cordance with the institutional guidelines of McGill University. Briefly, surgical anesthesia was obtained with 2.5% intravenous sodium thiopentone. Anesthesia and paralysis were maintained during recording with 7:3 NO_2/O_2, sodium pentobarbital (1 mg per kilogram of body weight per hour), and gallamine triethiodide (10 mg/kg per hour). End-tidal CO_2, temperature, electroencephalogram, and electrocardiogram were monitored and maintained at normal levels. Cells were classified as simple or complex according to conventional criteria [D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **160**, 106 (1962); B. C. Skottun *et al.*, *Vision Res.* **31**, 1079 (1991)]. A statistical significance test was used to compare each neuron's envelope response with its spontaneous activity and luminance response at the carrier spatial frequency.

9. Because the envelope-responsive cells included both simple and complex types, the nonlinearity mediating the envelope response must be distinct from the one distinguishing complex cells from simple cells.
10. In testing the envelope responses at different stimulus orientations, the carrier and envelope were always at the same orientation.
11. A pointwise nonlinearity is defined as: output(x,t) = N[input(x,t)] (x, distance; t, time). Candidates for such a transform include half-wave or full-wave rectification, a squaring operation, a logarithmic transformation, or any pointwise nonlinearity that can be expressed by a polynomial function with nonzero even terms.
12. D. G. Albrecht and R. L. De Valois [*J. Physiol. (London)* **319**, 497 (1981)] did not observe envelope-responsive cells in striate cortex of cat and monkey, using envelope stimuli with a fixed 5:1 ratio for carrier:envelope spatial frequency and a rigid motion between envelope and carrier. Our investigation indicated that this ratio varied from 4 to 30 for different neurons. Consequently, fixing the frequency ratio at 5:1 significantly reduced the chance of finding envelope-responsive cells, especially considering the small sample size (n = 24), the smaller percentage of envelope-responsive neurons in area 17 of cat (see text), and the very narrow range of effective carrier frequency for a given neuron.
13. We thank R. F. Hess for suggestions on the manuscript and M. Moscovitch for contributions to computer programming. Supported by the Canadian Medical Research Council (MA-9685) and Stairs Memorial grants to C.L.B. Gallomine triethiodide was donated by Rhone-Poulenc Pharma.

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G Protein–Coupled Signal Transduction Pathways for Interleukin-8

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Interleukin-8 (IL-8) is one of the major mediators of the inflammatory response. The pathways by which IL-8 activates inositide-specific phospholipase C (PLC) were investigated by co-expression of different components of the guanosine triphosphate binding protein (G protein) pathway in COS-7 cells. Two distinct IL-8 receptors reconstituted ligand-dependent activation of endogenous PLC when transfected together with the G protein α subunits $G\alpha_{14}$, $G\alpha_{15}$, or $G\alpha_{16}$. However, reconstitution was not observed with cells that overexpressed $G\alpha_q$ or $G\alpha_{11}$. Furthermore, IL-8 receptors interacted with endogenous pertussis toxin–sensitive G proteins or with the recombinant G protein G_i to release free βγ subunits that could then specifically activate the β2 isoform of PLC. These findings suggest that IL-8 acts through signal-transducing pathways that are limited to specific heterotrimeric G proteins and effectors. These may provide suitable targets for the development of anti-inflammatory agents.

A wide range of chemical signals are transduced into intracellular changes in metabolism through the coupling of receptors to heterotrimeric guanosine triphosphate (GTP) binding proteins (G proteins). The interaction of ligand with a G protein–coupled membrane receptor results in the exchange of guanosine diphosphate (GDP) bound to the G protein α subunit for GTP, which causes the subsequent dissociation of the heterotrimer into the α and βγ subunits (*1*, *2*). To examine the nature of the signal transduction pathways that take part in complex cellular responses such as inflammation, we sought to reconstitute signal transduction with white cell–specific receptors and to examine their requirements for specific G proteins and specific effectors. The peptide cytokine IL-8 is one of the most potent chemoattractants for neutrophils (*3*). IL-8 also induces angiogenesis, mediates cytokine-induced transendothelial neutrophil migration (*4*), and triggers a variety of other effects associated with the inflammatory response (*3*). The complementary DNAs (cDNAs) that encode two distinct types of IL-8 receptors, designated α and β, have been cloned and sequenced (*5–7*). These receptors appear to couple to G proteins and activate PLC in neutrophils (*3*).

