Biochem. J. (1992) 282, 429–434 (Printed in Great Britain)

G-protein activation by interleukin 8 and related cytokines in human neutrophil plasma membranes

Roland W. KUPPER,* Beatrice DEWALD,† Karl H. JAKOBS,* Marco BAGGIOLINI† and Peter GIERSCHIK*‡
*Pharmakologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, Federal Republic of Germany, and †Theodor Kocher Institut, Universität Bern, CH-3000 Bern 2, Switzerland

Interleukin 8 (IL-8) is a member of the rapidly growing superfamily of those cytokines which are thought to be involved in the regulation of inflammatory processes and cell proliferation. In neutrophils, IL-8 triggers a variety of cellular responses by interacting with specific cell-surface receptors. To examine whether IL-8 receptors are coupled to activation of guanine-nucleotide-binding proteins (G-proteins), we have investigated the influence of IL-8 on GTP hydrolysis by and guanosine 5′-[γ-[^{35}S]thio]triphosphate (GTP[^{35}S]) binding to purified human neutrophil plasma membranes. IL-8 stimulated high-affinity GTPase about 2-fold at 100 nM, and half-maximal stimulation was observed at 1 nM. The peptide-stimulated GTPase was confined to plasma membranes upon subcellular fractionation, and was due to an increase in $V_{max.}$ rather than a decrease in K_m. High-affinity binding of GTP[^{35}S] to neutrophil plasma membranes was stimulated half-maximally and maximally (up to 5-fold) by IL-8 at about 10 nM and 100 nM respectively. GTP[^{35}S] binding to the membranes was also stimulated by two IL-8-related cytokines, neutrophil-activating peptide 2 (NAP-2) and melanoma growth-stimulatory activity (*gro*/MGSA). Taken together, these results demonstrate that receptors for IL-8 and related cytokines are coupled to and activate G-proteins in neutrophil plasma membranes, indicating that G-protein activation is an important intermediate step in the induction of neutrophil functions by IL-8 and its congeners.

INTRODUCTION

The accumulation of neutrophils and mononuclear phagocytes after a disturbance of tissue homoeostasis is a major characteristic of many inflammatory processes. This phenomenon is initiated by chemotactic substances causing the amoeboid movement of these cells into the affected tissue, which is followed by phagocytosis and killing of the invading micro-organism and repair of the damaged tissue. *N*-Formylmethionyl-containing chemotactic peptides (e.g. fMet-Leu-Phe), complement component C5a, leukotriene B_4 (LTB_4) and platelet-activating factor (PAF) are some of the classical stimuli well known to be responsible for the described effects in neutrophils.

Chemotaxis and a variety of other cellular responses of neutrophils, including shape change, secretion of granular enzymes and release of oxygen radicals are also triggered by a 72-amino-acid cytokine termed neutrophil-activating peptide-1 (NAP-1) [1]. The cytokine has recently been named interleukin 8 [2] and will hence be referred to as IL-8 in the present paper. IL-8 is produced by a variety of cells, including monocytes, alveolar macrophages, endothelial cells, fibroblasts, epithelial cells and hepatoma cells after appropriate stimulation with agents such as IL-1α, IL-1β, tumour necrosis factor-α or lipopolysaccharide (for review and references see [1]. IL-8 has been purified [3,4] and sequenced [4,5]. Fully active IL-8 has been synthesized by conventional peptide synthesis [6,7], and by expression in *Escherichia coli* using the IL-8 cDNA [8,9] or a synthetic IL-8 gene [5].

Amino acid sequence analysis of IL-8 revealed that the peptide belongs to a rapidly expanding superfamily of cytokines. Members of this family are involved in regulating inflammatory responses and cell proliferation (for review see [10]). Specifically, amino acid sequence similarities have been found between IL-8 and a proteolytic product of platelet basic protein referred to as NAP-2 [11,12], the melanoma growth-stimulatory activity *gro*/MGSA [13–15], the type 2 macrophage inflammatory proteins (MIP-2a, MIP-2b) [16,17], an interferon-γ-inducible protein termed cIP-10 [18], and platelet factor 4 [19,20]. One of the common structural features of this branch of the family of IL-8-related cytokines is an *N*-terminally located pair of cysteines separated by a single amino acid (C-X-C subfamily). A second branch is characterized by two adjacent cysteines in the corresponding position (C-C subfamily). Members of this subfamily include the type 1 macrophage inflammatory proteins (MIP-1a, MIP-1b) [21,22], human monocyte chemotactic and activating factor (MCAF/MCP-1), also referred to as glioma-derived monocyte chemotactic factor 2 (GDCF-2) ([23,24]; for review see [25]), the monocyte and T-lymphocyte chemoattractant RANTES [26], and several other putative cytokines termed LD78, Act-2 and I-309 (for review and references see [10]).

