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EVIDENCE THAT A RECEPTOR-OPERATED EVENT ON THE NEUTROPHIL MEDIATES NEUTROPHIL ACCUMULATION IN VIVO

Pretreatment of ^{111}In-Neutrophils with Pertussis Toxin in Vitro Inhibits Their Accumulation in Vivo[1]

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The role of neutrophil chemoattractant receptors in neutrophil stimulation in vitro is well established, however, the precise mechanisms underlying local neutrophil accumulation at inflammatory sites in vivo have not been defined. A fundamental question that remains open is whether chemoattractants act on the endothelial cell or the neutrophil to initiate the process of neutrophil migration in vivo. To address this question we have investigated whether neutrophil accumulation in vivo can occur if chemoattractant receptor occupancy is uncoupled from neutrophil stimulation. For this purpose we have used pertussis toxin (PT) as the pharmacologic tool. We have investigated the effect of in vitro pretreatment of rabbit neutrophils with PT on their responses in vitro and on their accumulation in vivo. Pretreatment of rabbit neutrophils with PT inhibited FMLP- and C5a-, but not PMA- induced increases in CD18 expression, neutrophil adherence, and degranulation in vitro. This pretreatment procedure with PT inhibited the accumulation of radiolabeled neutrophils in vivo in response to intradermally injected FMLP, C5a, C5a des Arg, leukotriene B_4, IL-8, and zymosan in rabbit skin. Further, in contrast to the in vitro results, PT inhibited the PMA-induced ^{111}In-neutrophil accumulation in vivo. Interestingly, pretreatment of neutrophils with PT also inhibited accumulation in response to intradermally injected IL-1, despite the reports that IL-1 lacks neutrophil chemoattractant activity in vitro. Although the experimental techniques used cannot distinguish the different stages of neutrophil migration involved, these results suggest that the accumulation of neutrophils induced by local extravascular chemoattractants in vivo depends on a pertussis toxin-sensitive receptor operated event on the neutrophil itself. Further, PMA and IL-1 may release secondary chemoattractants in vivo.

At sites of inflammation the extravascular generation of chemoattractants, such as C5a, induce the adherence of neutrophils to venular endothelial cells. This is then followed by neutrophil migration across the vessel wall. Recent experimental and clinical observations have begun to define the molecules on the surface of neutrophils and endothelial cells mediating the adhesive component of neutrophil-endothelial cell interaction (1). With respect to the neutrophil, important adherence molecules are contained within the CD11/CD18 Ag complex (1). mAb recognizing the CD11/CD18 glycoproteins block stimulated neutrophil adhesion in vitro (1–4) and neutrophil accumulation in vivo (5–8). In addition, the murine MEL-14 Ag (9) or its human leukocyte equivalent, Leu 8 (10), appear to mediate the adherence of neutrophils to venular endothelial cells under basal conditions (11). With respect to the endothelial cell surface, the main adhesion molecules characterized are intercellular adhesion molecule-1 (1), endothelial leukocyte adhesion molecule-1 (12), and GMP-140 (13).

However, despite much progress, the exact mechanisms involved in localized neutrophil accumulation in vivo remains unclear and controversial. Topical application of a chemoattractant, such as C5a, to a microvascular bed in vivo induces a rapid attachment of neutrophils to the endothelium. A fundamental question that remains open is whether chemoattractants generated locally, or applied extravascularly act on the endothelial cell or the neutrophil to initiate this process. In most tissues neutrophil-endothelial cell interaction takes place selectively in venules (in the lung, capillaries are involved). This selectivity of adherence site implies either an active change in the surface of specialized endothelial cells induced by the chemoattractant, or a favored site for the adherence of activated neutrophils. With respect to the former, it is not clear whether such a rapid change in the endothelial cell surface can occur in response to chemoattractants. In vitro, the expression of high affinity chemoattractant receptors on neutrophils has been well characterized (14) but their presence on endothelial cells is contentious. It has been suggested that cultured endothelial cells show increased adhesive properties in response to chemoattractants and that this is associated with the presence of specific chemoattractant receptors (15–17). However, other studies have failed to detect such effects (18). As these experiments are normally carried out with cultured cells from major vessels rather than microvessels, they may not reflect the conditions in vivo. To answer whether chemoattractants act on the endo-