The G_q class of G protein α subunits can activate members of the β family of PLC isozymes to stimulate the release of inositides and diacylglycerol (*8*). Five cDNAs that encode these α subunits of this class have been characterized: $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$ (*2*). All of the α subunits activate the PLC-β1 isoform (*9*, *10*), and most of them activate the PLC-β2 isoform (*10*, *11*). Furthermore, the free βγ complex activates PLC-β2 but not PLC-β1 in cotransfection assays (*12*) and preferentially activates PLC-β2 in cell-free assays (*13*). The responses of cells to ligands that induce PLC activity have been divided into two groups: those that are sensitive to pertussis toxin inhibition and those that are resistant (*2*). Pertussis toxin covalently modifies certain Gα subunits so that ligand-induced exchange of GDP for GTP on the Gα subunit is blocked. Pertussis toxin–resistant activation of PLC is probably mediated by the α subunits of the G_q class. The $G\alpha_q$ subunits that activate PLC-β lack a site for modification by pertussis toxin (*2*), and a variety of specific antisera to the $G\alpha_q$ subunits block receptor-mediated responses that are resistant to pertussis toxin (*14*). The pertussis toxin–sensitive response can be reconstituted through receptor-mediated release of βγ subunits from members of the G_i class (*12*). The toxin apparently blocks the activation of PLC-β2 by interfering with the release of the βγ subunits from the trimeric G proteins.

The expression of PLC-β2 appears to be developmentally regulated and is expressed in the hematopoietic lineage (*15*). Expression of certain Gα subunits, such as $G\alpha_{16}$, is limited to cells derived from the hematopoietic lineage (*16*). To determine whether the two IL-8 receptors, IL-8Rα and IL-8Rβ, transduce signals through any of the known G_q family members, we cotransfected COS-7 cells with cDNAs encoding IL-8Rα or IL-8Rβ alone or together with $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, or $G\alpha_{16}$. Neither nontransfected COS-7 cells (Fig. 1) nor cells transfected with receptors alone showed any ligand-induced release of inositol phosphates (Fig. 1, A and B). However, ligand-induced release of inositol phosphates was detected in cells that co-expressed IL-8Rα or IL-8Rβ and $G\alpha_{16}$ or $G\alpha_{14}$ but not in cells cotransfected with either of the IL-8 receptors and $G\alpha_q$ or $G\alpha_{11}$. This indicates that both IL-8 receptors can activate PLC molecules that are endogenous to COS-7 cells by specifically coupling to $G\alpha_{14}$ and $G\alpha_{16}$ but not to $G\alpha_q$ or $G\alpha_{11}$. Tests with specific antibodies indicated that PLC-β1 was present in COS-7 cells but PLC-β2 was not (*12*). Both IL-8 receptors can also couple to $G\alpha_{15}$ (Fig. 1, A and B), the mouse counterpart of human $G\alpha_{16}$ (*17*). The dose-dependent responses to ligand indicate a mean effective concentration (EC_{50}) for both IL-8Rα and IL-8Rβ of about 2.5 nM (Fig. 1C), which is very close to the inhibition constant of IL-8 for

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both receptors (1.2 to 3.8 nM) measured in transfected COS-7 cells (*6*, *7*).

