Physical Association of $G\alpha_{12}$ with Interleukin-8 Receptors*

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Interleukin-8 (IL-8), one of the major mediators of the inflammatory response, belongs to a family of chemokines that includes NAP-2 (neutrophil-activating peptide-2) and Gro-α and whose biological activities are directed to a great extent toward neutrophils. Two distinct receptors have been described with overlapping, but not identical, binding affinities for IL-8, NAP-2, and Gro-α. This study was designed to examine the intracellular pathways activated upon the occupation of each of the IL-8 receptors (IL-8R). The formation of a physical coupling between IL-8 receptors and the $\alpha$-subunit of heterotrimeric G proteins was tested in neutrophils by examining the presence of the former in anti-G$\alpha$ immune precipitates. The addition of IL-8 to a suspension of human neutrophils led to a time-dependent detection of IL-8 in anti-G$\alpha_{12}$ (raised against amino acids 159–168 (LERIAQSDYI) of G$\alpha_{12}$) and anti-G$\alpha_{13}$ (raised against the COOH-terminal 10 amino acids (KENLKDGLFL) of G$\alpha_{13}$), but not anti-G$\alpha_{11}$ immunoprecipitates. Similar results were obtained in human 293 cells stably transfected with IL-8RA or IL-8RB. The peptide derived from the COOH-terminal sequence of G$\alpha_{12}$ inhibited the co-immunoprecipitation of IL-8R and G$\alpha_{12}$ observed in response to the anti-G$\alpha_{12}$ and anti-G$\alpha_{12}$ antibodies. On the other hand, the G$\alpha_{13}$ peptide only inhibited the immunoprecipitation induced by the anti-G$\alpha_{13}$ antibody. Peptides derived from G$\alpha_{11}$ or G$\alpha_{3}$ had no effect in this assay. The introduction of the anti-G$\alpha_{12}$ or anti-G$\alpha_{13}$ antibodies or their neutralizing peptides, but not the G$\alpha_{12}$ or G$\alpha_{13}$ peptides, into 293 IL-8RA or 293 IL-8RB cells completely blocked the calcium responses obtained upon stimulation with IL-8. These results demonstrate that the occupation of either type of IL-8 receptor leads to a physical coupling to the $\alpha$-subunit of G$\alpha_{12}$. In addition, the use of the subunit-specific peptides identified two functionally important but distinct regions of G$\alpha_{12}$, one involved in receptor/G$\alpha_{12}$ interaction (KENLKDGLFL) and the other mediating downstream signal transmission (LERIAQSDYI). Finally, the results of this study also validate the use of the transfected 293 cell line as a model for the study of the signal transduction pathway(s) initiated by IL-8.

Interleukin-8 (IL-8)$^\dagger$ is a small nonglycosylated protein produced as a precursor of 99 amino acids and secreted after cleavage of a signal sequence of 20 residues. The mature form of 79 residues is processed proteolytically at the amino terminus, yielding several variants with biological activity. The predominant form consists of 72 amino acids and has a molecular mass of 8383 Da (1–4).

IL-8 belongs to a family of small proteins of 8–10 kDa with four cysteine residues at virtually identical positions (5–7). Alignment of these residues distinguishes between two groups of peptides: one with the two cysteines separated by one amino acid (CXC) and the other with adjacent cysteines (CC). IL-8, NAP-2 (neutrophil-activating peptide-2), and Gro gene products (Gro-α, Gro-β, Gro-γ) belong to the first group (8, 9).

IL-8 was identified on the basis of two biological effects on human neutrophils, namely chemotaxis and release of granule enzymes. This cytokine and the two structurally related ligands, Gro-α and NAP-2, are known to be largely, but not exclusively, selective for neutrophils. IL-8 is produced by many cell types, including fibroblasts and epithelial cells (10–12) and monocytes (4) as well as neutrophils themselves (3, 13). These pro-inflammatory cytokines induce increases in the intracellular concentration of free cytoplasmic calcium (14), cause changes in intracellular pH and cell shape (15), and stimulate chemotaxis (16–20) as well as the release of lysozyme enzymes in human neutrophils (16). All of these responses are ablated by pretreating neutrophils with pertussis toxin (21, 22), which is known to inactivate specific subunits of heterotrimeric G proteins (23, 24).

