COMMENTARY

Roles of phospholipid signaling in chemoattractant-induced responses

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SUMMARY

Chemoattractants, including chemokines, play a central role in regulation of inflammatory reactions by attracting and activating leukocytes. These molecules have been found to regulate metabolism of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) via phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K). Recent studies of mouse lines that lack PLC-β2, PLC-β3, or PI3Kγ demonstrate that chemoattractants act through PLC-β2 and PLC-β3 to hydrolyze PtdIns(4,5)P2 and through PI3Kγ to phosphorylate PtdIns(4,5)P2 in mouse neutrophils. These studies also confirmed the importance and revealed new roles of these signaling pathways in chemoattractant-induced responses.

Key words: Chemoattractant, Chemokine, G protein, Chemotaxis, Phospholipase, Phosphoinositide 3-kinase, Gene targeting

INTRODUCTION

Many biologically active molecules function as chemoattractants and activators of leukocytes. These include bacterial by-products formyl-Met-Leu-Phe (fMLP) and its related peptides, lipid derivatives such as platelet-activating factor, complement proteolysis fragments such as C5a, and the superfamily of small (8-10 kDa), inducible, secreted, pro-inflammatory cytokines called chemokines. Chemokines are produced and secreted by various cell types, usually upon stimulation by injuries, infections, or other pro-inflammatory cytokines. Members of the chemokine family share amino acid sequence homology and are currently divided into four classes (CXC, CC, C and CX3C) on the basis of the arrangement of the conserved cysteine residues of the mature proteins (see recent reviews for details: Baggioioli et al., 1997; Zlotnik and Yoshie, 2000). These chemoattractants exert their effects by interacting with specific cell surface receptors. To date, a large number of chemoattractant receptors have been cloned and sequenced (Murphy, 1994; Schall, 1994; Zlotnik and Yoshie, 2000). They include the fMLP and C5a receptors, five CXC chemokine receptors, ten CC chemokine receptors, one C chemokine receptor and one CX3C chemokines receptor. All these receptors possess seven transmembrane domains, which are characteristic of G protein-coupled receptors.

Great attention has been paid to these chemotactic molecules because of their roles in inflammatory reactions and lymphocyte development and function. Although inflammation plays an important role in host defense, uncontrolled inflammatory reactions are responsible for a variety of pathological conditions, including rheumatoid arthritis, ischemia-reperfusion injury, arteriosclerosis, virus-induced myocarditis, psoriasis and other inflammatory skin conditions, and allergic reactions (Furie and Randolph, 1995). The understanding of signaling mechanisms that mediate the actions of chemoattractants should provide more suitable targets for novel therapeutic approaches and agents that combat inflammation. Evidence indicates that the chemoattractant receptors can couple to Gαi proteins, a class of G protein that is sensitive to Pertussis toxin (PTx). PTx is a bacterial toxin that specifically modifies a subset of Gt subunits including Goα, Goγ and transducin α subunits, but not the α subunits of the Gs, Gq or G12 classes, and prevents functional coupling of receptors to modified G proteins. The sensitivity of chemoattractant-induced responses to PTx suggests that the Gαi proteins might mediate chemoattractant signal transduction. Pharmacological, biochemical and transfection approaches have been used to characterize two signaling pathways for chemoattractants that are mediated by Gαi proteins. Both pathways involve metabolism of phosphatidylinositides, particularly phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Chemoattractants were shown to stimulate hydrolysis and phosphorylation of PtdIns(4,5)P2 via phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K), respectively, in various leukocytes. The finding that Gβγ can activate PLC-β2, PLC-β3 and PI3Kγ suggests that chemoattractant receptors activate PLC and PI3K probably through the Gβγ subunits that are released from the Gαi proteins. Pharmacological studies and studies using other approaches have implicated the PLC and PI3K pathways in a variety of chemoattractant-induced responses. Recent studies of mouse lines that lack these effector proteins have provided...
CHEMOATTRACTANTS ACTIVATE PLC-β2 AND PLC-β3 IN MOUSE NEUTROPHILS

