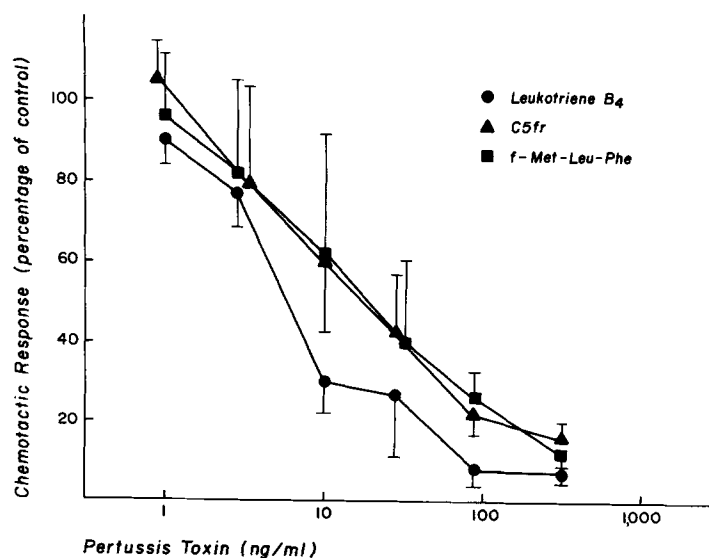


FIGURE 2. Concentration-dependence of inhibition of chemotaxis by pertussis toxin. Chemotactic migration of 21 ± 5 , 25 ± 8 , and 20 ± 4 net PMNL per hpf was elicited from control leukocytes by 3 nM LTB₄ (●), 100 nM fMLP (■), and a 1/20 dilution of C5fr (▲), respectively. The stimulated response from pertussis toxin-treated PMNL is expressed as a percentage of the response obtained from control PMNL. Each data point is the mean \pm SD of three or four experiments.

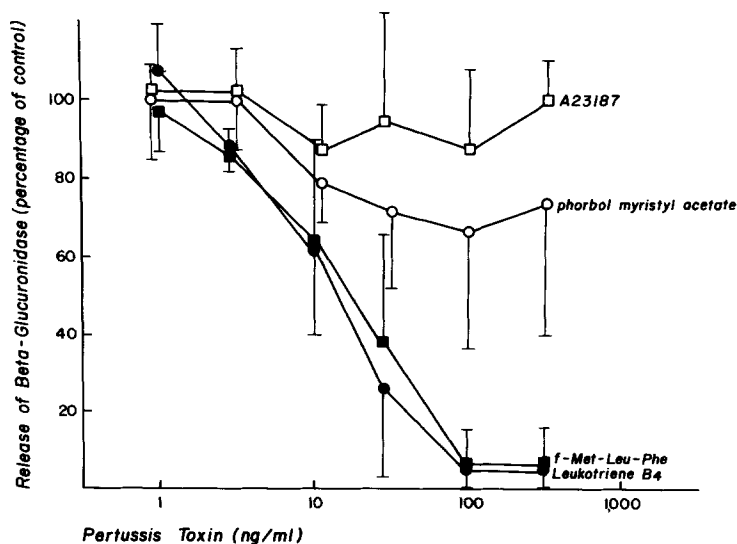


FIGURE 3. Concentration-dependence of inhibition of β -glucuronidase release by pertussis toxin. Control PMNL released $18.5 \pm 9.7\%$, $40.8 \pm 18.0\%$, $22.0 \pm 9.6\%$, and $66.7 \pm 13.7\%$ of the total β -glucuronidase activity in response to 3 nM LTB₄ (●), 1 μ M fMLP (■), 10 ng/ml PMA (○), and 2 μ M A23187 (□), respectively, in the presence of 5 μ g/ml of cytochalasin B. The stimulated response from pertussis toxin-treated PMNL is expressed as a percentage of the response obtained from control PMNL. Each data point is the mean \pm SD of four experiments.