Several reports have demonstrated that IL-8 binds with high affinity to human neutrophils and myeloid cell lines. For peripheral blood neutrophils, receptor numbers in the range of 20,000–75,000/cell and mean affinity constants ($K_d$) of 0.1–0.3 nM were obtained (23). An additional population of high affinity binding sites (5200 receptors, $K_d = 11–35$ pm) has been described (25). Competition studies showed that $\sim 70\%$ of the IL-8 receptors (named IL-8RB) also bind NAP-2 and Gro-α with high affinity ($K_d = 0.1–0.3$ nm) (19, 25). The remaining 30% of the receptors (designated IL-8RA) bind the latter two peptides with low affinity ($K_d = 100–300$ nm) (25). IL-8, on the other hand, binds to both receptors with similar high affinity, IL-8RA and IL-8RB have molecular masses of 70 kDa (5200 receptors/cell) and 44 kDa (34,200 receptors/cell), respectively, and share $\sim 77\%$ amino acid sequence identity, with most of the diversity concentrated in the amino acid carboxyl-terminal region. The amino acid sequence encoded by the cDNA clone of IL-8 recep-

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$\ddagger$ The abbreviations used are: IL-8, interleukin-8; IL-8R, interleukin-8 receptor; HBSS, Hank's balanced salt solution; FACS, fluorescence-activated cell sorting.
The mode of interaction of IL-8 with its receptors remains incompletely understood (26). Most of the recent studies have concentrated on the architecture of the ligand-binding domain of the receptor (27), and little is known about the details of the signal transduction pathways activated upon receptor occupancy.

The recent observation that of the three CXC chemokines, IL-8, NAP-2, and Gro-β, only IL-8 is capable of efficiently activating phospholipase D (28) as well as the pattern of desensitization of these agonists (19) both indicate that the signal transduction pathways activated by the two receptor types, although similar, are not identical. However, in spite of the two former observations, it has been difficult to systematically determine the extent of similarity or divergence between the signal transduction pathways used by IL-8RA and IL-8RB since human neutrophils express both receptor subtypes. Recently, the cDNAs coding for IL-8RA and IL-8RB have been expressed in a human embryonic kidney cell line (293) (27). These transfectants express large numbers of IL-8 receptors that display binding affinities similar to those of the native neutrophil receptors. We took advantage of the availability of these stably transfected cell lines expressing a single IL-8 receptor type to initiate a systematic study of the signal transduction pathways linked to their occupancy. The initial steps of this investigation were to define the involvement of G proteins in the activation of the cells mediated through either type of IL-8 receptor, namely IL-8RA and IL-8RB.

The results of this study (a) demonstrate that the two IL-8 receptors in human neutrophils and those expressed in the 293 cell line are coupled through a pertussis toxin-sensitive G protein to a mobilization of calcium; (b) provide unequivocal evidence for a stimulus-induced physical association between the IL-8 receptors and the α-subunit of the G protein subset of G proteins; and (c) identify specific regions of the α-subunits of the G proteins that are involved in the upstream and downstream transmission of the intracellular activation signal.

MATERIALS AND METHODS

Reagents and Antibodies—The rabbit anti-Giα antibody was raised against the COOH terminus (KENLKDGLCF) of transducin as described previously (29). The rabbit anti-Gαi2 antibody was raised against the COOH terminus (KENLKDCGLF) of transducin as described previously (27). The rabbit anti-Gtα antibody was raised against the COOH terminus (KENLKDCGLF) of transducin as described previously (30). Briefly, the cells were washed with HBSS, resuspended at 2.5 × 106/ml, and their fluorescence was monitored in a spectrofluorometer (SLM Aminco, Inc., Brockville, ON, Canada) at 37°C. The goat anti-human IL-8 antibody was affinity purified from goat serum (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and was used at a concentration of 10 μg/ml. The polyclonal goat anti-human IL-8 antibody was raised against the LERIAQSDYI sequence of Gi2α (amino acids 159–168). The monoclonal rat anti-human IL-8 antibody was raised against the LDRISQSNYI sequence of Giα1 (amino acids 159–168). The rabbit anti-Gi1 antibody was raised against the COOH terminus (KENLKDCGLF) of transducin as described previously (27).