Many chemoattractants induce rapid elevation of cytosolic Ca\(^{2+}\) levels, which presumably results from activation of PLC (Murphy, 1994). PLC hydrolyzes PtdIns(4,5)\(_{2}\) to generate two important second messengers, inositol 1,3,4-triphosphate (Ins(1,4,5)\(_{3}\)) and diacylglycerol (DAG; Berridge, 1989). The known phosphatidylinositol-specific PLC-molecules can be divided into three families: β, γ and δ (Rhee and Bae, 1997). The β family, which consists of four isoforms, PLC-β1-PLC-β4, is regulated by G proteins (Rhee and Bae, 1997). PLC-β2 has been detected primarily in hematopoietic cells thus far, whereas PLC-β3 and PLC-β1 are found in a wide range of cells and tissues (Rhee and Bae, 1997). PLC-β4 is predominantly expressed in certain neuronal cells (Jiang et al., 1996b; Kano et al., 1998). We have demonstrated using the cotransfection assays that many chemoattractant receptors, including CXCR-1 and CXCR-2 (Wu et al., 1993), CCR-1 and CCR-2 (Kuang et al., 1996) CCR-5, CXCR-4 (D. Wu, unpublished data), and the fMLP receptor and C5a receptor (Jiang et al., 1996a) can couple to the Gi proteins to stimulate PLC activities. Although the α subunits of Gi proteins cannot regulate PLC activity directly (Wu et al., 1992), the βγ subunits released from activated Gi proteins were shown to activate PLC-β2 (Jiang et al., 1996a; Katz et al., 1992; Kuang et al., 1996; Wu et al., 1993). This was further confirmed by the observations that purified Giβγ proteins activated recombinant PLC-β2 as well as PLC-β3 (Camps et al., 1992; Carozzi et al., 1993; Smrcka and Sternweis, 1993). Since responses to many chemoattractants in mature leukocytes are sensitive to PTx, the Gi-Gi pathway was postulated to occur in leukocytes.

We investigated the significance of the β2 and β3 isoforms of PLC in chemoattractant-induced PLC activation in mouse neutrophils, using gene targeting. Neutrophils isolated from mice lacking PLC-β2 showed significant reduction in PLC activation and Ca\(^{2+}\) efflux in response to fMLP and IL-8 (Jiang et al., 1997), which suggests that the PLC-β2 isoform can mediate chemoattractant-induced PLC activation. However, this study also suggested that PLC-β2 is not the only PLC isoform active in neutrophils. We observed residual levels of PLC activity and Ca\(^{2+}\) efflux in response to chemoattractants in neutrophils lacking PLC-β2 (Jiang et al., 1997). Another Giβγ-regulated PLC-β isoform, PLC-β3, might be responsible for the residual activities. Subsequent study of PLC-β3-deficient mice and those that lack both PLC-β2 and PLC-β3 confirmed this idea, showing that neutrophils lacking both PLC-β2 and PLC-β3 cannot respond to chemoattractants in PLC and Ca\(^{2+}\) efflux assays (Li et al., 2000). This result indicates that PLC-β2 and PLC-β3 are the only isoforms that are responsible for chemoattractant-induced PLC activation in mouse neutrophils. The fact that PLC-β3-deficiency alone does not markedly affect the PLC activities suggests that PLC-β2 is the major PLC isoform in neutrophils (Li et al., 2000).

CHEMOATTRACTANT-INDUCED PtdIns(3,4,5)P\(_3\) PRODUCTION IN MOUSE NEUTROPHILS

Chemoattractant receptors stimulate the production of phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)\(_{3}\)) in leukocytes. The finding (Stephens et al., 1997) that Giβγ potently activates PI3Kγ suggests that chemoattractant receptors may activate PI3Kγ through the Giβγ subunits released from Gi proteins. However, the results from two studies using tyrosine kinase inhibitors contradicted this hypothesis (Ptasznik et al., 1996; Thelen and Didichenko, 1997). These studies suggested that PI3Kγ is not the major PI3K isoform responsible for fMLP-induced production of PtdIns(3,4,5)\(_{3}\) in human neutrophils. The gene targeting experiments, however, clearly demonstrated that PI3Kγ is the major isoform in mouse neutrophils. Three groups have independently shown that mouse neutrophils lacking PI3Kγ do not produce detectable levels of PtdIns(3,4,5)\(_{3}\) in response to fMLP (Hirsch et al., 2000; Li et al., 2000; Sasaki et al., 2000), IL-8 and C5a (Hirsch et al., 2000). The apparent discrepancy between the results from human and mouse neutrophils may be due to the species difference, lack of specificity of the inhibitors, or possibility that other PI3K isoforms might be involved in amplification of the signal initiated by PI3Kγ.