Using radiolabelled recombinant IL-8, receptors for the cytokine have been identified and characterized on human neutrophils, monocytes and several cultured phagocytes [27–31]. Peripheral blood neutrophils clearly contain the highest number of binding sites (20000–90000 sites/cell), followed by monocytes and cultured phagocytes (1600–8000 sites/cell) and lymphocytes (150–1500 sites/cell). NAP-2 and *gro*/MGSA were shown to inhibit the binding of radiolabelled IL-8 to neutrophils [31]. Affinity-labelling experiments revealed that two forms of IL-8 receptors with molecular masses of 42–58 and 66–74 kDa occur on the neutrophil surface [27,30,31]. Agonist occupancy of the IL-8 receptor leads to rapid internalization of the receptor–ligand complex, followed by degradation of the ligand by lysosomal enzymes [28–30]. The internalized receptor protein is recycled and reappears on the cell surface within 10 min after removal of free ligand [29].

Abbreviations used: G-protein, signal-transducing guanine-nucleotide-binding protein; GTP[S], guanosine 5′-[γ-thio]triphosphate; NAP-2, neutrophil-activating peptide 2; *gro*/MGSA, melanoma growth-stimulatory activity; IL, interleukin; fMet-Leu-Phe, *N*-formylmethionyl-leucyl-phenylalanine; LTB_4, leukotriene B_4; PAF, platelet-activating factor; C5a, complement component C5a.

‡ To whom all correspondence should be addressed.

Despite the substantial amount of information on both IL-8 and its receptor, very little is known about the molecular mechanisms by which IL-8 transmits its signal across the plasma membrane. In one report, treatment of intact neutrophils with pertussis toxin has been shown to block several of the cellular responses to the cytokine, suggesting the involvement of a guanine-nucleotide-binding protein (G-protein) in IL-8 transmembrane signalling [32]. As pertussis toxin has also been shown to alter cell functions by mechanisms unrelated to G-protein modification [33–38], we have sought more direct evidence for the activation of G-proteins by IL-8. Here we report that IL-8 stimulates the hydrolysis of GTP by and the binding of guanosine 5′-[γ-[^{35}S]thio]triphosphate (GTP[^{35}S]) to purified human neutrophil plasma membranes. In addition, we show that two other members of the superfamily of IL-8-related cytokines, NAP-2 and *gro*/MGSA, also act via G-protein activation.

MATERIALS AND METHODS

Materials

GTP[^{35}S] and [γ-^{32}P]GTP were obtained from New England Nuclear (Bad Homburg, Germany). Unlabelled nucleotides, creatine kinase and phosphocreatine were from Boehringer (Mannheim, Germany). Human recombinant IL-8, prepared in accordance with [5], was obtained from Sandoz Research Institute (Vienna, Austria). IL-8 was diluted in buffer containing 50 mM-Mes, pH 6.5, 430 mM-NaCl and 1.6% (w/v) BSA (A-6003; Sigma, Deisenhofen, Germany). Synthetic NAP-2 and *gro*/MGSA were kindly provided by Dr. I. Clark-Lewis, Biomedical Research Centre, University of British Columbia, Vancouver, B.C., Canada. All other materials were from sources previously described [39,40].

Preparation of human neutrophils

Human neutrophils were prepared from donor blood stored overnight at 4–10 °C (Swiss Red Cross Laboratory, Bern, Switzerland). Neutrophils were isolated from buffy coats by Hypaque/Ficoll gradient centrifugation, followed by lysis of erythrocytes with iso-osmotic NH_4Cl as described previously [40]. The cells were finally suspended at a density of 10^8 cells/ml in 154 mM-NaCl supplemented with 0.05 mM-$CaCl_2$. These suspensions contained on average 98% granulocytes.

Cell homogenization

Purified neutrophils obtained from several donors were pooled and harvested by centrifugation at 300 *g* for 5 min at 4 °C. The pellets were resuspended at a density of 2×10^8 cells/ml in lysis buffer containing 0.25 M-sucrose, 20 mM-Tris/HCl, pH 7.5 at 20 °C, 1.5 mM-$MgCl_2$, 1 mM-ATP, 3 mM-benzamidine, 1 μM-leupeptin, 1 mM-phenylmethanesulphonyl fluoride and 2 μg of soybean trypsin inhibitor/ml. Cells were homogenized by nitrogen cavitation as described previously [39], except that a Kontes Mini-Bomb (Kontes, Vineland, NJ, U.S.A.) was used and 10 ml of the cell suspension was pressurized at a time. The homogenate was supplemented with 1.25 mM-EGTA and held on ice for 15 min. The foamy layer on top of the homogenate and coarse particles were removed by filtration through one layer of cheesecloth. The filtrate was centrifuged at 300 *g* for 5 min at 4 °C to remove unbroken cells and nuclei.