RESULTS

Neutrophil Studies

The strategy used to detect the potential presence and/or formation of complexes between IL-8 receptors and G protein subunits was as follows. Human neutrophils, incubated in the same culture conditions as the parental cell lines except for the addition of Genetin (800 μg/ml).

Immunoprecipitation—Cell suspensions (20 × 10^6 cells/sample) were incubated at 37°C and, where indicated, stimulated with 10 nM IL-8. Cells were lysed by adding an equal volume of immunoprecipitation buffer (50 mM Tris, pH 7.4, 0.1 mM NaCl, 0.25 mM phenylmethanesulfonyl fluoride, 1 mM Na3VO4) containing 1% octyl-β-D-glucopyranoside. The samples were vortexed and placed on ice for 30 min. The insoluble material was removed by centrifugation at 12,000 × g for 15 min. The supernatants were diluted with immunoprecipitation buffer to obtain a final octyl-β-D-glucopyranoside concentration of 0.413%. Protein A-Sepharose preincubated for 30 min at 37°C with the antibody (15 μg/ml) was added, and immunoprecipitations were carried out for 90 min on ice. The immunocomplexes were recovered by centrifugation at 12,000 × g for 2 min; washed three times with 50 mM Tris, pH 7.4, 0.2 mM NaCl, 2 mM phenylmethanesulfonyl fluoride, 2 mM Na3VO4, and finally resuspended in SDS sample buffer (60 mM Tris, pH 8.0, 2% SDS, 2% dithiothreitol, 10% glycerol, 0.01% bromphenol blue). Samples were boiled for 10 min before being subjected to SDS-polyacrylamide gel electrophoresis on 15% gels.

Immunoblotting—This was performed as described previously (31). Briefly, electrophoretic transfer cells (Fisher Scientific Instruments, Ontario) were used to transfer proteins from the gels to Immobilon polyvinylidene difluoride membranes. Nonspecific sites were blocked using 5% skim milk in TBS-Tween (25 mM Tris·HCl, pH 7.4, 0.15% Tween 20) for 1 h at 37°C. The polyoclonal goat anti-human IL-8 antibody was then incubated with the membranes for 1 h at 37°C for a final dilution of 1:1000 in fresh blocking solution. The membranes were washed three times at room temperature in TBS-Tween and then incubated with a horseradish peroxidase-linked swine anti-goat IgG antibody for 45 min at 37°C in a final dilution of 1:10,000 in fresh blocking solution. The membranes were washed three times with TBS/Tween, and the bands were revealed using the ECL Western blotting system.
presence or absence of IL-8 for different times and at different temperatures, were lysed, and the lysates were then incubated with anti-G_{\alpha} antibodies, anti-G_{\alpha} antibodies, or preimmune serum. The antibodies were then precipitated by centrifugation following incubation with protein A-Sepharose beads. After electrophoresis and transfer to Immobilon polyvinylidene difluoride membranes, the transferred materials were reacted with anti-IL-8 antibodies. The detection of IL-8 on the transfer membrane is thus an indication of the formation of a complex containing at least the cytokine, its receptors, and the \( \alpha \)-subunit of the G protein under study. The anti-G_{\alpha} antibody recognizes the \( \alpha \)-subunits of transducin, G_{\alpha}, and G_{\alpha} (35), whereas the anti-G_{\alpha} antibody recognizes amino acids 159–168 of G_{\alpha} only. The results obtained using the anti-G_{\alpha} antibodies are illustrated in Fig. 1. In this experiment, the cells were stimulated for various times before being lysed and subjected to the immunoprecipitation protocol. The amount of IL-8 recovered in the anti-G_{\alpha} immunoprecipitate increased in a time-dependent manner, attaining a maximal level by 1 min and then gradually declining until little, if any, IL-8 was detected by 10 min. In addition, IL-8 was detected in the anti-G_{\alpha} immunoprecipitates only if the chemokine was added to the cells before their lysis. This latter result demonstrated that the IL-8 detected in the immunoprecipitates did not represent a preformed complex between the receptor and the G protein or a carry-over of soluble IL-8. Furthermore, IL-8-preabsorbed antiserum (used as a negative control) failed to react with the transferred material on the polyvinylidene difluoride membranes (data not shown). The formation of the IL-8-G_{\alpha} complexes was temperature-dependent. No IL-8 could be detected in the anti-G_{\alpha} immunoprecipitates if the stimulation of cells was carried out at 4°C, whereas there was little, if any, difference in the amount of IL-8 recovered in the immunoprecipitates when the cells were stimulated for 1 min at room temperature or at 37°C. Similar results were found when the immunoprecipitation was carried out with the anti-G_{\alpha} antibodies (data not shown).