ROLES OF PHOSPHOLIPID SIGNALING IN CHEMOATTRACTANT-INDUCED CHEMOTAXIS

Chemoattractants induce a wide range of responses in leukocytes, including chemotaxis, superoxide production, cytoskeleton reorganization, and upregulation of cell surface adhesion molecules. The PLC-β2/PLC-β3-null and PI3Kγ-null mouse lines, in which two prominent phospholipid signaling pathways are largely abrogated in neutrophils, allowed us to examine genetically the roles of these pathways in these chemoattractant-elicited responses. Chemotaxis attracts the most attention because this process plays a key role in inflammatory responses. Previous studies have shown that clamping intracellular Ca\(^{2+}\) with Ca\(^{2+}\) chelators does not appear to affect chemoattractant-induced chemotaxis of neutrophils in the absence of adhesive molecules (Alteraifi and Zhelev, 1997; Elferink et al., 1992; Fabbri et al., 1997; Kuijpers et al., 1992; Marks et al., 1991). However, some of these studies also indicated that chemoattractant-induced chemotaxis of neutrophils on surfaces coated with adhesive molecules appears to depend on intracellular Ca\(^{2+}\) (Alteraifi and Zhelev, 1997; Hofman et al., 1999; Mandeville and Maxfield, 1997; Marks et al., 1991). The idea that chemoattractant-induced increases in cytosolic Ca\(^{2+}\) levels are not required for chemotaxis in the absence of adhesive molecules is consistent with the finding that neutrophils lacking both PLC-β2 and PLC-β3, despite showing no fMLP-induced Ca\(^{2+}\) efflux, respond to fMLP as well as do wild-type cells in the modified Boyden-chamber assay (Jiang et al., 1997; Li et al., 2000). We have also examined two important chemotactic parameters – speed and orientation – using Zigmond chambers containing gradients of fMLP and time-lapsed video microscopy. We did not observe significant differences between wild-type and PLC-β2/PLC-β3-deficient neutrophils in these two chemotactic parameters (Huang et al., unpublished data).
These results further confirm the conclusion above. Studies of PLC-deficient neutrophils also suggest that chemoattractant-induced changes in intracellular Ca²⁺ levels are not required for neutrophil chemotaxis in vivo, because PLC-deficiency does not impair the infiltration of neutrophils into peritonea in a bacteria-induced peritonitis model (Li et al., 2000). This result appears to contradict those from the Ca²⁺-clamping studies (Alteraifi and Zhelev, 1997; Hofman et al., 1999; Mandeville and Maxfield, 1997; Marks et al., 1991). Knowing that Ca²⁺ chelators used in the Ca²⁺-clamping studies may not only block ligand-induced increases in intracellular Ca²⁺ concentrations, but also reduce the intracellular Ca²⁺ concentrations below the basal levels (those in the absence of ligands), one may interpret the apparent discrepancy to suggest that the basal, but not chemoattractant-elevated, levels of Ca²⁺ are critical for adhesion-dependent chemotaxis.

In contrast to lack of impairment of IL-8- or fMLP-induced chemotaxis in cells lacking PLC-β isoforms, MIP1α-induced chemotaxis of neutrophils lacking PLC-β2 is enhanced (Jiang et al., 1997), suggesting that the PLC-β2 pathway selectively downregulates chemoattractant-induced chemotactic activities. Similar results were also observed for neutrophils lacking both PLC-β2 and PLC-β3 (Li et al., 2000). In addition, PLC-β2-deficiency also enhances chemotaxis of eosinophils in response to Eotaxin and of T cells in response to MIP1α and RANTES (Jiang et al., 1997). The finding that PLC deficiency results in an enhanced response is not restricted to hematopoietic cells; PLC-β3 appears to inhibit the effects of the µ-opioid receptor, another G_i/o-coupled receptor, as determined by both behavioral studies and electrophysiological study of dorsal root ganglion neurons (Xie et al., 1999). All these results suggest that PLC-β2 and PLC-β3 may underlie physiological pathways that inhibit certain responses induced by some G_i-coupled receptors. The molecular mechanisms of these PLC-mediated inhibitory effects, especially those involved in chemotaxis, will be particular interesting. The selective effect of the PLC-deficiency on some of the chemokines suggests that different chemoattractant receptors have different sensitivities to PLC-mediated modification. A plausible hypothesis is that the PLC-pathway leads, via PKC, to phosphorylation of chemoattractant receptors, resulting in enhanced desensitization of some of the receptors (Ali et al., 1999). This hypothesis needs to be investigated experimentally.