Subcellular fractionation

For subcellular fractionation, the post-nuclear supernatant (5 ml per gradient) was layered on top of continuous Percoll gradients prepared as previously described [39]. Centrifugation, collection of subcellular fractions (cytosol, plasma membranes, and a mixture of azurophil and specific granules), and removal of Percoll from the particulate fractions were done as described in [41]. The membranes and granules were washed three times by homogenization through a 0.9 mm × 40 mm needle with buffer containing 20 mM-Tris/HCl, pH 7.5 at 20 °C, 1 mM-dithiothreitol, 1 mM-EDTA, 3 mM-benzamidine, 1 μM-leupeptin, 1 mM-phenylmethanesulphonyl fluoride and 2 μg of soybean trypsin inhibitor/ml, and centrifugation in a Beckman JA 20 rotor ($r_{av.}$ = 7.0 cm) at 20000 rev./min for 15 min at 4 °C. They were then resuspended in the same buffer at ~ 1.5 and 4 mg of protein/ml respectively, and stored at −70 °C.

GTPase assay

Hydrolysis of [γ-^{32}P]GTP (0.1 μCi/tube) was determined in a reaction mixture (100 μl) containing 50 mM-triethanolamine/HCl, pH 7.3 at 30 °C, 5 mM-Mes, 1 mM-EDTA, 5 mM-$MgCl_2$, 143 mM-NaCl, 0.1 μM-GTP, 0.8 mM-adenosine 5′-[βγ-imido]-triphosphate, 0.1 mM-ATP, 0.4 mg of creatine kinase/ml, 5 mM-phosphocreatine (disodium salt) and 0.16% (w/v) BSA. Reactions were started by addition of membranes (1.6–2.6 μg of protein) to the prewarmed (5 min, 30 °C) incubation mixture and conducted for 15 min at 30 °C. Reactions were terminated by addition of 700 μl of ice-cold sodium phosphate (10 mM, pH 2.0) containing 5% (w/v) activated charcoal. After centrifugation for 30 min at 10000 *g*, 500 μl of the supernatant was removed and used for determination of Čerenkov radiation. High-affinity GTPase activity was calculated by subtracting low-affinity GTPase activity determined at 50 μM-GTP from total GTPase activity. Low-affinity GTPase activity was typically about 20% of total basal GTPase activity. Note that the GTPase assay is specific for hydrolysis of GTP, since the assay is performed in the presence of high concentrations of adenine nucleotides and only high-affinity GTPase activity is reported.

GTP[^{35}S]-binding assay

Binding assays were performed essentially as described for membranes of myeloid differentiated human leukaemia (HL-60) cells [42]. In brief, membranes (0.5–2 μg of protein/assay) were incubated at 30 °C in a mixture (100 μl) containing 50 mM-triethanolamine/HCl, pH 7.3 at 30 °C, 5 mM-Mes, 1 mM-EDTA, 5 mM-$MgCl_2$, 143 mM-NaCl, 0.16% BSA, 10 μM-GDP and 0.4 nM-GTP[^{35}S] (1200–1400 Ci/mmol). Reactions were started by addition of membranes to the prewarmed (5 min, 30 °C) reaction mixture (60 μl). The incubation was terminated by rapid filtration through Whatman GF/C filters. The filters were washed with 3 × 5 ml of ice-cold buffer containing 50 mM-Tris/HCl, pH 7.3, 1 mM-EDTA and 5 mM-$MgCl_2$, then dried, and radioactivity was determined by liquid-scintillation counting in 2.5 ml of scintillation fluid (Quicksafe A; Zinsser Analytic, Frankfurt, Germany). Binding of GTP[^{35}S] to human neutrophil plasma membranes exhibited strict guanine nucleotide specificity in competition experiments (GTP[S] > GTP ≈ GDP ⋙ GMP > ATP; results not shown). Non-specific binding was defined as the binding not competed for by 50 μM unlabelled GTP[S] and was generally less than 5% of total basal GTP[^{35}S] binding. Only the specific binding is reported.

Data presentation

All experiments were performed at least three times using two different preparations of neutrophil plasma membranes. Similar results were obtained in all cases. Data from representative experiments are shown as means ± S.D. of triplicate measurements.

Miscellaneous

Protein was determined as described by Bradford [43], with bovine IgG as standard.

RESULTS

Fig. 1 shows that IL-8-stimulated GTP hydrolysis was readily detectable in plasma-membrane preparations from human peripheral neutrophils. The basal accumulation of P_i owing to hydrolysis of GTP increased linearly with time up to at least 30 min. Addition of IL-8 (0.3 μM) stimulated the hydrolysis of GTP by ~2.4-fold without any apparent lag phase. This stimulation was constant for up to 30 min under the assay conditions used. A 15 min incubation period was therefore used for all subsequent experiments. The data shown in Table 1 demonstrate that high-affinity GTPase activity is localized in the plasma-membrane compartment of human neutrophils. Basal GTP hydrolysis was about 100-fold higher in the plasma-membrane fraction than in the granule fraction. Only the activity in the plasma membranes could be stimulated with IL-8. The peptide had no effect on GTP hydrolysis by components of the granule fraction. Purified plasma membranes were therefore used to examine IL-8-dependent G-protein activation throughout this study.