The specificity of the immunoprecipitation was determined next using a series of peptides including those against which the antibodies were raised (Fig. 2). In these experiments, the cells were stimulated for 1 min with IL-8 and then lysed. The immunoprecipitations were carried out with the anti-G_{\alpha} or anti-G_{\alpha} antibodies as in Fig. 1 or with antibodies preincubated with the indicated peptides. As shown in Fig. 2 (A and B), only the immunizing peptide of each antibody prevented the immunoprecipitation of the IL-8-G_{\alpha} complex with either antibody. The other three peptides were without effect. An irrelevant peptide derived from the structure of the Lolp1 antigen of the rye grass pollen was similarly without effect (data not shown).

The G protein-derived peptides were next introduced into the cells using the transient saponin-based permeabilization protocol described under "Materials and Methods." Prior to stimulation with IL-8, co-immunoprecipitation of G_{\alpha} and IL-8 with the anti-G_{\alpha} antibody was prevented by the addition of the immunizing peptide, but not the G_{\alpha}, G_{\alpha}, and G_{\alpha} peptides (Fig. 3A). On the other hand, when the immunoprecipitation was carried out with the anti-G_{\alpha} antibodies, the addition of either of the G_{\alpha} and G_{\alpha} peptides prevented the formation of the IL-8-G_{\alpha} complex (Fig. 3B). The peptides derived from G_{\alpha} or G_{\alpha} had no effect on the immunoprecipitation with either of the two antibodies (Fig. 3, A and B).

Studies in Transfected 293 Cells

The presence of two IL-8 receptors in human neutrophils makes it difficult, if not impossible, to determine unambiguously whether one or both are involved in the physical coupling between IL-8R and G proteins illustrated in Figs. 1–3. To resolve this ambiguity, we turned next to 293 cells stably transfected with individual IL-8 receptors.

Co-immunoprecipitation—The strategy utilized to detect the potential presence and/or formation of complexes between G proteins and IL-8RA or IL-8RB in transfected 293 cells was the same as that used for human neutrophils and described under "Neutrophil Studies." IL-8 was recovered in the anti-G_{\alpha} protein immunoprecipitates of transfected 293 cells stimulated for 1 min with the chemokine (Fig. 4), and this precipitation was blocked if the antibodies were neutralized with the peptide against which the antibody was raised, but not with the other peptides (data not shown). In the next series of experiments, the cells were permeabilized using saponin, and the peptides were introduced into the cells prior to stimulation with IL-8. IL-8 was recovered in the anti-G_{\alpha} and anti-G_{\alpha} precipitates of both 293 IL-8RA and 293 IL-8RB cells stimulated with the...
chekmoke (Fig. 4). The optimal time of stimulation with IL-8 prior to lysis was 1 min (data not shown). Furthermore, IL-8 was detected in anti-Gtα and anti-Giα immunoprecipitates only if the chemokine was added to the cells before their lysis (data not shown). No IL-8 could be detected in immunoprecipitates derived from the preimmune sera (data not shown). Finally, the formation of the two IL-8Rα complexes was also found to be temperature-dependent. No IL-8 could be detected in the anti-Gtα immunoprecipitates if the incubation with the cells was carried out at 4°C, whereas it was readily detected at room temperature (data not shown). Co-immunoprecipitation of Gtα and IL-8 with anti-Gtα antibody was prevented by the addition of the immunizing peptide, but not the Gtα, Gi2α, and Gi3α peptides (Fig. 4A). On the other hand, when the immunoprecipitation was carried out with the anti-Gtα antibodies, the addition of either of the Gtα- and Giα-derived peptides prevented the formation of the IL-8Rα-Gtα complex (Fig. 4B). The peptides derived from Gt1 or Gt3 had no effect on the immunoprecipitation with either of the two antibodies (Fig. 4, A and B).