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thelial cell or the neutrophil to induce cell attachment in situ will depend on experiments carried out in vivo. To investigate this we have carried out the present study, using pertussis toxin as the pharmacologic tool. Pretreatment of radiolabeled neutrophils with pertussis toxin, which inhibited receptor-mediated responses induced by chemoattractants in vitro, inhibited neutrophil accumulation in vivo. The results of this study indicate that a pertussis toxin-sensitive receptor operated event on the neutrophil is crucial in mediating neutrophil accumulation in vivo.

MATERIALS AND METHODS

Animals. Male NZW rabbits (2.5 to 2.8 kg) were purchased from Froxfield Farm, Hampshire, England and Hacking and Churchill, Huntingdon, Cambridgeshire, England.

Materials. Sagatal (pentobarbitone sodium, 60 mg/ml) was from May and Baker, Dagenham, Essex, England. PMA, 4α-phorbol, BSA, zymosan, 2-mercaptopyridine-N-oxide, FMLP, and cytochalasin B were from Sigma Chemical Co., Poole, Dorset, England. FITC-rabbit anti-mouse Ig was obtained from DAKO Ltd, Buckinghamshire, England. ^{125}I-human serum albumin (20 mg albumin per ml of sterile isotonic saline, 50 μCi/ml) and ^{111}Indium chloride ($^{111}InCl_3$, 2mCi in 0.2 ml sterile pyrogen-free 0.04 N hydrochloric acid) were from Amersham International, Amersham, Buckinghamshire, England. Hespan (6% hydroxyethyl starch in 0.9% NaCl) was from American Hospital Supply, Didcot, Oxfordshire, England. Percoll was from Pharmacia Fine Chemicals, Uppsala, Sweden. The 96-well flexible polyvinyl chloride assay plates were from Becton Dickinson, Mountain View, CA. Evans blue dye and gelatin were from British Drug Houses, Poole, Dorset, England. Purified pertussis toxin derived from *Bordetella pertussis* was from Porton Products Ltd, Maidenhead, Berkshire, England. rHIL-1α and leukotriene B_4 were gifts from Dr. D. Westmacott, Roche Products Ltd, Welwyn Garden City, Hertfordshire, England and Dr. S. Foster, Imperial Chemical Industries, Macclesfield, England, respectively. rHC5a was a gift from Dr. H. J. Showell, Pfizer Central Research, Groton, CT. rHIL-8 was a gift from Dr. K. Matsushima and Dr. J. J. Oppenheim from National Cancer Institute, Frederick, MD. mAb 60.3 (mouse IgG2a), that recognizes the CD18 Ag was a gift from Dr. J. M. Harlan, University of Washington, Seattle, WA.

ZAP[3] as a source of rabbit C5a des Arg (RC5a des Arg) was prepared by incubating heparinized (10 U/ml) plasma with zymosan (5 mg/ml) for 30 min at 37°C. Zymosan was removed by centrifugation (2 × 10 min, 3000 × *g*) and ZAP was stored in aliquots at −25°C. The RC5a des arg content of ZAP was 3.5×10^{-7} M as measured by RIA (19).

Separation and ^{111}In-labelling of rabbit neutrophils. Rabbits were anaesthetised with i.v. Sagatal and bled via the carotid artery into acid citrate dextrose. Neutrophils were isolated and labeled with ^{111}In as previously described (8, 20). Before the final wash, ^{111}In-neutrophils from each donor rabbit were divided into two aliquots one of which was treated with pertussis toxin (1.0 μg/ml of cell suspension containing 2 to 5×10^7 neutrophils) for 60 min at 27°C. The second cell aliquot was incubated with the appropriate vehicle. Cells were then washed and resuspended in autologous plasma or HBSS containing 10 mM HEPES and 0.25% BSA (HBSS/HEPES/BSA buffer, pH 7.4). The percentages of radioactivity retained by the cells during the final washing procedure were 95.6 ± 1.3 and 97.8 ± 2.6 (mean ± SEM, n = 8 pairs of rabbits) for control and pertussis toxin-treated neutrophils, respectively.