TABLE I
Binding of LTB₄ to PMNL Treated With and Without Pertussis Toxin

Treatment	High-affinity receptors		Low-affinity receptors	
	K_d ($\times 10^{-10}$)	Receptors/PMNL	K_d ($\times 10^{-7}$)	Receptors/PMNL
	<i>M</i>		<i>M</i>	
Control	9.6 ± 4.9	$9,900 \pm 3,100$	3.5 ± 2.6	$440,000 \pm 190,000$
Pertussis toxin	3.3 ± 2.9	$3,900 \pm 900$	2.0 ± 0.7	$440,000 \pm 200,000$
<i>P</i>	0.061	0.026	0.27	0.47

PMNL were incubated without (control) or with 100 ng/ml pertussis toxin for 2 h. The binding of [³H]LTB₄ was then assessed at 0°C. The binding parameters represent the mean \pm SD obtained from three experiments. The *P* values were determined by a paired sample *t*-test.

membranes. Homogenates prepared from control PMNL incorporated 2.2 pm of ADP-ribose per milligram of protein, while homogenates of PMNL treated with 100 ng/ml of pertussis toxin incorporated 0.9 pm of ADP-ribose per milligram of protein. Preincubation of PMNL with 300 ng/ml of pertussis toxin for 3 h did not further reduce the amount of [³²P]-labeled ADP-ribose subsequently incorporated into the homogenate. Increasing the amount of NAD in the reaction mixture and adding a second aliquot of [³²P]NAD 15 min after initiating the ADP-ribosylation incubation did not increase the amount of ADP-ribose incorporated; this suggested that the incubation conditions caused complete ADP-ribosylation of the 41 kD protein. Thus, only 59% of the 41 kD

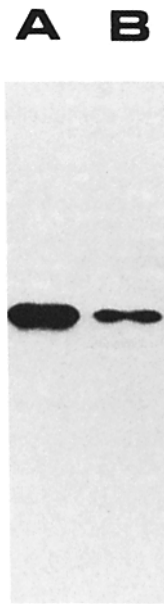


FIGURE 4. Pertussis toxin-catalyzed ADP-ribosylation of proteins of homogenates of PMNL. PMNL were incubated without (A) or with (B) 100 ng/ml pertussis toxin for 2 h. Homogenates prepared from treated PMNL were incubated with activated pertussis toxin and 10 μ M α - 32 P]NAD, and the ADP-ribosylated proteins were analyzed on SDS-PAGE.

proteins in PMNL homogenates are accessible for pertussis toxin-stimulated ADP-ribosylation in intact PMNL. Pertussis toxin-catalyzed modification of these accessible sites is associated with inhibition of stimulated PMNL function.

Discussion

The possible involvement of a guanine nucleotide-binding protein in the signal transduction process initiated by occupancy of chemotactic factor receptors in PMNL (15, 16), combined with the inhibition by pertussis toxin of calcium-dependent release of histamine in mast cells (23), suggested that pertussis toxin may inhibit the increase in $[Ca^{+2}]_{in}$ stimulated by chemotactic factors in human PMNL. The use of Quin 2 to measure $[Ca^{+2}]_{in}$ permitted a direct demonstration of the inhibitory effects of pertussis toxin on chemotactic factor-induced stimulation of $[Ca^{+2}]_{in}$ (Fig. 1). The increase in $[Ca^{+2}]_{in}$ induced by LTB₄ and fMLP in PMNL consists of two components, an increased influx of extracellular calcium, and calcium release from intracellular stores (11, 12). Human PMNL incubated in calcium-free media containing 5 mM EGTA will respond to LTB₄ and fMLP with an increase in Quin 2 fluorescence, but the increase is only 10%–15% of that observed in the presence of extracellular calcium (D. W. Goldman, unpublished observation), suggesting that the inhibition of Quin 2 fluorescence changes are in part due to the inhibition of calcium influx. Recent studies have shown that the influx of $^{45}Ca^{+2}$ induced by fMLP in guinea pig neutrophils (42) and the changes in $[Ca^{+2}]_{in}$ induced by fMLP and LTB₄ in rabbit neutrophils (43) are inhibited by pertussis toxin.