Fig. 2 illustrates that the stimulation of high-affinity GTPase activity of neutrophil plasma membranes by IL-8 was concentration-dependent. Half-maximal and maximal stimulation were observed at approx. 1 nM- and 100 nM-IL-8 respectively.

To determine the kinetic parameters of both the basal and the IL-8-stimulated GTPase, the hydrolysis of [γ-^{32}P]GTP by human neutrophil plasma membranes was measured in the presence of increasing concentrations of unlabelled GTP (0.1–5 μM). Fig. 3 shows that double-reciprocal plots of the high-affinity GTPase activity versus GTP concentration were linear. The basal high-affinity GTPase exhibited an apparent K_m value for GTP of ~0.3 μM and a $V_{max.}$ of ~85 pmol of GTP hydrolysed/min per mg of protein. IL-8 at 0.3 μM had no effect on the apparent affinity of the enzyme for GTP, but increased the $V_{max.}$ value by about 2.2-fold.

We have recently developed methods for assessing the formyl-peptide-receptor-stimulated binding of the poorly hydrolysable GTP analogue GTP[^{35}S] to membranes of cultured human HL-60 granulocytes [42]. The results shown in Table 2 demonstrate that this methodology could be adapted to examine IL-8-stimulated binding of GTP[^{35}S] to human neutrophil plasma membranes. Thus no effect of IL-8 on GTP[^{35}S] association with the membranes was observed when GTP[S] was the only nucleotide present in the incubation medium (results not shown). In contrast, addition of IL-8 (1 μM) markedly stimulated GTP[^{35}S] binding when GDP was present in high (> 1000-fold) excess over GTP[^{35}S] (Table 2). Thus at 1 mM-GDP a > 5-fold stimulation was observed. Since addition of 10 μM-GDP led to a marked increase in the fold stimulation of GTP[^{35}S] binding by IL-8 without decreasing the absolute stimulation, GDP was used at this concentration in all subsequent GTP[^{35}S]-binding assays.

Fig. 4 shows the effect of IL-8 on the time course of GTP[^{35}S] binding to human neutrophil plasma membranes. Note that, under the conditions used here, both basal and IL-8-stimulated GTP[^{35}S] binding to the membranes were relatively slow processes. Thus neither basal nor IL-8-stimulated binding of GTP[^{35}S] reached a plateau within the 4 h incubation period of the experiment shown in Fig. 4.

The data shown in Fig. 5 illustrate that stimulation of GTP[^{35}S] binding by IL-8 was concentration-dependent. Half-maximal and maximal stimulation was observed at ~1 nM- and 100 nM-IL-8 respectively. Note that these values are somewhat higher than those observed for the stimulation of high-affinity GTPase (cf. Fig. 2). It is important to keep in mind, however, that the conditions used to assay GTPase activity and the optimal conditions for determining receptor-mediated GTP[^{35}S] binding

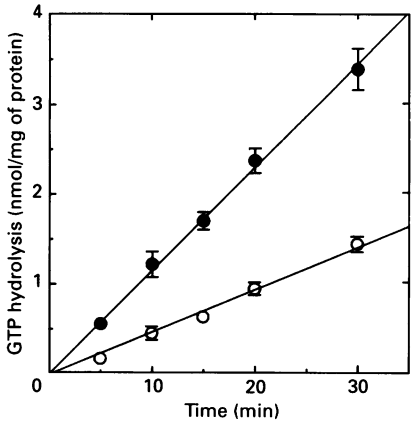

Fig. 1. Effect of IL-8 on GTP hydrolysis by purified human neutrophil plasma membranes

High-affinity GTPase was determined in human neutrophil plasma membranes in the absence (○) or presence (●) of 0.3 μM-IL-8 as described in the Materials and methods section.

Table 1. Subcellular distribution of IL-8-stimulated high-affinity GTPase in human neutrophils

Human neutrophils were homogenized by nitrogen cavitation, subjected to subcellular fractionation by Percoll-gradient centrifugation, and high-affinity GTPase activity was determined in the two membrane fractions in the absence or presence of 1 μM-IL-8 as described in the Materials and methods section. Each value represents the mean ± S.D. of triplicate determinations.

	GTP hydrolysis (pmol/min per mg of protein)	
Fraction	Control	IL-8
Plasma membranes	68.7±3.8	129.8±6.9
Granules	0.5±0.4	0.3±0.8

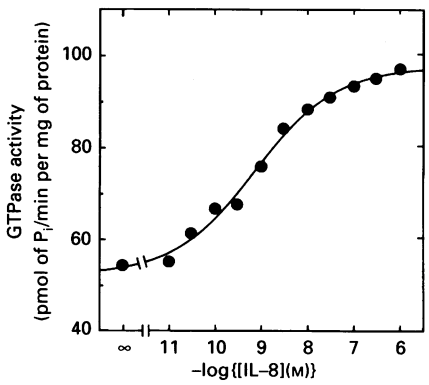

Fig. 2. Concentration-dependence of IL-8-stimulated GTP hydrolysis by neutrophil plasma membranes

High-affinity GTPase activity was determined by incubating neutrophil plasma membranes for 15 min in the presence of the indicated concentrations of IL-8.

