Involvement of PI3Kγ in Superoxide Anion Production in Response to IL-8, RANTES, and fMLP in Human Peripheral Blood Neutrophils

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Abstract

Neutrophils are essential components of the immune system and have a critical role in combating bacterial and fungal infections. A key weapon in the neutrophil armory is the "respiratory burst," which is the generation of reactive oxygen species (ROS) by a multicomponent oxidase complex. It is well established that preexposure of human neutrophils to proinflammatory cytokines and chemokines markedly augments the production of reactive oxygen species (ROS) to subsequent stimuli. In inflammatory reactions, there are complex interactions of protein mediators (cytokines) and mediators derived from lipids. An important event in inflammation is superoxide production, in relation to microbicidal activity as well as tissue damage. A better understanding of phenomena involved in the regulation of NADPH oxidase could help developing novel therapeutic agents for inflammatory diseases involving abnormal neutrophil superoxide. Therefore, stimulating superoxide production by human neutrophils was investigated for this reason and because it sheds a light on intracellular signals that activate this response. Pretreatment of human neutrophils with N-formylmethionyl-leucyl-phenylalanine (fMLP), interleukin-8 (IL-8), and regulated on activation, normal T-cell expressed and secreted (RANTES) markedly augmented the amount of superoxide anion produced, which was inhibited completely (IL-8 and fMLP) or partially (RANTES) by a specific isoform of phosphoinositide 3-kinase (PI3K), the PI3KγII inhibitor.

Keywords: fMLP, IL-8, Neutrophil, PI3K, RANTES, Superoxide anion, PI3KγII.

1 Introduction

Human polymorphonuclear neutrophils play a key role in host defenses against invading microorganisms [1]. In response to a variety of stimuli, neutrophils release large quantities of superoxide anion ($O_2^{\cdot-}$) in a phenomenon known as the respiratory burst. The $O_2^{\cdot-}$ anion is the precursor of effective oxidants, which are important for bacterial killing and also potentiate inflammatory reactions. Regulation of this production is consequently critical to kill pathogens without inducing tissue injury. Neutrophil production of $O_2^{\cdot-}$ is dependent on the respiratory burst oxidase, or NADPH oxidase, a multicomponent enzyme system that catalyzes NADPH-dependent reduction of oxygen to $O_2^{\cdot-}$. NADPH oxidase is activated and regulated by various neutrophil stimuli at infection or inflammatory sites. Proinflammatory cytokines such as granulocyte mac-

rophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF) and IL-8 modulate NADPH oxidase activity through a priming phenomenon. The neutrophil oxidase is regulated by the combined action of several intracellular signaling pathways, including those driven by PI3K, phospholipase C (PLC)/Ca^{2+}/protein kinase C (PKC), phospholipase D (PLD), phospholipase A2 (PLA2), and p38/$Erk_{2/3}$ [2, 3]. Presumably, this diversity reflects the need for the oxidase to respond to multiple families of cell-surface receptors (e.g., receptors for Fc, integrins, lipopolysaccharide, chemokines, cytokines, and bacterial fragments) that function through different proximal signal-transducing elements such as heterotrimeric G proteins or protein tyrosine kinases. It is clear from the use of specific catalytic site inhibitors and certain mouse knockouts that the PI3K signaling pathway is important for the mechanisms by which Gi-coupled receptors regulate ROS production in neutrophils, for example, downstream of receptors for fMLP, C5a, platelet-activating factor (PAF), IL-8, Leukotriene B4 (LTB4), and adenosine triphosphate (ATP) [4, 5, 6, 7].

Priming phenomena are implicated in normal innate immune defense and in some inflammatory diseases. The mechanisms underlying the priming process are poorly understood, even though some studies have suggested that priming with various agonists is regulated at the receptor and post-receptor levels. Resolution of inflammation involves desensitization phenomena and cytokines are involved in this process by various mechanisms [8].