Pertussis toxin inhibited chemotactic factor-induced $[Ca^{+2}]_{in}$ increases, chemotaxis, and lysosomal enzyme release, but not non-receptor-stimulated enzyme release (Figs. 2 and 3), demonstrating that pertussis toxin acts at an early stage in the signal transduction process. The decrease in high-affinity LTB₄ receptor number induced by pertussis toxin (Table I) implies a direct link between the site of pertussis toxin action and the LTB₄ receptor. Guanine nucleotides also decrease the number of high-affinity fMLP (15) and LTB₄ (D. W. Goldman, unpublished observation) receptors on PMNL plasma membranes. That pertussis toxin may modify a guanine nucleotide-binding protein is suggested by the similar effects of guanine nucleotides and pertussis toxin on chemotactic factor receptors and on α_2 adrenergic receptors. In neuroblastoma \times glioma hybrid cell membranes (44) and in rat renal cortical membranes (45), α_2 adrenergic receptor affinity for agonist binding is decreased, consistent with a redistribution of receptors between high- and low-affinity states. Pertussis toxin attenuated agonist-induced inhibition of adenylate cyclase, with a concomitant decrease in average affinity of agonist binding of α_2 adrenergic receptors.

Reagents that increase cAMP levels in PMNL inhibit chemotaxis and lysosomal enzyme release (46). Pertussis toxin-catalyzed ADP-ribosylation of the α subunit of G_i uncouples adenylate cyclase from inhibition by α_2 adrenergic agonists (18, 19, 21, 44), with a resultant enhancement of cAMP levels in response to agonists that stimulate adenylate cyclase. However, pertussis toxin had no effect on cAMP levels in mast cells (22), and has been recently reported not to alter cAMP levels (42) or to substantially modify the fMLP-induced cAMP rise (47) in guinea pig neutrophils. This suggests that the inhibitory effects of pertussis toxin on PMNL function are not secondary to a change in cAMP content.

A 41 kD protein, which comigrated with the α subunit of G_i from human erythrocyte membranes on SDS-PAGE, was ADP-ribosylated by pertussis toxin in PMNL homogenates (Fig. 4). Although only 59% of the total sites available for ADP-ribosylation in the homogenates were accessible in intact PMNL, PMNL function was almost completely inhibited. Several studies (48) have identified subpopulations of PMNL that are functionally nonresponsive to chemotactic factors. If the 41 kD protein were accessible for ADP-ribosylation by pertussis toxin only in responding PMNL, then ADP-ribosylation of only a fraction of the total sites detected in homogenates could account for the almost complete inhibition of PMNL function. In that case, the defect in the nonresponding PMNL subpopulation might be caused by uncoupling of the 41 kD protein from the chemotactic factor receptors; the uncoupling phenomenon could reflect the same biochemical defect that makes the protein inaccessible to pertussis toxin in intact PMNL. Alternatively, all the PMNL may be sensitive to pertussis toxin, but a fraction of the 41 kD protein may be sequestered in an intracellular compartment that is not accessible to pertussis toxin, or is not involved in the signal transduction process.

Summary

Chemotactic factors stimulate a rapid increase in the cytosolic concentration of intracellular calcium ions ($[Ca^{2+}]_{in}$) in human polymorphonuclear leukocytes (PMNL), which may be an event that is critical to the expression of chemotaxis

and other PMNL functions. Treatment of PMNL with pertussis toxin catalyzes ADP-ribosylation of a protein similar or identical to the inhibiting regulatory protein of adenylate cyclase, G_i , and suppresses the increase in $[Ca^{2+}]_{in}$ elicited by leukotriene B_4 (LTB_4) and formyl-methionyl-leucyl-phenylalanine. Chemotactic migration and lysosomal enzyme release elicited by chemotactic factors were inhibited by pertussis toxin with a concentration-dependence similar to that for inhibition of the increase in $[Ca^{2+}]_{in}$, without an effect on lysosomal enzyme release induced by the ionophore A23187 and phorbol myristate acetate. Activated pertussis toxin catalyzed the $[^{32}P]$ ADP-ribosylation of a 41 kD protein in homogenates of PMNL. The extent of $[^{32}P]$ ADP-ribosylation of this protein was reduced 59% by pretreatment of intact PMNL with pertussis toxin. Pertussis toxin selectively decreased the number of high-affinity receptors for LTB_4 on PMNL by 60% without altering the number or binding properties of the low-affinity subset of receptors. Pertussis toxin modification of a membrane protein of PMNL analogous to G_i thus simultaneously alters chemotactic receptors and attenuates the changes in cytosolic calcium concentration and PMNL function caused by chemotactic factors.