With regard to the involvement of PI3K in chemotaxis, there seem to be contradictory results from various pharmacological studies. Treatment with PI3K inhibitors, including wortmannin and LY-294002, inhibited chemotaxis in some studies (Coffler et al., 1998; Harakawa et al., 1997; Knall et al., 1997) but not in others (Neptune and Bourne, 1997; Thelen et al., 1995). Studies of mice lacking PI3Kγ showed that PI3Kγ appears to play an important role in neutrophil chemotaxis (Hirsch et al., 2000; Li et al., 2000; Sasaki et al., 2000). Neutrophils purified from either the bone marrow or the peritoneal cavity of PI3Kγ-null mice showed a approximately 50% reduction in activities measured in the modified Boyden assay in response to fMLP, MIP1α, IL-8 and C5a in comparison with those from wild-type animals. The activities of neutrophils were also impaired in vivo infiltration assays. In addition, macrophages from the PI3Kγ-deficient mice appear less chemotactic (Hirsch et al., 2000). Interestingly, in all these assays, cells lacking PI3Kγ still show chemotactic motility, although reduced. It seems that PtdIns(3,4,5)P₃ plays a regulatory role in chemotaxis, rather than functioning as a primary mediator for chemoattractant-mediated motility.

What is the precise role of PI3Kγ in neutrophil chemotaxis? Recent findings (Jin et al., 2000; Servant et al., 2000) that revealed the polarized nature of neutrophils have implicated PI3K in polarization. They showed that pleckstrin homology (PH)-domain-containing proteins relocate to the leading edges of stimulated chemotactic cells (Parent et al., 1998; Servant et al., 2000). Since PH domains are believed to be involved in binding to PtdIns(3,4,5)P₃, it is reasonable to hypothesize that activation of PI3K has a role in translocation of these PH-domain proteins to the leading edges of polarized cells. Our recent observations support the idea that PI3Kγ is involved in neutrophil polarization. We examined, using confocal microscopy with serial optical sectioning, the subcellular localization of Akt and F-actin in neutrophils lacking PI3Kγ undergoing chemotaxis in Zigmond chambers containing gradients of fMLP. In wildtype cells, Akt and cortical actin were colocalized at the leading edges of chemotactic cells at both the top and bottom parts of the cells. However, in the PI3Kγ-null cells, although Akt and cortical actin are still colocalized at the leading edges following the gradient at the bottom optical section that is close to the cell attachment site, these two proteins no longer locate at the leading edges of the top part of cells that follow the fMLP gradient. It seems that more than one factor regulates cell polarization: something related to cell attachment and PI3Kγ are required for establishing cell polarity and each factor appear to have predominant effect on different part of cells. Thus, it is not difficult to image that PI3Kγ deficiency should cause problems in direction sensing. In fact, when cell movement was examined in the Zigmond chamber, the PI3Kγ-null cells clear lack a sense of direction, i.e. PI3K-null cells moved in all directions while wild-type cells generally move following the gradient of chemoattractants. In addition, we found that the mutant cells moved at approximately half of the speed of wild-type cells (Huang et al., unpublished data). The slower movement speed of the mutant cells may be caused by conflicting polarization signals or modulation of motility by PI3Kγ. The idea that multiple factors regulate cell polarization is supported by the finding that RhoA G proteins might also be involved in cell polarization (Servant et al., 2000).

It is also worth noting that the importance of PI3Kγ in chemotaxis appears to be restricted to the myeloid lineage, because PI3Kγ deficiency does not alter chemoattractant activities of spleen B cells in response to SDF-1, MIP3β or fractalkine (D. Wu et al., unpublished results). There are two possible explanations for the lack of effects on B cells: (1) there might be PI3Kγ homologs in B cells that compensate for the lack of PI3Kγ (determination of SDF-1-induced PtdIns(3,4,5)P₃ formation in B cells would provide an answer); and (2) PI3K might not be essential for B cell chemotaxis.