All of the Gα proteins tested were expressed in cotransfected COS-7 cells. The expressed proteins were detected with an antibody (anti-$G\alpha_q$ common) to a peptide corresponding to a common sequence shared by all of the α subunit proteins in the G_q class; thus, we would expect them to have approximately the same immunoreactivity. It is clear that the amount of expressed $G\alpha_{16}$ was less than that of $G\alpha_{15}$, $G\alpha_q$, or $G\alpha_{11}$. We could not clearly detect $G\alpha_{14}$ with anti-$G\alpha_q$ common because of the basal amounts of endogenous $G\alpha_{11}$ and $G\alpha_q$ (*9*). However, $G\alpha_{14}$ was indeed expressed because it was detected with a specific antibody to $G\alpha_{14}$ (Fig. 1D). The lower reactivity of $G\alpha_{14}$ with IL-8R could be attributed to either a lower level of expression or a poorer coupling to IL-8R or both. Furthermore, expression of $G\alpha_{16}$ or $G\alpha_q$ was not affected by co-expression of IL-8 receptors (Fig. 1E) nor was the amount of recombinant receptor proteins affected by co-expression of Gα subunits (*18*).

The IL-8 receptors activate PLC and raise the cytosolic Ca^{2+} concentration through a pertussis toxin–sensitive pathway in neutrophils (*3*, *19*), in which PLC-β2 is likely to be present (*15*). To test whether IL-8 receptors specifically activate PLC-β2 by interacting with endogenous G proteins and releasing βγ subunits, we cotransfected COS-7 cells with cDNAs that corresponded to the IL-8 receptors and PLC-β2, which is not present in COS-7 cells (*12*). The cotransfected cells displayed ligand-dependent release of inositol phosphates with an EC_{50} of about 2.5 nM (Fig. 2A). These responses, however, were sensitive to pertussis toxin treatment, which is in contrast to the responses in cells cotransfected with $G\alpha_{16}$ (Fig. 2, B and C). Cells cotransfected with PLC-β1 did not respond to IL-8 (Fig. 2A). The simplest explanation of these results is that IL-8 receptors may interact with endogenous heterotrimeric G proteins that are sensitive to pertussis toxin (*20*) and release βγ subunits. These free βγ subunits could then activate the recombinant PLC-β2.

To test whether the IL-8 receptors interacted with the G_{i2} or G_{i3} proteins and released Gβγ to activate PLC-β2, we established an assay system in which all the relevant components were co-expressed. Cells that co-expressed IL-8Rα, $G\alpha_{i2}$, and PLC-β2 displayed some ligand-mediated release of inositol phosphates (Fig. 3A) (*21*). However, cells that co-expressed IL-8Rα, PLC-β2, and $G\alpha_{i2}$ or $G\alpha_{i3}$ together with Gβ1 and Gγ2 showed a much higher IL-8–induced release of inositol phosphates, and this ligand-induced activity was sensitive to pertussis toxin (Fig. 3A). To confirm that the ligand-induced response in this five-component cotransfection assay was the result of recombinant proteins and not markedly affected by endogenous G proteins, we used a $G\alpha_{i3}$ mutant, $G\alpha_{i3}$*, instead of the wild-type $G\alpha_{i3}$. This mutant cannot be modified by pertussis toxin because Cys^{351}, which is the substrate for pertussis toxin action, is replaced by a Ser residue. The ligand-induced response in cells that co-expressed $G\alpha_{i3}$* with Gβ1 and Gγ2, PLC-β2, and IL-8Rα was resistant to pertussis toxin (Fig. 3A). This result, taken together with the results that showed that each of the given components was expressed in similar amounts (Fig. 3C), suggests that the ligand-induced response resulted from expression of the recombinant $G\alpha_i$ proteins. Because neither $G\alpha_{i3}$ nor