Measurement of ^{111}In-neutrophil accumulation and plasma exudation in rabbit skin. Rabbits were anaesthetized with Sagatal and their backs closely clipped. Neutrophil infiltration and edema formation in the rabbit dorsal skin were simultaneously measured as the local accumulation of i.v. injected ^{111}In-labeled neutrophils (2.5×10^7 cells/recipient, approximately 5 to 30 μCi/kg, prepared as described above) and ^{125}I-human serum albumin (5 μCi/kg) mixed with sterile Evans blue dye solution in saline (10 mg/kg) as previously described (8, 20). The agents under investigation were injected intradermally in 0.1 ml volumes according to a balanced site injection plan, each treatment having six replicates. After 4 h, a 10-ml cardiac blood sample was collected into heparin for the preparation of plasma and for determining the percentage of cell-associated and cell-free radioactivity in plasma at the end of the in vivo test period. The animal was then killed by an overdose of sodium pentobarbitone, the back skin removed, and the injection sites punched out with a 17-mm diameter punch. Skin and plasma samples were counted in a 5-head gamma-counter with automatic spill-over and cross talk correction (Packard Cobra, Meridien, CT). The ^{111}In-counts per neutrophil was determined and used to express neutrophil accumulation in each skin site in terms of number of labeled leukocytes. Exudate volumes were expressed as μl of plasma by dividing skin sample ^{125}I-counts by ^{125}I-counts in 1 μl of plasma.

Calculation of the percentage of radiolabeled neutrophils circulating at the end of the experiment was based on total cell-associated activity injected and the recipient rabbit's total blood volume. The latter was determined by the isotope dilution method using an i.v. injection of ^{125}I-albumin. When expressed as percentage of the body weight, the total blood volume ranged from 6.9 to 7.9% (mean 7.4 ± 0.2, n = 6 rabbits).

Immunofluorescence flow cytometry. Immunofluorescence flow cytometry was carried out as previously described (8). Briefly, neutrophils (5×10^6/ml in HBSS/HEPES/BSA buffer) were incubated with buffer or stimuli for 20 min at 37°C before being treated with a saturating concentration of mAb 60.3 (88 μg/ml) for 30 min at 4°C. After two washes, the cells were treated with a saturating concentration of FITC-rabbit antimouse Ig for 30 min at 4°C. Finally, the cells were resuspended in fresh buffer and analyzed using a Becton Dickinson FACStar Plus flow cytometer. The results are represented as percent increase in CD18 expression, as determined by the binding of mAb 60.3, over unstimulated basal levels.

Measurement of ^{111}In-neutrophil adherence to gelatin-coated plates. Rabbit ^{111}In-neutrophils were suspended in HBSS/HEPES/BSA buffer at a concentration of 5×10^6 cells/ml. Aliquots of cells (50 μl/well) were then added to gelatin (5.0%)-coated 96-well flexible assay plates followed immediately by the addition of 50 μl of buffer (control), FMLP (final concentration 10^{-9} M), rHC5a (final concentration 10^{-9} M), or PMA (final concentration 10^{-10} M). Plates were incubated at 37°C for 15 min after which nonadherent neutrophils were removed by two washes with HBSS. Wells were then cut out of the plates and counted in a gamma-counter. Stimulated adherence of neutrophils was expressed as percentage increase over unstimulated control levels. All tests were carried out in quadruplicate.