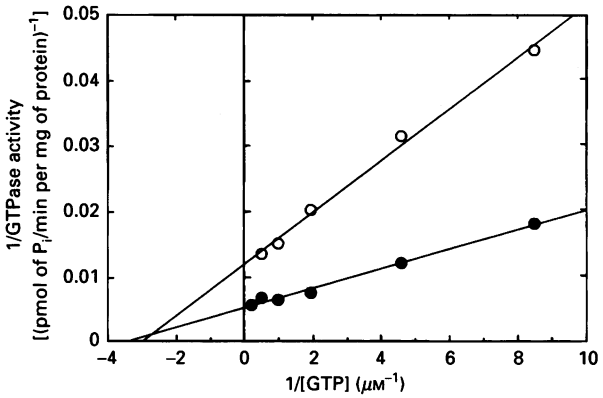

Fig. 3. Effect of IL-8 on the kinetic parameters of high-affinity GTPase in neutrophil plasma membranes

High-affinity GTPase activity was determined at the indicated concentrations of GTP in the absence (○) or presence (●) of 0.3 μM-IL-8. Double-reciprocal plots of high-affinity GTPase activity versus GTP concentration are shown.

Table 2. Effect of GDP on IL-8 stimulation of GTP[^{35}S] binding to neutrophil plasma membranes

Purified neutrophil plasma membranes were incubated for 45 min in the absence or presence of 1 μM-IL-8 with GTP[^{35}S] and GDP at the indicated concentrations, and then analysed for bound GTP[^{35}S] as described in the Materials and methods section. Each value represents the mean ± S.D. of triplicate determinations. The absolute and the relative (fold) stimulation of GTP[^{35}S] binding by IL-8 is given in parentheses (absolute/relative).

GDP (μM)	GTP[^{35}S] bound (pmol/mg of protein)	
	Control	IL-8
0.1	6.89±0.25	8.65±0.21 (1.76/1.26)
1	4.09±0.18	5.49±0.12 (1.41/1.34)
10	1.15±0.26	2.97±0.09 (1.82/2.58)
100	0.28±0.01	1.08±0.08 (0.80/3.86)
1000	0.03±0.01	0.16±0.03 (0.13/5.48)

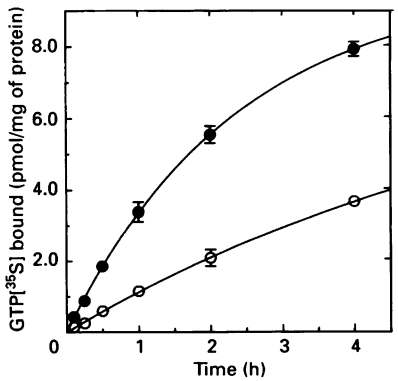

Fig. 4. Effect of IL-8 on the time course of GTP[^{35}S] binding to neutrophil plasma membranes

Neutrophil plasma membranes were incubated in the absence (○) or presence (●) of 1 μM-IL-8 with GTP[^{35}S]. At the times indicated on the abscissa, samples were analysed for specifically bound GTP[^{35}S] as described in the Materials and methods section.

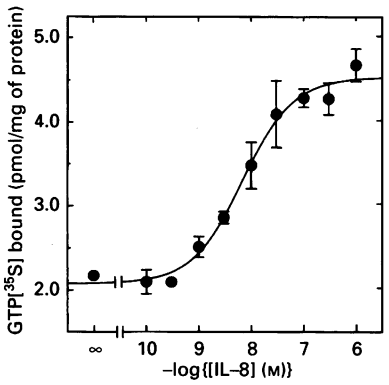

Fig. 5. Concentration-dependence of the IL-8-induced stimulation of GTP[^{35}S] binding to purified human neutrophil plasma membranes

Human neutrophil plasma membranes were incubated for 120 min with GTP[^{35}S] and IL-8 at concentrations indicated at the abscissa.

Table 3. Stimulation of GTP[^{35}S] binding to neutrophil plasma membranes by IL-8, NAP-2 and *gro*/MGSA

Human neutrophil plasma membranes were incubated for 30 min in the absence or presence of IL-8, NAP-2 or *gro*/MGSA (each at 1 μM) with GTP[^{35}S], and then analysed for bound GTP[^{35}S]. Each value represents the mean ± S.D. of triplicate determinations. The fold stimulation of [^{35}S]GTP[S] binding induced by the cytokines is given in parentheses.