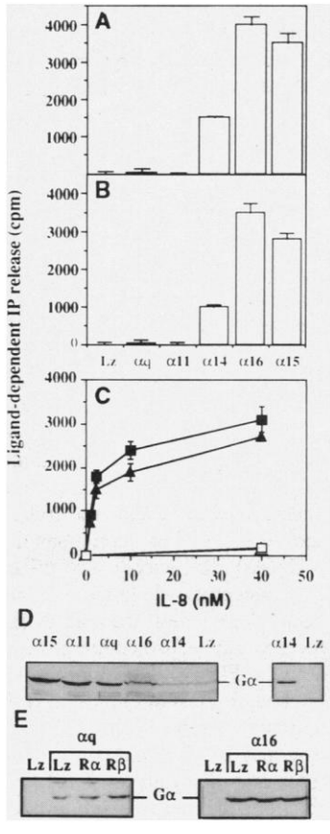

Fig. 1. IL-8–induced activation of endogenous PLC in COS-7 cells that co-expressed IL-8 receptors and Gα subunits. (**A** and **B**) COS-7 cells were cotransfected with cDNA (0.5 μg per well) encoding IL-8Rα (A) or IL-8Rβ (B) and cDNA (0.5 μg per well) encoding β-galactosidase (Lz) or the α subunits of the G_q class, $G\alpha_q$ (αq), $G\alpha_{11}$ (α11), $G\alpha_{14}$ (α14), $G\alpha_{15}$ (α15), or $G\alpha_{16}$ (α16). (**C**) COS-7 cells were cotransfected with the $G\alpha_{16}$ cDNA (0.5 μg per well) and cDNA (0.5 μg per well) encoding β-galactosidase (Lz, open symbols), IL-8Rα (closed triangles), or IL-8Rβ (closed squares). The amount of inositol phosphate (IP) in the cells was measured 25 min after the addition of 100 nM IL-8 (A and B) or various amounts of it (C) (*27*). (**D**) COS-7 cells were transfected with the cDNA [indicated as in (A)] (1 μg per well). Cells were lysed in SDS sample buffer 48 hours after transfection and subjected to protein immunoblotting with the anti-$G\alpha_q$ common (left panel) or with an antibody specific for $G\alpha_{14}$ (right panel). The anti-$G\alpha_q$ common was raised against the synthetic peptide GESGKSTFIKQMRII. (Abbreviations for the amino acid residues are: E, Glu; F, Phe; G, Gly; I, Ile; K, Lys; M, Met; Q, Gln; R, Arg; S, Ser; and T, Thr.) (**E**) COS-7 cells were cotransfected with cDNAs (0.5 μg per well per component) as indicated. The same amounts of cell extract were analyzed by protein immunoblotting with antibodies specific for $G\alpha_q$ (left panel) or $G\alpha_{16}$ (right panel). IL-8Rα, Rα; IL-8Rβ, Rβ.

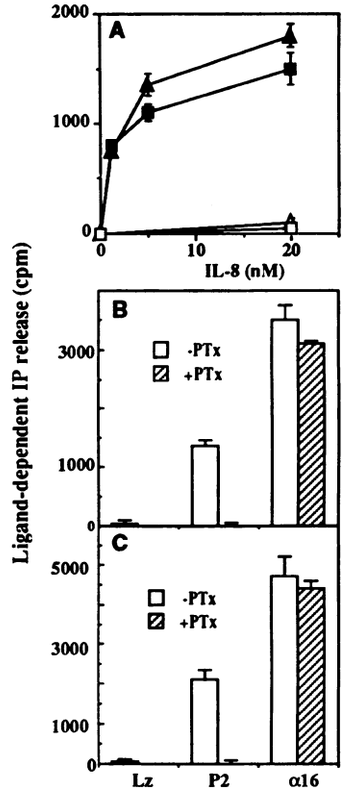

Fig. 2. IL-8–induced release of inositol phosphate (IP) from cells that co-expressed IL-8 receptors and PLC-β2. (**A**) COS-7 cells were cotransfected with cDNA (0.5 μg per well) encoding IL-8Rα (squares) or IL-8Rβ (triangles) and cDNA (0.5 μg per well) encoding PLC-β2 (closed symbols) or PLC-β1 (open symbols). IL-8–induced release of inositol phosphates was measured as described (Fig. 1). (**B** and **C**) COS-7 cells were cotransfected with cDNA (0.5 μg per well) encoding IL-8Rα (B) or IL-8Rβ (C) and cDNA (0.5 μg per well) corresponding to β-galactosidase (Lz), PLC-β2 (P2), or $G\alpha_{16}$ (α16). The release of inositol phosphates, induced by 100 nM IL-8, was measured 4 hours after the treatment in the presence (shaded bars) or absence (open bars) of pertussis toxin (PTx) (400 μg/ml).