Stimulation of neutrophil granular enzyme release. Aliquots of rabbit neutrophils (10^6 cells/ml), suspended in HBSS/HEPES/BSA buffer, were warmed to 37°C and incubated with cytochalasin B (5 μg/ml) for 5 min. The cells were then stimulated with rHC5a or PMA. All reactions were carried out in duplicate. After 10 min the reaction tubes were centrifuged at 800 × *g* for 15 min at 4°C. The cell-free supernatants were assayed for the presence of the azurophil granular enzyme β-N-acetylhexoseaminidase and the cytoplasmic enzyme lactate dehydrogenase. The enzymes were assayed fluorimetrically in microtitre plates using the reagents described by Morgan et al. (21). Enzyme release was expressed as the percentage of total cellular enzyme activity as measured in incubation tubes containing 0.2% Triton X-100.

Statistical analysis. Data are presented as mean ± SEM and have been analyzed using the unpaired Student's t-test. A p value of <0.05 was considered statistically significant.

RESULTS

Effect of pertussis toxin on stimulated increase in neutrophil CD18 expression and adherence to gelatin-coated plates. FMLP (10^{-7} M) and PMA (10^{-8} M) induced a significant increase in CD18 expression, as determined by the binding of mAb 60.3, on rabbit neutrophils (Fig. 1). Pretreatment of neutrophils with pertussis toxin inhibited the FMLP-induced increase in CD18 expression. Similar results were obtained when the leukocytes were stimulated with rHC5a (results not presented). However, pertussis toxin had no affect on the increased CD18 expression in response to PMA (Fig. 1).

The above stimuli were also potent in inducing ^{111}In-neutrophil adherence to gelatin-coated plates. FMLP (10^{-9} M), rHC5a (10^{-9} M), and PMA (10^{-10} M) induced 54.3 ± 4.1 (n = 4), 78.9 ± 7.3 (n = 4), and 113 ± 11.4 (n = 4) percent increases in adherence over basal levels. With pertussis toxin-treated leukocytes, the percent increase in adherence levels were 5.6 ± 4.1 (n = 4), 6.1 ± 4.2 (n = 4), and 125.0 ± 13.5 (n = 4) for FMLP, rHC5a, and PMA, respectively. Hence, as found with increased CD18

[3] Abbreviations used in this paper: ZAP, zymosan-activated rabbit plasma; H, human; i.d., intradermal; Gi, GTP-binding regulatory protein.

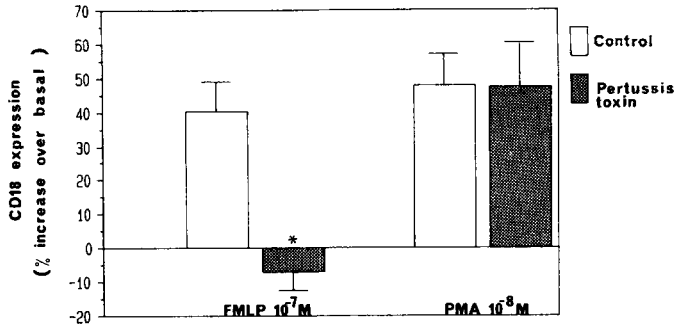

Figure 1. Effect of pertussis toxin on FMLP and PMA-induced increase in CD18 expression on rabbit neutrophils. CD18 expression was determined by the binding of mAb 60.3. Results are mean ± SEM for $n = 3$ to 5 experiments and are presented as percent increase over unstimulated control levels. *Asterisks* indicate a significant difference from control. $^*p < 0.05$.

expression, pertussis toxin inhibited the response to FMLP and rHC5a but not to PMA. In these experiments, pertussis toxin had no significant affect on basal CD18 expression or adherence.

Effect of pertussis toxin on rHC5a-induced and PMA-induced neutrophil degranulation in vitro and ^{111}In-neutrophil accumulation in vivo. rHC5a and PMA induced the release of the granular enzyme β-N-acetylhexoseaminidase from cytochalasin B-treated neutrophils in a dose-dependent manner (Fig. 2 *A* and *C*). Further, intradermal administration of these stimuli-induced ^{111}In-neutrophil accumulation over the 4h test period in rabbit skin (Fig. 2 *B* and *D*). In this in vivo assay, intradermal injection of 4α-phorbol (2×10^{-10} mol/site), an inactive analogue of PMA, did not induce neutrophil accumulation (results not presented).