	GTP[^{35}S] bound (pmol/mg of protein)
Control	0.82±0.07 (1.00)
NAP-2	1.29±0.12 (1.57)
gro/MGSA	1.39±0.06 (1.70)
IL-8	1.99±0.11 (2.43)

were different. Specifically, the incubation mixture for the latter assay contains high levels of GDP (10 μM), which decreases the potency of agonists to initiate G-protein activation in membrane preparations (P. Gierschik & K. H. Jakobs, unpublished work).

To investigate whether other members of the family of IL-8-related cytokines utilize signal-transduction pathways similar to those used by IL-8, we examined the effects of NAP-2 and *gro*/MGSA on GTP[^{35}S] binding to human neutrophil plasma membranes. As shown in Table 3, both NAP-2 and *gro*/MGSA (each at 1 μM) induced a considerable stimulation of GTP[^{35}S] binding, which was ~40% and 50%, respectively, of the stimulation observed in response to 1 μM-IL-8.

DISCUSSION

The results presented in this paper demonstrate directly that receptors for IL-8 interact functionally with G-proteins present in the plasma membrane of human neutrophils, since IL-8 markedly stimulates GTP hydrolysis by and GTP[^{35}S] binding to the membrane preparation. Both activities were strictly specific for guanine nucleotides (see the Materials and methods section). Binding of GTP[S] to G-proteins in the presence of Mg^{2+} has previously been shown to coincide with G-protein activation [44,45]. The data presented here therefore clearly indicate that the IL-8 receptor is coupled to and activates G-proteins in human neutrophil plasma membranes.

The second important outcome of this study is that the ability to elicit G-protein activation is not limited to IL-8, but may also

be observed with other members of the superfamily of IL-8-related cytokines. Thus significant stimulation of GTP[^{35}S] binding was also observed with both NAP-2 and *gro*/MGSA, although the degree of stimulation was lower than that observed for IL-8. It is important to note, however, that both NAP-2 and *gro*/MGSA were also less effective than IL-8 when their effects were examined on a variety of cellular functions of intact neutrophils. Specifically, both NAP-2 and *gro*/MGSA were less effective in inducing elastase release from cytochalasin B-treated human neutrophils [11,46]. Furthermore, in contrast with IL-8, *gro*/MGSA showed only marginal stimulation of the respiratory burst, and was slightly less effective as a chemoattractant than IL-8 [46]. One potential explanation for this phenomenon is that both NAP-2 and *gro*/MGSA only act as partial agonists at IL-8-binding sites. On the other hand, recent studies have shown that more than one type of receptors exist on the neutrophil surface to interact with IL-8, NAP-2 and *gro*/MGSA [31]. In the latter study, IL-8 bound to all receptors with high affinity. However, only two-thirds of the IL-8 receptors exhibited high affinity for NAP-2 and *gro*/MGSA, whereas one-third showed 300–1000-fold lower affinity. In addition, two distinct neutrophil surface proteins were specifically radiolabelled by chemical cross-linking with iodinated IL-8, and this labelling was inhibited in the presence of an excess of unlabelled NAP-2 or *gro*/MGSA [27,31]. It therefore appears likely that neutrophils express at least two receptors which recognize all three cytokines, albeit with different affinities, and that these differences in the binding parameters give rise to the lower efficacy of NAP-2 and *gro*/MGSA to elicit G-protein activation and induce cellular functions.

The nature of the G-protein involved interacting with the receptor(s) for IL-8 and its congeners is unknown and requires further investigation. It is very likely, however, that the IL-8 receptor interacts with G-proteins similar to, or even identical with, the G-proteins coupling to the formyl-peptide receptor and probably other chemoattractant receptors as well. This suggestion is supported by the similar patterns of cellular functions that are induced in neutrophils of IL-8 and classical chemotactic stimuli such as fMet-Leu-Phe, C5a, PAF and LTB_4 (for review see [1]). In addition, many of the parameters of receptor-stimulated GTPase activity and GTP[^{35}S] binding to membrane-bound G-proteins are very similar for IL-8, fMet-Leu-Phe, LTB_4 and C5a ([42,47,48]; R. W. Kupper & P. Gierschik, unpublished work). Finally, pertussis-toxin treatment similarly inhibits agonist stimulation of neutrophil functions regardless of whether IL-8 or one of the classical chemoattractants is used as stimulus [32,49]. Myeloid cells have previously been shown to express G_{i2} and G_{i3}, but lack G_{i1} and G_o-type G-proteins [50–52]. The formyl-peptide receptor of myeloid differentiated HL-60 cells interacts with both G_{i2} and G_{i3} [53]. It is thus tempting to speculate that the agonist-activated IL-8 receptor also interacts with the two G_i-proteins present in human neutrophils. Selective coupling of the receptors responsive to *gro*/MGSA to only one of these G-proteins would certainly be an intriguing possibility, because this might offer an explanation for the lack of stimulation of the respiratory burst by this cytokine.