Received for publication 7 January 1985 and in revised form 27 March 1985.

References

1. Snyderman, R., and E. J. Goetzl. 1981. Molecular and cellular mechanisms of leukocyte chemotaxis. *Science (Wash. DC)* 213:830.
2. Tauber, A. I. 1981. Current views of neutrophil dysfunction: An integrated clinical perspective. *Am. J. Med.* 70:1237.
3. Snyderman, R., and M. C. Pike. 1984. Chemoattractant receptors on phagocytic cells. *Annu. Rev. Immunol.* 2:257.
4. Snyderman, R., M. C. Pike, and N. M. Kredich. 1980. Role of transmethylation reactions in cellular motility and phagocytosis. *Mol. Immunol.* 17:209.
5. Hirata, F., B. A. Corcoran, K. Venkatasubramanian, E. Schiffman, and J. Axelrod. 1979. Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. *Proc. Natl. Acad. Sci. USA.* 76:2640.
6. O'Dea, R. F., O. H. Viveros, J. Axelrod, S. Aswanikumar, E. Schiffman, and B. A. Corcoran. 1978. Rapid stimulation of protein carboxymethylation in leukocytes by a chemotactic peptide. *Nature (Lond.)* 272:462.
7. Bokoch, G. M., and P. W. Reed. 1980. Stimulation of arachidonic acid metabolism in the polymorphonuclear leukocyte by an *N*-formylated peptide: Comparison with ionophore A23187. *J. Biol. Chem.* 255:10223.
8. Volpi, M., R. Yassin, W. Tao, T. F. P. Molski, P. H. Naccache, and R. I. Sha'afi. 1984. Leukotriene B_4 mobilizes calcium without the breakdown of polyphosphoinositides and the production of phosphatidic acid in neutrophils. *Proc. Natl. Acad. Sci. USA.* 81:5966.
9. Cockcroft, S., J. P. Bennett, and B. D. Gomperts. 1981. The dependence on Ca^{2+} of phosphatidylinositol breakdown and enzyme secretion in rabbit neutrophils stimulated by formylmethionylleucylphenylalanine or ionomycin. *Biochem. J.* 200:501.
10. Rubin, R. P., L. E. Sink, and R. J. Freer. 1981. On the relationship between formylmethionyl-leucyl-phenylalanine stimulation of arachidonyl phosphatidylinositol turnover and lysosomal enzyme secretion by rabbit neutrophils. *Mol. Pharmacol.* 19:31.