Calcium Mobilization—The increase in the level of intracellular free calcium was chosen as a relevant and convenient measure of fibroblast activation by IL-8. The effect of the addition of IL-8 on the mobilization of calcium in 293 IL-8RA, 293 IL-8RB, and wild-type 293 cells was investigated. As shown in Fig. 5, a significant increase in the level of intracellular calcium was observed when IL-8 (100 nM) was added to 293 IL-8RA or 293 IL-8RB cells. In contrast, IL-8 failed to elicit a mobilization of calcium in the parental nontransfected cell line (data not shown). Furthermore, NAP-2 and Gro-α only induced an increase in the concentration of free calcium in 293 IL-8RB cells and were without effect in 293 IL-8RA cells.

The effect of pertussis toxin on the ability of IL-8 to induce a mobilization of calcium in 293 IL-8RA and 293 IL-8RB cells was examined (Fig. 6). Preincubation of 293 IL-8RA and 293 IL-8RB cells with pertussis toxin (1 μg/ml, 2 h, 37°C) virtually abolished the calcium response to IL-8 (Fig. 6), NAP-2, and Gro-α (293 IL-8RB only; data not shown). This effect was not observed with the B oligomer of pertussis toxin. In addition, preincubation with cholera toxin (1 μg/ml, 2 h, 37°C) did not affect significantly the magnitude or the shape of the calcium response to IL-8 (data not shown).

Inhibition of IL-8-induced Mobilization of Calcium—Evidence of the functional significance of the formation of the IL-8Rα-Gtα complex described in Figs. 1–4 was sought by examining the effects of the anti-Gtα antibodies and of the G protein-derived peptides on the mobilization of calcium induced by IL-8 in 293 IL-8RA (Fig. 7A) and 293 IL-8RB (data not shown) cells. The antibodies and the peptides were introduced into the cells during a transient permeabilization with saponin as described under "Materials and Methods." Under these conditions, ~90% of the cells were found to incorporate fluorescein isothiocyanate-labeled OKT3 antibody (Fig. 7A), and 70% of the cells remained capable of excluding trypan blue following the removal of saponin (data not shown). Furthermore, the cells retained their ability to transduce normal functional responses as verified by the calcium mobilization assay (Fig. 7B). As shown in Fig. 8, the inclusion of the preimmune serum or anti-Gtα antibodies during the permeabilization step did not affect the calcium response to IL-8. In contrast, the introduction of the anti-Gtα or anti-Giα antibodies into the cells significantly inhibited the subsequent ability of IL-8 to stimulate calcium mobilization in either transfected cell type (Fig. 8).

The saponin permeabilization procedure was also utilized to introduce Gtα, Gi2α, Gi3α, and Gtα peptides or an irrelevant peptide derived from the sequence of the Lolp1 allergen into the cells. The effect of the introduction of these peptides on the subsequent ability of IL-8 to induce a mobilization of calcium in 293 IL-8RA and 293 IL-8RB cells is shown in Fig. 9. These data demonstrate that the calcium mobilization response to IL-8 was significantly inhibited by the Gtα and Giα peptides, but was unaffected by the Giα, Giα, or Lolp1 peptide (latter not shown). Furthermore, none of the peptides had any effect on the mobilization of calcium induced by IL-8 if added to the control untreated cells in the absence of permeabilization (data not shown).
and a functional assay (Ca$^{2+}$ mobilization) with the transient nature of the association of IL-8 with Gi subunits. This complex was not observed if IL-8 was added to transfected 293 cells, or if the cells were incubated with a 1 μg/ml concentration of the toxins for 2 h at 37°C. The basal calcium levels were 150 ± 25 nM, and the increases in calcium represent the differences between the basal and peak levels of the cytoplasmic concentrations of calcium reached upon stimulation. The data represent the mean ± S.E. of the number of experiments indicated in parentheses. PT, pertussis toxin.