Pretreatment of neutrophils with pertussis toxin significantly inhibited neutrophil degranulation in vitro and neutrophil accumulation in vivo induced by rHC5a. As found with increased CD18 expression (Fig. 1) and adherence, pertussis toxin had no affect on neutrophil degranulation induced by PMA. However, pertussis toxin pretreatment significantly suppressed PMA-stimulated ^{111}In-neutrophil accumulation in vivo (Fig. 2*D*). The results in Figure 2 indicate the similarities and differences of behavior of identical cells in vitro and in vivo demonstrating the need for caution when extrapolating in vitro results to the in vivo situation.

The inhibitory effect of pertussis toxin on neutrophil accumulation in vivo was not due to a reduction in the number of ^{111}In-neutrophils circulating in the rabbits. The percentage of control labeled neutrophils and pertussis toxin-treated ^{111}In-neutrophils circulating at the end of the 4 h in vivo test period were 42.6 ± 4.1 and 38.9 ± 5.1 (mean ± SEM, $n = 8$ pairs of rabbits), respectively. In these experiments the percentages of radioactivity in cell-free plasma in animals receiving control and pertussis toxin-treated radiolabelled neutrophils were 3.9 ± 0.3 and 4.2 ± 0.4 respectively. These numbers demonstrate that the extent of ^{111}In leakage was the same for both cell populations. In the in vitro degranulation experiments, no significant levels of lactate dehydrogenase were detected in any of the samples.

Effect of pertussis toxin on ^{111}In-neutrophil accumulation in response to inflammatory mediators and zymosan in vivo. i.d. injections of FMLP, LTB_4, RC5a des Arg, and zymosan-induced ^{111}In-neutrophil accumulation over the 4-h test period in rabbit skin (Fig. 3). As observed with rHC5a and PMA, the pretreatment of labeled neutrophils with pertussis toxin significantly inhibited their accumulation in response to the above stimuli. Plasma protein leakage induced by the mediators used is neutrophil dependent. However, in the rabbits receiving pertussis toxin-treated ^{111}In-neutrophils, there was no significant affect on edema formation in response to i.d. inflammatory mediators or zymosan (results not presented). This is presumably because the labeled cells represent less than 3% of the recipient rabbit's circulating neutrophils and therefore do not make a major functional contribution to oedema formation.

Effect of pertussis toxin on ^{111}In-neutrophil accumulation induced by intradermal rHIL-1α and rHIL-8. i.d. administration of rHIL-1α and rHIL-8 induced dose-dependent ^{111}In-neutrophil accumulation over the 4 h in vivo test period (Fig. 4). In this assay, rHIL-1α was found to be approximately 1000 times more potent than rHIL-8 and all the other inflammatory mediators tested. As