ROLES OF PHOSPHOLIPID SIGNALING IN CHEMOTRACTANT-INDUCED SUPEROXIDE FORMATION

Superoxide production plays an important role in innate immunity. Many stimuli, including chemoattractants, can
stimulate its production (Babior, 1999; Baggioni et al., 1993; Bokoch, 1995). Pharmacological studies have implicated both PI3K and PLC-PKC pathways in regulation of superoxide production (Lew, 1990; Park and Babior, 1992; Thelen et al., 1994; Vlahos et al., 1995). These results were in fact confirmed by the studies of the knockout mice. Chemotactant-induced production of superoxides is blocked in neutrophils isolated from PI3Kγ-deficient (Hirsch et al., 2000; Li et al., 2000; Sasaki et al., 2000) or PLC-β2/PLC-β3-deficient mice (Jiang et al., 1997; Li et al., 2000). However, cells from these mice can still produce superoxides in response to other stimuli such as lipopolysaccharide (LPS; D. Wu et al., unpublished data) and phorbol esters (Sasaki et al., 2000). The apparent lack of any increase in fatality due to spontaneous microbial infection of PLC-β2/PLC-β3- or PI3Kγ-deficient mice that are housed in non-germ-free environments suggests that chemotactant-elicted superoxide production plays an auxiliary role in host defense.

The small GTP-binding protein Rac regulates superoxide production by directly interacting with the NADPH oxidase (Dagher et al., 1995; Knaus et al., 1991; Umeki, 1994). Some studies using transfection and pharmacological reagents suggest that the PI3K pathway may be involved in regulation of small GTP-binding proteins including Rac (Akasaki et al., 1999; Bacon, 1999; Parker, 1995). However, PI3Kγ deficiency does not significantly block fMLP-induced activation of Rac in mouse neutrophils. In addition, the time course for ligand-induced Rac activation, which peaks within 5 seconds (Li et al., 2000), is faster than that for ligand-induced increases in the PtdIns(3,4,5)P3 levels, which peaks at about 45-60 seconds (Hirsch et al., 2000). The short time course for Rac regulation suggests that chemotactant receptors may regulate the small GTP-binding protein via a more direct pathway in neutrophils. These results, however, appear to be in disparity with those obtained from studies of human neutrophils using PI3K inhibitors (Akasaki et al., 1999; Benard et al., 1999). Benard et al. also showed that a tyrosine inhibitor could block fMLP-induced Rac activation. These discrepancies reminisce those described above for which PI3K isoforms are predominantly involved in fMLP-induced production of PtdIns(3,4,5)P3, and may be explained by the same possibilities.

Although the mechanisms by which PI3Kγ regulates superoxide production remain to be characterized, the involvement of the PLC-pathway in production of superoxides appears to involve at least the translocation of p47phox, one of the components of the NADPH oxidase. The PLC-β2/PLC-β3 deficiency, but not the PI3Kγ deficiency, impairs the translocation of the protein from the cytosol to membranes (Li et al., 2000). Because PKC-mediated phosphorylation of p47phox regulates this translocation process (Park and Babior, 1992) and PLC deficiency blocks chemotactant-induced PKC activation (Li et al., 2000), it is reasonable to believe that the PLC pathway regulates p47phox translocation via PKC.

**INVolvEMENT OF PHOSPHOLIPID SIGNALING IN OTHER CHEMOATTRACTANT-ELICITED RESPONSES**

Chemoattractants regulate many protein kinases, including members of the MAPK superfamily – ERK, JNK and p38 kinase. The PLC-pathway appears to play a more significant role in mediating the regulation of these kinases by chemoattractants, because neutrophils lacking PLC-β2 and