11. Sha'afi, R. I., P. H. Naccache, T. F. P. Molski, P. Borgeat, and E. J. Goetzl. 1981. Cellular regulatory role of leukotriene B₄: Its effect on cation homeostasis in rabbit neutrophils. *J. Cell. Physiol.* 108:401.
12. Naccache, P. H., H. J. Showell, E. L. Becker, and R. I. Sha'afi. 1977. Transport of sodium, potassium and calcium across rabbit polymorphonuclear leukocyte membranes. *J. Cell Biol.* 73:428.
13. McCall, C. E., D. Bass, S. Cousart, and L. R. DeChatelet. 1979. Enhancement of hexose uptake in human polymorphonuclear leukocytes by activated complement component C5a. *Proc. Natl. Acad. Sci. USA.* 76:5896.
14. Seligmann, B., E. K. Gallin, D. L. Martin, W. Shain, and J. I. Gallin. 1980. Interaction of chemotactic factors with human polymorphonuclear leukocytes: Studies using a membrane potential sensitive cyanine dye. *J. Membr. Biol.* 52:257.
15. Koo, C., R. J. Lefkowitz, and R. Snyderman. 1983. Guanine nucleotides modulate the binding affinity of the oligopeptide chemoattractant receptor on human polymorphonuclear leukocytes. *J. Clin. Invest.* 72:748.
16. Hyslop, P. A., Z. G. Oades, A. J. Jesaitis, R. G. Painter, C. G. Cochrane, and L. A. Sklar. 1984. Evidence for *N*-formyl chemotactic peptide-stimulated GTPase activity in human neutrophil homogenates. *FEBS Lett.* 166:165.
17. Manning, D. R., and A. G. Gilman. 1983. The regulatory components of adenylate cyclase and transducin. A family of structurally homologous guanine nucleotide-binding proteins. *J. Biol. Chem.* 258:7059.
18. Bokoch, G. M., T. Katada, J. K. Northup, and A. G. Gilman. 1983. Identification of the predominant substrate for ADP-ribosylation by islet activating protein. *J. Biol. Chem.* 258:2072.
19. Codina, J., J. Hildebrandt, R. Iyengar, L. Birnbaumer, R. D. Sekura, and C. R. Manclark. 1983. Pertussis toxin substrate, the putative Ni component of adenylate cyclases, is an alpha-beta heterodimer regulated by guanine nucleotide and magnesium. *Proc. Natl. Acad. Sci. USA.* 80:4276.
20. Cassel, D., and Z. Selinger. 1977. Mechanism of adenylate cyclase activation by cholera toxin: Inhibition of GTP hydrolysis at the regulatory site. *Proc. Natl. Acad. Sci. USA.* 74:3307.
21. Murayama, T., and M. Ui. 1983. Loss of the inhibitory function of the guanine nucleotide regulatory component of adenylate cyclase due to its ADP-ribosylation by islet-activating protein, pertussis toxin, in adipocyte membranes. *J. Biol. Chem.* 258:3319.
22. Nakamura, T., and M. Ui. 1983. Suppression of passive cutaneous anaphylaxis by pertussis toxin, an islet-activating protein, as a result of inhibition of histamine release from mast cells. *Biochem. Pharmacol.* 32:3435.
23. Nakamura, T., and M. Ui. 1984. Islet-activating protein, pertussis toxin, inhibits Ca²⁺-induced and guanine nucleotide-dependent releases of histamine and arachidonic acid from rat mast cells. *FEBS Lett.* 173:414.
24. Gomperts, B. D. 1983. Involvement of guanine nucleotide-binding protein in the gating of Ca²⁺ by receptors. *Nature (Lond.)* 306:64.
25. Fernandez, J. M., E. Neher, and B. D. Gomperts. 1984. Capacitance measurements reveal stepwise fusion events in degranulating mast cells. *Nature (Lond.)* 312:453.
26. Goetzl, E. J., and W. C. Pickett. 1980. The human PMN leukocyte chemotactic activity of complex hydroxy-eicosatetraenoic acids (HETEs). *J. Immunol.* 125:1789.
27. Sekura, R. D., F. Fish, C. R. Manclark, B. Meade, and Y. Zhang. 1983. Pertussis toxin: Affinity purification of a new ADP-ribosyltransferase. *J. Biol. Chem.* 258:14647.
28. Goetzl, E. J., and K. F. Austen. 1972. A neutrophil-immobilizing factor derived from human leukocytes. I. Generation and partial characterization. *J. Exp. Med.* 136:1564.