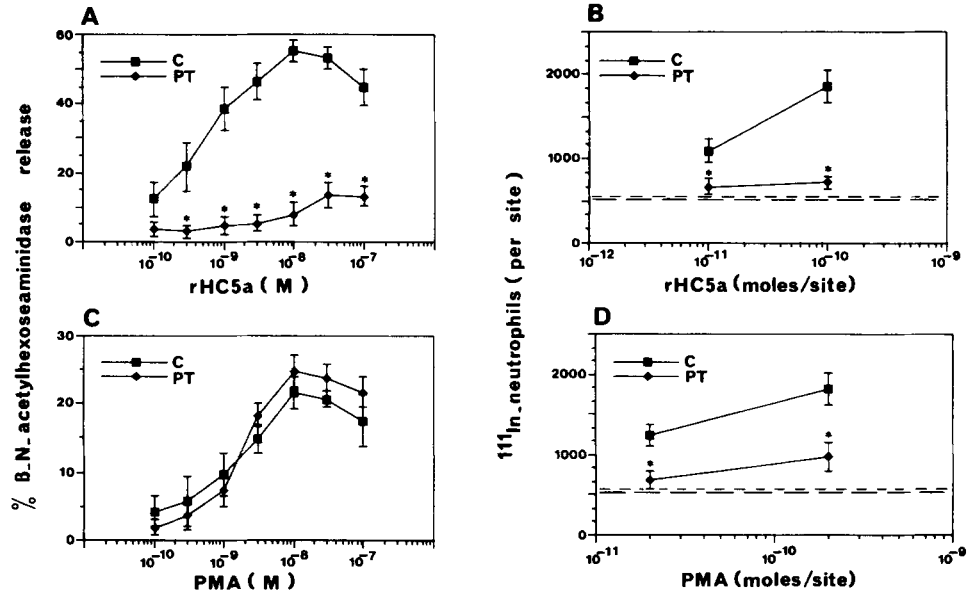

Figure 2. Effect of pertussis toxin on rHC5a-induced and PMA-induced neutrophil degranulation in vitro (*A* and *C*) and ^{111}In-neutrophil accumulation in vivo (*B* and *D*). The results are from control (*C*) and pertussis toxin-treated (*PT*) neutrophils. In *B* and *D*, the stimuli were administered intradermally and the *dashed lines* represent levels determined after i.d. saline for control (– – –) and PT-treated neutrophils (—— ——). For experimental details see *Materials and Methods*. Results are the mean ± SEM for $n = 4$ cell preparations in *A* and *C* and $n = 6$ pairs of rabbits in *B* and *D*. *Asterisks* indicate a significant difference from control, $^*p < 0.05$.

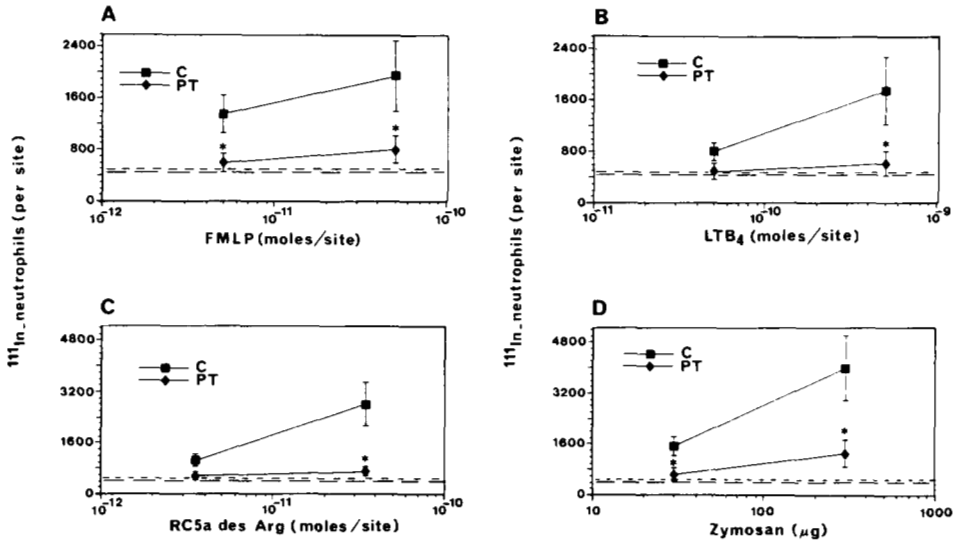

Figure 3. Effect of pertussis toxin on ^{111}In-neutrophil accumulation in response to intradermal injections of FMLP, leukotriene B_4, RC5a des Arg, and zymosan. The results are from control (*C*) and pertussis toxin (*PT*)-treated ^{111}In-neutrophils. *Dashed lines* represent levels determined after i.d. saline for control (– – –) and PT-treated neutrophils (—— ——). Results are the mean ± SEM for $n = 5$ pairs of rabbits. *Asterisks* indicate a significant difference from control. $^*p < 0.05$.