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**Fig. 1.** Roles of PLC and PI3K pathways in chemoattractant-induced responses. This figure is intended for illustration of only the pathways that were characterized in these recent transgenic studies. Chemoattractants stimulate Ins(3,4,5)P3 production and increase intracellular Ca2+ concentrations by activating PLC-β2 and PLC-β3 and stimulate PtdIns(3,4,5)P3 production by activating PI3Kγ in mouse neutrophils. PI3Kγ plays a significant role in chemotaxis and CD4+ T cell survival and is required for chemoattractant-induced superoxide production and for production of antibodies containing the λ light chains in response to T-cell-independent antigens. The PLC-pathway plays a major role in chemoattractant-mediated regulation of protein kinases including PKC, ERK, JNK and p38. The PLC-pathway is also required for upregulation of CD11b and superoxide production in response to chemoattractants. In addition, the PLC-pathway inhibits chemotaxis induced by certain chemoattractants and the production of antibodies containing the λ light chains in response to T cell-independent antigens. Thick lines represent significant pathways, and the thin lines represent minor pathways or those whose significance need to be further investigated.
PLC-β3 showed a more significant reduction in fMLP-induced activation of JNK (Li et al., 2000) as well as ERK and p38 kinase (D. Wu et al., unpublished data) than did those lacking PI3Kγ. The PI3Kγ pathway might also be involved in regulation of ERK, because neutrophils lacking PI3Kγ showed some reduction in fMLP-induced ERK activation (Sasaki et al., 2000). The fact that the PLC-deficiency blocked JNK activation, but not Rac activation, suggest that Rac is not the primary mediator for chemoattractant-induced JNK activation, although Rac activates JNK in some transfection assays (Cosso et al., 1995; Minden et al., 1995). Thus, the signaling pathways that mediate the regulation of MAPK kinases by extracellular stimuli might be cell-type dependent.

Studies of PLC-β2/PLCβ3 and PI3Kγ knockout mice also revealed that the PLC-pathway, but not the PI3K pathway, mediates chemoattractant-induced upregulation of cell surface adhesion molecule CD11b (Jiang et al., 1997; D. Wu et al., unpublished data). In addition, neither pathway appears to be involved in regulation of actin polymerization, because the fMLP-stimulated binding of F-actin-binding phallolidin to neutrophils isolated from mutant mice is similar to that of wild-type cells (Li et al., 2000). This observation is consistent with the assertion that neither the PLC-β- nor PI3Kγ-pathway is the primary mediator for regulation of Rac, which is known to regulate actin polymerization.

**FUTURE DIRECTIONS**

Studies of knockout mice have clearly confirmed the previous belief that phospholipid signaling mediated by PLC and PI3K has important roles in chemoattractant-induced responses. Fig. 1 summarizes the roles of these two prominent phospholipid-signaling pathways that these recent studies have revealed or confirmed. Although many results from the transgenic studies are in agreement with those obtained from pharmacological studies, which mainly use human leukocytes, there are a few apparent discrepancies. Further studies are warranted to clarify these differences.

Despite the impairment of chemotaxis by PI3Kγ deficiency, the PI3K pathway appears to play a more important role in determining the direction of the movement than in movement itself. Thus, primary mechanisms by which chemoattractants induce increase in motility still remain elusive, and the understanding of these mechanisms will be undoubtedly of great interest and could provide additional therapeutic targets for anti-inflammatory drugs. The fact that chemoattractant-mediated chemotaxis, like other chemoattractant-induced responses, is sensitive to PTx suggests that G proteins is involved. Two recent studies demonstrated that chemoattractant receptors might use the Gβγ subunits, rather than the Gαi subunits, to activate chemotaxis (Arai et al., 1997; Neptune and Bourne, 1997). In addition, our studies of the PLC-β2/PLC-β3-deficient mice have eliminated any direct involvement of PLC-β2 or PLC-β3 in mediation of chemoattractant-induced chemotaxis. Thus, it is reasonable to hypothesize that chemoattractant receptors use yet-to-be-characterized Gβγ-mediated pathways in stimulating motility. The missing link might lie in the mechanism by which Gβγ regulates the RhoA family of small GTP-binding proteins, which includes Rac, Cdc42 and RhoA. These proteins are known to regulate cytoskeleton reorganization, which is essential for cell movement. Recent study of Rac2-null mice further confirmed the importance of small G proteins in chemoattractant-induced responses including chemotaxis, superoxide production, and actin polymerization (Roberts et al., 1999).

Thus far, the studies of these mouse lines have largely focused on neutrophils. Chemoattractants, especially many chemokines, also play significant roles in lymphocyte development and function. Although there is no profound effects of PLC-β2/PLC-β3 deficiency or PI3Kγ deficiency on lymphocyte development and function, differences in the number of CD4+ T cells in spleens from the mice lacking PI3Kγ (Sasaki et al., 2000) and in production of antibodies containing the λ light chains in response of immunization of T cell-independent antigens in mice lacking PI3Kγ or PLC-β3 (Li et al., 2000) have been observed. However, the physiological significance and mechanistic basis for the involvement of PI3Kγ and/or PLC-molecules in these processes need to be studied further.

**REFERENCES**