An important question raised by this study is whether binding of other IL-8-related cytokines to receptors present on other target cells, e.g. lymphocytes, monocytes or melanoma cells, also elicits G-protein activation, and whether distinct receptors exist for these cytokines to mediate G-protein activation. If true, this would add a considerable number of new members to the already enormous list of G-protein-coupled receptors and would establish once again that these receptors are involved in regulating a wide variety of important biological functions, including normal and abnormal cell growth. It is clear that the availability of the individual cytokines in pure form, together with the highly sensitive methodology to assay cytokine-receptor–G-protein interaction outlined in this paper, will allow one to address these intriguing questions in the near future.

The expert technical assistance of Christina and Peter Stannek is greatly appreciated. This work was supported by grants from the Fritz Thyssen Stiftung and the Deutsche Forschungsgemeinschaft (Gi 138/3-2).

REFERENCES

1. Baggiolini, M., Walz, A. & Kunkel, S. L. (1989) J. Clin. Invest. **84**, 1045–1049
2. Westwick, J., Li, S. W. & Camp, R. D. (1989) Immunol. Today **10**, 146–147
3. Walz, A., Peveri, P., Aschauer, H. & Baggiolini, M. (1987) Biochem. Biophys. Res. Commun. **149**, 755–761
4. Yoshimura, T., Matsushima, K., Tanaka, S., Robinson, E. A., Appella, E., Oppenheim, J. J. & Leonard, E. J. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 9233–9237
5. Lindley, I., Aschauer, H., Seifert, J.-M., Lam, C., Brunowsky, W., Kownatzki, E., Thelen, M., Peveri, P., Dewald, B., von Tscharner, V., Walz, A. & Baggiolini, M. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 9199–9203
6. Tanaka, S., Robinson, E. A., Yoshimura, T., Matsushima, K., Leonard, E. J. & Appella, E. (1988) FEBS Lett. **236**, 467–470
7. Clark-Lewis, I., Moser, B., Walz, A., Baggiolini, M., Scott, G. J. & Aebersold, R. (1991) Biochemistry **30**, 3128–3135
8. Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Lew, W., Appella, E., Kung, H. F., Leonard, E. J. & Oppenheim, J. J. (1988) J. Exp. Med. **167**, 1883–1893
9. Furuta, R., Yamagishi, J., Kotani, H., Sakamoto, F., Fukui, T., Matsui, Y., Sohmura, Y., Yamada, M., Yoshimura, T., Larsen, C. G., Oppenheim, J. J. & Matsushima, K. (1989) J. Biochem. (Tokyo) **106**, 436–441
10. Wolpe, S. D. & Cerami, A. (1989) FASEB J. **3**, 2565–2573
11. Walz, A. & Baggiolini, M. (1989) Biochem. Biophys. Res. Commun. **159**, 969–975
12. Walz, A. & Baggiolini, M. (1990) J. Exp. Med. **171**, 449–454
13. Anisowicz, A., Bardwell, L. & Sager, R. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 7188–7192
14. Richmond, A., Balentien, E., Thomas, H. G., Flaggs, G., Barton, D. E., Spiess, J., Bordoni, R., Francke, U. & Derynck, R. (1988) EMBO J. **7**, 2025–2033
15. Derynck, R., Balentien, E., Han, J. H., Thomas, H. G., Wen, D., Samanta, A. K., Zachariae, C. O., Griffin, P. R., Brachmann, R., Wong, W. L., Matsushima, K. & Richmond, A. (1990) Biochemistry **29**, 10225–10233
16. Wolpe, S. D., Sherry, B., Juers, D., Davatelis, G., Yurt, R. W. & Cerami, A. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 612–616
17. Tekamp-Olson, P., Gallegos, C., Bauer, D., McClain, J., Sheery, B., Fabre, M., Van Deventer, S. & Cerami, A. (1990) J. Exp. Med. **172**, 911–919
18. Luster, A. D., Unkeless, J. C. & Ravetch, J. V. (1985) Nature (London) **315**, 672–676
19. Doi, T., Greenberg, S. M. & Rosenberg, R. D. (1987) Mol. Cell. Biol. **7**, 898–904
20. Poncz, M., Surrey, S., LaRocco, P., Weiss, M. J., Rappaport, E. F., Conway, T. M. & Schwartz, E. (1987) Blood **69**, 219–223
21. Sherry, B., Tekamp-Olson, P., Gallegos, C., Bauer, D., Davatelis, G., Wolpe, S. D., Masiarz, F., Coit, D. & Cerami, A. (1988) J. Exp. Med. **168**, 2251–2259
22. Davatelis, G., Tekamp-Olson, P., Wolpe, S. D., Hermsen, K., Luedke, C., Gallegos, C., Coit, D., Merryweather, J. Cerami, A. (1988) J. Exp. Med. **167**, 1939–1945
23. Furutani, Y., Nomura, H., Notake, M., Oyamada, Y., Fukui, T., Yamada, M., Larsen, C. G., Oppenheim, J. J. & Matsushima, K. (1989) Biochem. Biophys. Res. Commun. **159**, 249–255
24. Robinson, E. A., Yoshimura, T., Leonard, E. J., Tanaka, S., Griffin, P. R., Shabanowitz, J., Hunt, D. F. & Appella, E. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 1850–1854
25. Leonard, E. J. & Yoshimura, T. (1990) Immunol. Today **11**, 97–101
26. Schall, T. J., Bacon, K., Toy, K. J. & Goeddel, D. V. (1990) Nature (London) **347**, 669–671