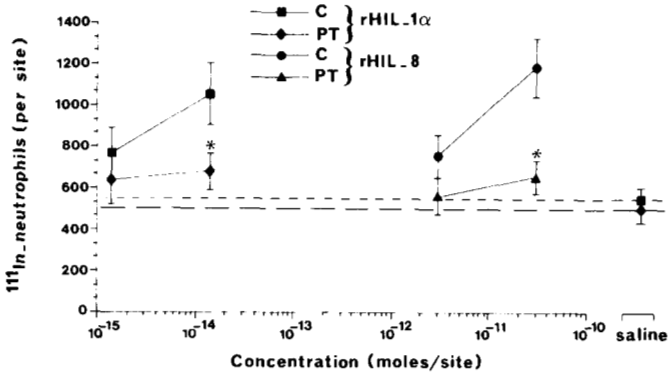

Figure 4. Effect of pertussis toxin on ^{111}In-neutrophil accumulation in response to intradermal injections of rHIL-1α and rHIL-8. The results are from control (C) and pertussis toxin (*PT*)-treated ^{111}In-neutrophils. *Dashed lines* represent levels determined after intradermal injection of saline for control (– – –) and PT-treated neutrophils (—— ——). Results are the mean ± SEM for $n = 5$ pairs of rabbits. *Asterisks* indicate a significant difference from control. $^*p < 0.05$.

found with the other stimuli, labeled neutrophils pretreated with pertussis toxin failed to accumulate in response to i.d. rHIL-1α and rHIL-8.

DISCUSSION

In response to an inflammatory stimulus, neutrophils adhere to microvascular endothelial cells, migrate through the vessel wall and enter the surrounding tissue. The aim of this study was to investigate whether a pertussis toxin-sensitive receptor operated mechanism on the neutrophil was involved in this process. For this purpose, we investigated the effect of pretreatment of radiolabelled neutrophils with pertussis toxin on their accumulation in vivo.

Pertussis toxin interacts with a Gi that couples neutrophil chemoattractant receptor occupancy to the phospholipase C mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (22). The inhibition of Gi by pertussis toxin prevents the chemoattractant-induced production of second messenger molecules such as inositol 1,4,5-trisphosphate and 1,2-diacylglycerol resulting in suppression of neutrophil activation (22). In vitro, pertussis toxin inhibits all chemoattractant-induced neutrophil responses, but has no affect on neutrophil stimulation in response to the protein kinase C activator PMA which by passes the Gi step (22). In the present study, pretreatment of rabbit neutrophils with pertussis toxin inhibited FMLP- and C5a-induced neutrophil degranulation, adherence, and increased CD18 expression in vitro. Under the same experimental conditions, pertussis toxin had no affect on neutrophil activation induced by PMA. Having achieved conditions under which pertussis toxin caused a selective inhibition of chemoattractant receptor-mediated neutrophil responses in vitro, the effect of pretreatment with pertussis toxin was tested on ^{111}In-neutrophil accumulation in vivo.

Pretreatment of radiolabeled neutrophils with pertussis toxin in vitro inhibited their accumulation in response to intradermally administered neutrophil chemoattractants C5a, C5a des Arg, LTB_4, FMLP, and IL-8 in vivo. The fact that pretreatment virtually abolished localization of ^{111}In-neutrophils in skin sites suggests that PT blocks the initial adherence of the neutrophils to venular endothelial cells. We used the same pretreatment procedure to investigate neutrophil accumulation induced by endogenously generated C5a in response to i.d. zymosan. As found with the preformed mediators, pertussis toxin pretreatment of neutrophils inhibited their accumulation in response to zymosan. Further, Spangrude et al. (23) found that pertussis toxin inhibited neutrophil accumulation in a delayed-type hypersensitivity reaction in mice. The suppression of cell accumulation we observed was not due to a reduced number of circulating leukocytes as there was no significant difference between the percentage of circulating control and toxin-treated neutrophils. In addition, the treated neutrophils responded normally to PMA in vitro.