**DISCUSSION**

An approach combining a co-immunoprecipitation protocol and a functional assay (Ca$^{2+}$ mobilization) was used to demonstrate the coupling of IL-8 receptors to the α-subunit of G proteins of the G$_2$ family. Evidence was obtained indicating that both IL-8 receptors interact in a dynamic fashion with G$_{2}$α and identifying sequences of G$_{2}$α involved in upstream and downstream coupling.

The basic result obtained during this investigation is that the occupancy of IL-8 receptors leads to the formation of a multimeric complex containing at least the receptors themselves and the α-subunits of G$_2$ proteins. This complex can be detected using the co-immunoprecipitation protocol in human neutrophils as well as in 293 cells transfected with IL-8RA or IL-8RB. This result is consistent with the known sensitivity of the biological effects of CXC chemokines on neutrophils to pertussis toxin. The IL-8-G$_{2}$α complex is apparently not preformed in human neutrophils or transfected 293 cells. Its detection in the co-immunoprecipitation experiments was dependent on the addition of IL-8 to the cells. Preincorporation of the G$_{2}$α peptide into the cells prevented its formation. Furthermore, this complex was not observed if IL-8 was added to the cells at 4°C or after lysis. These latter observations, coupled with the transient nature of the association of IL-8 with G$_{2}$α, indicate that the interaction between the two proteins is a dynamic phenomenon driven by the occupancy of IL-8 receptors. It is worth pointing out that an association between G$_{2}$α and the formyl-Met-Leu-Phe receptors (transfected L cells (36, 37) and mouse fibroblasts (38)) and C5a receptors in human neutrophils (39, 40) has recently been demonstrated. It is therefore likely that the formation of this multimeric protein complex is a common element of the chemotactic factor receptor signaling mechanisms.

The neutrophil responses to IL-8 are unique among those to chemoattractants in that these cells express two distinct receptor subtypes, both of which bind IL-8 with high affinity (25, 41, 42). In the absence of highly specific receptor antagonists, it is therefore difficult to unambiguously assign a particular response to IL-8 in neutrophils to one or the other receptor subtype or both receptors. To circumvent this difficulty, we took advantage of the recent availability of 293 cells stably transfected with IL-8RA or IL-8RB. Several lines of evidence indicate that the two IL-8 receptors are not only expressed in 293 cells with close to normal binding affinities (27), but that they are also functionally coupled to at least one relevant effector system, namely the mobilization of calcium. The transfected cell lines responded to physiologically relevant concentrations of IL-8 with a mobilization of calcium and exhibited the expected selectivity toward the three CXC chemokines. Furthermore, the responses to all three chemokines were inhibited by pertussis toxin. This latter result demonstrates that pertussis toxin-sensitive G proteins are present in 293 cells and suggests that, as in human neutrophils, they play a coupling role in the mediation of the transduction pathways stimulated by interleukin-8 in these cells. This conclusion is supported by the detection of proteins reacting with anti-G$_{2}$α antibodies in 293 cells and by the demonstration of the formation of an immunoprecipitable complex between IL-8RA, IL-8RB, and G$_{2}$α in transfected 293 cells.