27. Samanta, A. K., Oppenheim, J. J. & Matsushima, K. (1969) J. Exp. Med. **169**, 1185–1189
28. Besemer, J., Hujber, A. & Kuhn, B. (1989) J. Biol. Chem. **264**, 17409–17415
29. Samanta, A. K., Oppenheim, J. J. & Matsushima, K. (1990) J. Biol. Chem. **265**, 183–189
30. Grob, P. M., David, E., Warren, T. C., DeLeon, R. P., Farina, P. R. & Homon, C. A. (1990) J. Biol. Chem. **265**, 8311–8316
31. Moser, B., Schumacher, C., von Tscharner, V., Clark-Lewis, I. & Baggiolini, M. (1991) J. Biol. Chem. **266**, 10666–10671
32. Thelen, M., Peveri, P., Kernen, P., von Tscharner, V., Walz, A. & Baggiolini, M. (1988) FASEB J. **2**, 2702–2706
33. Tamura, M., Nogimori, K., Yajima, M., Ase, K. & Ui, M. (1983) J. Biol. Chem. **258**, 6756–6761
34. Nogimori, K., Ito, K., Tamura, M., Satoh, S., Ishii, S. & Ui, M. (1984) Biochim. Biophys. Acta **801**, 220–231
35. Nogimori, K., Tamura, M., Yajima, M., Ito, K., Nakamura, T., Kajikawa, N., Maruyama, Y. & Ui, M. (1984) Biochim. Biophys. Acta **801**, 232–243
36. Strnad, C. F. & Carchman, R. A. (1987) FEBS Lett. **225**, 16–20
37. Banga, H. S., Walker, R. K., Winberry, L. K. & Rittenhouse, S. E. (1987) J. Biol. Chem. **262**, 14871–14874
38. Gray, L. S., Huber, K. S., Gray, M. C., Hewlett, E. L. & Engelhard, V. H. (1989) J. Immunol. **142**, 1631–1638
39. Gierschik, P., Sidiropoulos, D., Spiegel, A. & Jakobs, K. H. (1987) Eur. J. Biochem. **165**, 185–194
40. Dewald, B. & Baggiolini, M. (1986) Methods Enzymol. **132**, 267–277
41. Borregaard, N., Heiple, J. M., Simons, E. R. & Clark, R. A. (1983) J. Cell Biol. **97**, 52–61
42. Gierschik, P., Moghtader, R., Straub, C., Dieterich, K. & Jakobs, K. H. (1991) Eur. J. Biochem. **197**, 725–732
43. Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
44. Nothup, J. K., Smigel, M. D. & Gilman, A. G. (1982) J. Biol. Chem. **257**, 11416–11423
45. Higashijima, T., Ferguson, K. M., Sternweis, P. C., Ross, E. M., Smigel, M. D. & Gilman, A. G. (1987) J. Biol. Chem. **262**, 752–756
46. Moser, B., Clark-Lewis, I., Zwahlen, R. & Baggiolini, M. (1990) J. Exp. Med. **171**, 1797–1802
47. Gierschik, P., Sidiropoulos, D., Steisslinger, M. & Jakobs, K. H. (1989) J. Pharmacol. **172**, 481–492
48. McLeish, K. R., Gierschik, P., Schepers, T., Sidiropoulos, D. & Jakobs, K. H. (1989) Biochem. J. **260**, 427–434
49. Becker, E. L., Kermode, J. C., Naccache, P. H., Yassin, R., Munoz, J. J., Marsh, M. L., Huang, C.-K. & Sha'afi, R. I. (1986) Fed. Proc. Fed. Am. Soc. Exp. Biol. **45**, 2151–2155
50. Gierschik, P., Falloon, J., Milligan, G., Pines, M., Gallin, J. I. & Spiegel, A. M. (1986) J. Biol. Chem. **261**, 8058–8062
51. Goldsmith, P. K., Gierschik, P., Milligan, G., Unson, C. G., Vinitsky, R., Malech, H. L. & Spiegel, A. M. (1987) J. Biol. Chem. **262**, 14683–14688
52. Goldsmith, P. K., Rossiter, K., Carter, A. D., Simonds, W. F., Unsen, C. G., Vinitsky, R. & Spiegel, A. M. (1988) J. Biol. Chem. **263**, 6476–6479
53. Gierschik, P., Sidiropoulos, D. & Jakobs, K. H. (1989) J. Biol. Chem. **264**, 21470–21473

Received 19 July 1991; accepted 4 October 1991