$G\alpha_{i2}$ can directly activate the endogenous PLC or PLC-β2 (*22*), it is reasonable to conclude that IL-8Rα interacts with the recombinant G_i proteins and causes the release of the βγ subunits, which then activate PLC-β2. The experiments were repeated with IL-8Rβ, and the results were similar (Fig. 3B).

The ability of these receptors to couple only to specific Gα subunits may explain why cells transfected with IL-8 receptors alone, unlike cells transfected with α_1-adrenergic receptors (*9*), do not respond to IL-8. $G\alpha_{14}$, $G\alpha_{15}$, or $G\alpha_{16}$ were not detected in COS-7 cells, even though $G\alpha_q$ and $G\alpha_{11}$ were found (*9*). Most of the components we reconstituted in COS-7 cells are likely to be found in neutrophils or related cells. PLC-β2 was cloned from human myeloid leukemia cell line (HL-60) cells (*15*) that also contain Gβ1 (*16*), $G\alpha_{i3}$, and $G\alpha_{i2}$ (*23*). The amount of $G\alpha_{i2}$ protein increases after the differentiation of HL-60 cells to the neutrophil phenotype, whereas the amount of $G\alpha_{16}$ decreases by 90% (*16*). This may explain why responses to IL-8 in mature neutrophils are mostly sensitive to pertussis toxin (*3*, *19*). Furthermore, $G\alpha_{16}$ and Gβγ subunits may act cooperatively to activate PLC (*11*). The synergistic action of multiple activation pathways could produce a large and rapid increase in the amount of second messengers, which may be necessary for the induction of the inflammatory responses.

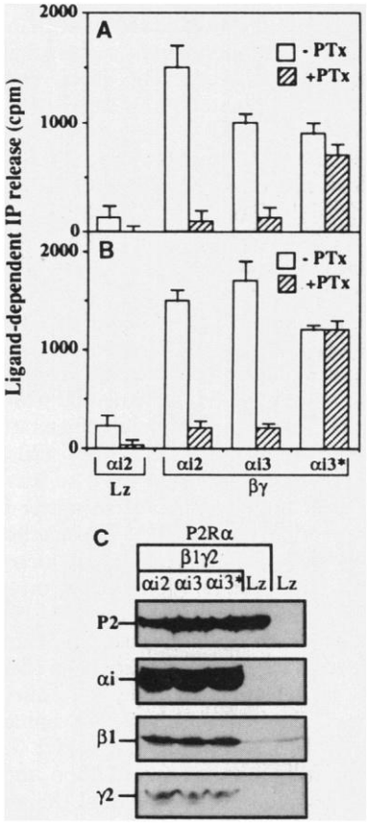

Fig. 3. Activation of PLC-β2 by IL-8 receptors through G_{i2} or G_{i3} proteins. (**A** and **B**) COS-7 cells were cotransfected with cDNAs (1 μg per well) encoding PLC-β2, Gβ1 (β), Gγ2 (γ), $G\alpha_{i2}$ (αi2), $G\alpha_{i3}$ (αi3), or the $G\alpha_{i3}$ pertussis toxin–resistant mutant (αi3*) and either IL-8Rα (A) or IL-8Rβ (B). Equal amounts of cDNA (0.2 μg per well per component) were transfected except for β-galactosidase (0.4 μg per well). The 100 nM IL-8–induced release of inositol phosphates was measured 4 hours after treatment in the presence (shaded bars) or absence (open bars) of pertussis toxin (PTx) (400 μg/ml). (**C**) The amounts of PLC-β2 (P2), Gβ1 (β1), Gγ2 (γ2), and $G\alpha_i$ (αi) subunits expressed were analyzed by protein immunoblotting. PLC-β2, Gβ1, and Gγ2 were detected with the appropriate specific antibodies, whereas $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_{i3}$* were detected with an antibody to the sequence that they share. IL-8Rα, Rα.