The co-immunoprecipitation experiments were complemented by examining the downstream consequences (i.e. mobilization of calcium) of the introduction of anti-G$_{2}$α antibodies or of peptides specific for various G protein subunits into transfected 293 cells. Transient permeabilization of 293 cells transfected with IL-8RA or IL-8RB in the presence of the anti-G$_{2}$α antibodies (which recognize G$_{2}$α and G$_{2}$β) or the anti-G$_{2}$β antibodies significantly inhibited the mobilization of calcium induced by IL-8. The anti-G$_{2}$α antibodies were ineffective in this assay, a result in accord with the apparent absence of G$_{2}$α in 293 cells as judged by Western blotting (data not shown). The introduction of the G$_{2}$α peptides into transfected 293 cells yielded data consistent with those derived from the antibody experiments. Peptides derived from sequences specific for G$_{2}$α or G$_{2}$β were without effect. On the other hand, the peptides derived from the COOH terminus of transducin (common to G$_{2}$α, G$_{4}$α, and G$_{2}$α) or from an internal and specific sequence of G$_{2}$β$_{2}$α inhibited the mobilization of calcium induced by IL-8 in...
293 IL-8RA and 293 IL-8RB cells. These results provide evidence supporting a functional relevance of the formation of the IL-8R

G \alpha complex that goes significantly beyond that derived from the use of pertussis toxin.

The use of G protein-specific peptides and antibodies provides detailed information about the nature of the G protein(s) coupled to the IL-8 receptor. The results are in accordance with the known pertussis toxin sensitivity of the responses elicited by CXC chemokines in neutrophils (15). However, they are at variance with the previously reported ability of members of the G\alpha family to reconstitute an IL-8-sensitivestimulation of phospholipase C in COS-7 cells transfected with IL-8 receptors (43), as in the present study, anti-G\alpha antibodies had no effect on the co-immunoprecipitation of IL-8R and G\alpha, and no immunoreactive material could be detected with the anti-G\alpha antibodies in human neutrophils or 293 cells (data not shown). Furthermore, no IL-8 could be recovered in immunoprecipitates obtained using the anti-G\alpha antibodies. The differences in these results may be accounted for by the known interchangeability that exists among \alpha-subunits of heterotrimeric G proteins. In addition, the only peptides that inhibited IL-8-induced calcium mobilization were those derived from the COOH-terminal tail of transducin (shared by G\alpha1 and G\alpha2) or from a sequence specific for G\alpha2. Equivalent peptides from sequences specific for G\alpha1 and G\alpha3 were without effect. Taken together, the pertussis toxin sensitivity and the antibody and peptide specificities all support the hypothesis that at least one of the physiologically relevant G proteins coupled to IL-8 receptors in neutrophils as well as in transfected 293 cells is G\alpha1, or a very closely related member of the same family.

Examination of the results obtained with the two peptides that inhibited the responses of the cells to IL-8 provides partial insights about the functional topology of the G protein involved.
in IL-8R signal transduction. The ability of the peptide derived from the COOH-terminal region of transducin (which is shared by Gt) to inhibit the co-immunoprecipitation of IL-8R (A and B) and Gt by both the anti-Gt and anti-Gtα antibodies as well as the mobilization of calcium induced by IL-8 indicates that this sequence interacts directly with the receptor. On the other hand, the peptide derived from the amino acid sequence of Gtα inhibited only the mobilization of calcium, but did not affect the co-immunoprecipitation observed with the anti-Gtα antibody. The simplest explanation of these results is that this latter sequence (amino acids 159–168 of Gtα) is exposed to the downstream effector side of the G protein. The identification of the effector systems linked to Gtα goes beyond the scope of this investigation. While it is unclear which of the subunits of Gtα is responsible for activating downstream events (including phospholipase C), the demonstration of a stimulated association between Gtα and IL-8R strongly suggests that the former plays at least a linking role in the signal transduction sequence initiated upon occupation of these receptors.

In summary, the demonstration of a physical coupling between the IL-8 receptors and Gtα as well as the validation of the transfected 293 cells as a model provide 1) significant information about the molecular components of the IL-8R complex, 2) information on the binding affinity of Gtα to IL-8 receptors, 3) insight on the topology of the G proteins and the identification of Gtα amino acid sequences interacting with IL-8 receptors or downstream effector systems, and finally, 4) the necessary experimental background to further these studies by examining the nature of other components of this complex as well as its linkage to other effector systems.

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