Neutrophils contain members of each of the currently defined families of PI3K-classes I, II, and III. The class I family of PI3Ks is responsible for cell-surface receptor–generated phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P3 or PIP3) and phosphatidylinositol (3,4)-bisphosphate (PtdIns(3,4)P2 or PIP2) and is thought to play the major role in the regulation of the oxidase [9]. Class I PI3Ks are subdivided into class IA and class IB based on the nomenclature of their p110 catalytic subunits (α, β, δ, γ) and mode of regulation; hence, PI3Kγ is subdivided into class IB, and PI3Kα, β and δ are subdivided into class IA [9]. The class IB enzyme has a p101 regulatory subunit and is activated by G-protein–coupled receptors. The class IA enzymes have p55-85 regulatory subunits and are classically activated by tyrosine kinase–coupl receptors [9]. Recent studies using mouse PI3Kγ knockouts have defined the class IB isoform as the major player in fMLP-driven PtdIns(3,4,5)P3 synthesis and ROS production [5, 6, 7].

Protein kinase B (PKB) was revealed as a mediator of the PI3K pathway. PKB, also known as Akt (a product of akt proto-oncogene), is a serine/threonine protein kinase and has an important role in many physiological processes which include protein synthesis, glucose transport and cell survival. Since PKB/Akt has emerged as a key effector of survival, growth and death responses, it is possible that oxidative stress-induced activation of this important signaling pathway contributes to various abnormalities associated with many chronic diseases. The PI3K-PKB/Akt pathway is highly conserved, and its activation is tightly controlled via a multistep process. Activated receptors directly stimulate class 1A PI3Ks bound via their regulatory subunit or adapter molecules such as the insulin receptor substrate (IRS) proteins [10].

On the other hand, IL-8, known as CXCL8, is a proinflammatory CXC chemokine [11]. It is a member of the α-chemokine family that attracts neutrophils, basophils, and T-cells, but not monocytes. It is also involved in neutrophil activation. It is released from several cell types in response to an inflammatory stimulus. Furthermore, IL-8 promotes the adhesion and transmigration of neutrophils across the endothelium into tissues [11]. The biological effects of IL-8 are mediated through the binding of IL-8 to two cell-surface G protein–coupled receptors, termed CXCR1 and CXCR2 [12, 13]. These receptors share considerable structural similarity suggesting that these genes arose through gene duplication [12, 13]. A chemotactic cytokine, IL-8 is capable of attracting neutrophils to the joints and activating their specific functions, and it may play a major role in neutrophil-mediated tissue damage in rheumatoid arthritis (RA). The action of IL-8 is pleiotropic in nature. For example, in vitro it has been shown to: (1) be chemotactic for neutrophils and lymphocytes (2) degranulate polymorphonuclear cells; (3) enhance neutrophil superoxide production; (4) enhance neutrophil phagocytosis; (5) increase neutrophil CR1 and CR3 receptor expression [14]. However, the release of IL-8 by the wrong cells at the wrong time, or at too high a concentration can lead to undesired pathologies, such as RA, inflammatory bowel disease, idiopathic pulmonary fibrosis, and cerebral nd myocardial ischemia [15].

N-formylated peptides like fMLP play a major role as

potent chemoattractants. They are believed to originate from either degraded bacterial or mitochondrial proteins [16, 17]. The N-formyl peptide receptor is G-protein coupled and mediates anti-inflammatory reactions in human neutrophils and other tissues [18] such as the production of reactive oxygen derivatives (e.g. hydrogen peroxide) upon stimulation with fMLP.

In humans, there are two different formyl-peptide receptors [19]. Both have 350 amino acids and an expected molecular mass of 38 kDa, but differ from each other by two residue changes at positions 101 and 346. Important differences are clear at the 3' and 5'- untranslated regions [19] from some experiments suggesting that the fMLP receptors form a family of closely related proteins. The interaction of fMLP with its receptor expressed on neutrophils triggers multiple second messengers through the activation of PLC, PLD and PLA2 and rapidly stimulates PI3K, as well as activating tyrosine phosphorylation [20]. A study showed that FMLP-sensitive PIP3 formation in human neutrophils involves the FMLP receptor, heterotrimeric G-proteins of the Gi type, PI3Kγ and phosphatidylinositol transfer protein (PITP) [21].