Because IL-8 has been found to act as a chemoattractant for some human peripheral T cells (*24*) and $G\alpha_{16}$ has been found in T lymphocytes (*16*), $G\alpha_{16}$ could mediate IL-8 effects in these cells. Furthermore, the IL-8 homolog GRO/MGSA is a potent autocrine factor for melanoma cells and specifically binds to IL-8Rβ but not to IL-8Rα (*7*). GRO/MGSA could also activate PLC through $G\alpha_{16}$, $G\alpha_{14}$, or Gβγ subunits (*25*).

Although IL-8 receptors are distinct from the C5a and *f*-Met-Leu-Phe (*f*MLP) receptors, they are thought to share some signal transduction pathways because in neutrophils they all activate PLC in a pertussis toxin–sensitive manner (*3*, *19*). C5a specifically couples to $G\alpha_{16}$ but not to $G\alpha_q$ (*26*). C5a and *f*MLP can also interact with G_{i2} or G_{i3} proteins to release βγ subunits and activate PLC-β2 in a pertussis toxin–sensitive manner (*25*). Therefore, the signal transduction pathways that we have described may be used by other chemotactic cytokines, and PLC-β2 is a potential target for the action of anti-inflammatory drugs. The cotransfection assay system in COS-7 cells may facilitate the search for such compounds.

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18. The binding of ^{125}I-labeled IL-8 to cells that co-expressed IL-8 receptors and $G\alpha_{16}$, $G\alpha_q$, or β-galactosidase was measured and analyzed by Scatchard analysis. Cells transfected with IL-8Rα and $G\alpha_{16}$, $G\alpha_q$, or β-galactosidase had 101, 34, and 86 fmol of receptor per 1×10^5 cells, respectively, whereas cells that co-expressed IL-8Rβ had 35, 30, and 53 fmol of receptor per 1×10^5 cells, respectively. These values are not corrected for transfection efficiency. The calculated dissociation constant values range from 1.8 to 3.8 nM.
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20. COS-7 cells have readily detectable amounts of the $G\alpha_{i2}$ protein, whereas the $G\alpha_o$ subunits were not detected (*25*).
21. The ligand-dependent response in cells cotransfected with the IL-8 receptors, PLC-β2, $G\alpha_i$, and two parts of β-galactosidase (Fig. 3, A and B) was smaller than that in cells transfected with only the receptor and PLC-β2 (Fig. 2). This may reflect the fact that less DNA (0.2 μg per well) was used for the experiments for Fig. 3 than in those for Fig. 2 (0.5 μg per well).
22. $G\alpha_{i2}$ or $G\alpha_{i3}$ subunits did not activate PLC-β1 or PLC-β2 in the cotransfection assay (*9*), and transfection with the activated mutants of $G\alpha_{i2}$ or $G\alpha_{i3}$ did not increase PLC activities in COS-7 cells.
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27. For transfection, COS-7 cells (1×10^5 cells per well) were seeded in 12-well plates the day before transfection with lipofectin. The cells were then labeled with [^{3}H]inositol (10 μCi/ml) 24 hours later. The next day, the release of inositol phosphates was measured 25 min after treatment of IL-8 (100 nM). Cells were lysed in 10% perchloric acid and neutralized with 2 N KOH. After centrifugation, the supernatant was transferred to columns that contained 0.5 ml of AG1-X8 anion exchange resin. The columns were washed with 6 ml of borax buffer and eluted with 0.3 ml of formic acid (0.1 M). Portions (0.5 ml) of eluted samples were mixed with a scintillation cocktail and counted in a scintillation counter. The basal amount of inositol phosphate in cells transfected with β-galactosidase is about 1000 cpm. Cells transfected with $G\alpha_{11}$, $G\alpha_q$, and $G\alpha_{15}$ showed 1000 cpm over the basal level, and cells transfected with $G\alpha_{16}$ or PLC-β2 showed 300 cpm over the basal release. Transfection with other cDNAs did not show any change in the basal release.

28. We thank A. Katz for providing the Gβγ plasmids and T. Daly and J. Barry for purified IL-8 and GRO/MGSA. Supported by a U.S. Public Health Service grant (M.I.S.) and Repligen Corporation.

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