These results suggest that a pertussis toxin-sensitive GTP-binding protein on the neutrophil is essential for chemoattractant-induced neutrophil accumulation in vivo. Thus, it appears that such a receptor-mediated event on the neutrophil is involved, but the exact mechanisms involved remain to be established. A chemoattractant in extravascular tissue fluid may have to diffuse into the lumen of the microvessel and act on the receptors of passing neutrophils. Receptor stimulation would then result in increased adhesiveness of the neutrophil surface owing to a change in its CD11/CD18

complexes, likely to be a change in their conformation (8). Although attachment of neutrophils to endothelial cells is localized in venules in most tissues, there is recent evidence that the chemoattractant signal may be picked up by the neutrophil as it passes through the capillary. Nagai and Katori (24) reported that in the hamster cheek pouch, administration of LTB_4 or FMLP by a glass capillary pipette into the interstitial space close to capillaries resulted in neutrophil adhesion to venules downstream. Application of these stimuli close to the venule did not cause adhesion of leukocytes.

The above findings are apparently in conflict with the observations of Colditz and Movat (25) who demonstrated that skin sites in the rabbit could be specifically desensitized to a particular chemoattractant by a previous i.d. injection of that substance. This study suggested that receptors for chemoattractants may be present upon endothelial cells or adjacent tissue cells. A possible explanation for these results is that chemoattractant receptors on the ablumenal surface of capillary endothelial cells may mediate the translocation of chemoattractant molecules across the capillary wall (26). Such a mechanism, for which there is now in vitro evidence (27), supports the possibility of an active role for vascular endothelial cells in chemoattractant-induced neutrophil accumulation in vivo.

Intradermal administration of PMA induced ^{111}In-neutrophil accumulation in rabbit skin. Pertussis toxin pretreatment of neutrophils had no affect on their responses to PMA in vitro, however, in contrast, pretreatment of the cells inhibited their accumulation in response to i.d. injected PMA in vivo. A possible explanation for this is that neutrophil accumulation in response to PMA in vivo may be mediated by the generation and release of a secondary chemoattractant. This proposal is supported by the observation that the PMA-induced neutrophil accumulation in vivo is protein synthesis dependent (S. Nourshargh and T. J. Williams, unpublished observations).

In vitro, IL-1 lacks neutrophil chemoattractant activity (28) but enhances neutrophil/endothelial cell interaction by inducing the expression of adhesive glycoproteins on cultured endothelial cells (1, 12). Despite the lack of neutrophil-stimulating activity of IL-1 in vitro, pretreatment of neutrophils with pertussis toxin inhibited their accumulation in response to i.d. injected IL-1 in vivo. Inasmuch as the IL-1-induced neutrophil accumulation is protein synthesis dependent (20, 29), the results suggest that the IL-1 response as suggested for PMA, may be mediated by the release of a secondary neutrophil chemoattractant. A possible candidate for this role is the newly characterized IL-8 (30). IL-8 that can be released from cultured endothelial cells stimulated with IL-1 (31), is a potent neutrophil chemoattractant. Further, the actions of IL-8 are sensitive to pertussis toxin in vitro (32) and in vivo (as shown). However, the possible involvement of other inflammatory mediators such as TNF in inflammatory responses induced by IL-1 can not be ruled out.

Pertussis toxin did not compromise the neutrophil's ability to adhere to gelatin-coated plates as it had no affect on the PMA-induced response in this test. However, in vivo, pertussis toxin may inhibit the stimulation of neutrophils via their adhesive molecules after an interaction with their ligands on endothelial cells. Hence, an alternative explanation for the in vivo results presented is that the initial interaction between adhesive molecules on the neutrophil surface and ligands on the endothelium in vivo leads itself to activation of neutrophil transduction mechanisms via a Gi protein. This activation could be necessary for the neutrophil to remain attached to the endothelial cell once contact has been made, for example to stimulate flattening of the neutrophils. This possibility will be explored in future experiments.

In conclusion, our report provides evidence that a pertussis toxin-sensitive receptor-coupled event in the neutrophil is essential for the local accumulation of neutrophils at an inflammatory site in vivo. Such an event appears to be necessary for neutrophil accumulation in vivo induced by chemoattractants, such as C5a, by the cytokine IL-1 and by the protein kinase C activator, PMA. These observations throw some light upon the fundamental mechanisms underlying local leukocyte accumulation during inflammatory reactions.

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