29. Boyum, A. 1968. Isolation of leukocytes from human blood: further observations. *Scand. J. Clin. Lab. Invest.* 21(Suppl 97):31.
30. Tsien, R. Y., T. Pozzan, and T. J. Rink. 1982. Calcium homeostasis in intact lymphocytes: Cytoplasmic free calcium monitored with a new, intracellularly trapped fluorescent indicator. *J. Cell Biol.* 94:325.
31. Goldman, D. W., L. A. Gifford, D. M. Olson, and E. J. Goetzl. 1985. Transduction by leukotriene B₄ receptors of increases in cytosolic calcium in human polymorphonuclear leukocytes. *J. Immunol.* In press.
32. Munson, P. J., and D. Rodbard. 1980. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107:220.
33. Goldman, D. W., and E. J. Goetzl. 1984. Heterogeneity of human polymorphonuclear leukocyte receptors for leukotriene B₄. *J. Exp. Med.* 159:1027.
34. Klass, H. J., J. Hopkins, G. Neale, and T. J. Peters. 1977. The estimation of serum lysozyme: A comparison of four assay methods. *Biochem. Med.* 18:52.
35. Amrein, P. C., and T. P. Stossel. 1980. Prevention of degradation of human polymorphonuclear leukocyte proteins by diisopropylfluorophosphate. *Blood* 56:442.
36. Van Dop, C., G. Yamanaka, F. Steinberg, R. D. Sekura, C. R. Manclark, L. Stryer, and H. R. Bourne. 1984. ADP-ribosylation of transducin by pertussis toxin blocks the light-stimulated hydrolysis of GTP and cGMP in retinal photoreceptors. *J. Biol. Chem.* 259:23.
37. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680.
38. Katada, T., and M. Ui. 1980. Slow interaction of islet-activating protein with pancreatic islets during primary culture to cause reversal of alpha-adrenergic inhibition of insulin secretion. *J. Biol. Chem.* 255:9580.
39. Murayama, T., T. Katada, and M. Ui. 1983. Guanine nucleotide activation and inhibition of adenylate cyclase as modified by islet-activating protein, pertussis toxin, in mouse 3T3 fibroblasts. *Arch. Biochem. Biophys.* 221:381.
40. Tamura, M., K. Nogimori, S. Murai, M. Yajma, K. Ito, T. Katada, M. Ui, and S. Ishii. 1982. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. *Biochemistry.* 21:5516.
41. Lagast, H., T. Pozzan, F. A. Waldvogel, and P. D. Lew. 1984. Phorbol myristate acetate stimulates ATP-dependent calcium transport by the plasma membrane of neutrophils. *J. Clin. Invest.* 73:878.
42. Okajima, F., and M. Ui. 1984. ADP-ribosylation of the specific membrane protein by islet-activating protein, pertussis toxin, associated with inhibition of a chemotactic peptide-induced arachidonate release in neutrophils: A possible role of the toxin substrate in Ca²⁺-mobilizing biosignaling. *J. Biol. Chem.* 259:13863.
43. Molski, T. F. P., P. H. Naccache, M. L. Marsh, J. Kermodé, E. L. Becker, and R. I. Sha'afi. 1984. Pertussis toxin inhibits the rise in the intracellular concentration of free calcium that is induced by chemotactic factors in rabbit neutrophils: Possible role of the "G proteins" in calcium mobilization. *Biochem. Biophys. Res. Commun.* 124:644.
44. Kurose, H., T. Katada, T. Amano, and M. Ui. 1983. Specific uncoupling by islet-activating protein, pertussis toxin, of negative signal transduction via alpha-adrenergic, cholinergic, and opiate receptors in neuroblastoma × glioma hybrid cells. *J. Biol. Chem.* 258:4870.
45. Boyer, J. L., A. Garcia, C. Posadas, and J. A. Garcia-Sainz. 1984. Differential effect of pertussis toxin on the affinity state for agonists of renal alpha₁- and alpha₂-adrenoceptors. *J. Biol. Chem.* 259:8076.
46. Weissmann, G., J. E. Smolen, and H. Korchak. 1980. Prostaglandins and inflamma-

- tion: Receptor/cyclase coupling as an explanation of why PGE's and PGI₂ inhibit functions of inflammatory cells. *Adv. Prostaglandin Thromboxane Res.* 8:1637.
47. Bokoch, G. M., and A. G. Gilman. 1984. Inhibition of receptor-mediated release of arachidonic acid by pertussis toxin. *Cell.* 39:301.
 48. Gallin, J. I. 1984. Human neutrophil heterogeneity exists, but is it meaningful? *Blood.* 63:977.