Regulated on activation normal T-cell expressed and secreted (RANTES) is a small protein of 68 amino acids that belongs to the rapidly growing chemokine family [22]. RANTES induces leukocyte migration by binding to specific receptors in the seven-transmembrane G protein-coupled receptor (GPCR) family, namely CCR1, CCR3, CCR4 and CCR5. Increased RANTES expression has been associated with a wide range of inflammatory disorders and pathologies, including allogeneic transplant rejection, atherosclerosis, arthritis, atopic dermatitis, inflammatory airway disorders such as asthma, delayed-type hypersensivity reactions, glomerulonephritis, endometriosis, some neurological disorders (such as Alzheimer's disease) and certain malignancies [23]. In all of these pathologies, RANTES is thought to act by promoting leukocyte infiltration to sites of inflammation [24]. Increased RANTES expression, as observed during respiratory viral infections, may play an important role in the associated neutrophilia and exacerbations of asthma. The role of RANTES in the pathogenesis of disease processes is not well understood [25].

2 Materials and Methods

2.1 Materials

Dextran Type IV, powder, Phosphate Buffer Saline (PBS X1), Bovine Serum Albumin (Fraction V, Powder), Hanks' Balanced Salt solution (HPSS) Modified, with sodium bicarbonate, calcium chloride and magnesium sulphate liquid, without phenol red, sterile-filtered, and suitable for cell culture. N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES solution), Trypan Blue solution 0.4%, liquid, sterile-filtered, suitable for cell culture, Cytochrome C from equine heart, Superoxide Dismutase (SOD) from bovine erythrocytes (lyophilized powder) suitable for cell culture, Ni-Formyl-Met-Leu-Phe (fMLP) BioXtra > 99.0% (TLe) were all obtained from Sigma, UK. Recombinant Human Interleukin-8 (IL-8) (72 amino acids) was from Peprotech, USA, Phorbol-l l-myristate-l I-acetate (PMA) was from Merck, USA, 5- (2,2 -Dijl uoro-benzo] l,3/dioxol-5- ylmethylene)-thiazolidine-2,4-dione (PI3Kγ Inhibitor II) was from Merck, USA, and Regulated on activation normal T-cell expressed and secreted (RANTES) was purchased from Peprotech Ltd., London, UK.

2.2 Isolation of human neutrophils

This study was conducted on 30 normal healthy subjects, with an age range of 18-50 years. Blood was collected in heparin tubes. The anti-coagulated blood was mixed with 1/5 volume of dextran (6% wt/vol) solution in normal saline. Samples were left to stand for 45-60 min at room temperature to allow sedimentation of erythrocytes. The leukocyte-rich supernatant was decanted and layered onto 10 ml of cushions of Lymphoprep in 50 ml conical polypropylene tubes and centrifuged at 800 g for 25 minutes at 18-20°C. This procedure separated granulocytes, which were found in the resultant pellets. The granulocyte preparations contained predominantly neutrophils. The pellets were resuspended in chemotaxis buffer (49 ml HBSS, 0.5 ml HEPES, and 0.5 ml 0.25% BSA) at a density of 20 X 10^5 cell/ml. Total cell number and viability (> 99%) were quantified by the exclusion of trypan blue dye (0.1%) and the cells were used immediately for assessment of superoxide production.

2.3 Respiratory burst measurement

Superoxide anion generation was measured as the superoxide dismutase (SOD)-inhibited reduction of ferricytochrome *c*. Neutrophils (20×10^5) were incubated in 225 µl of chemotaxis buffer containing 100 µM cytochrome *c*, with or without SOD.

PI3KγII inhibitor at different concentrations (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) was added and incubated at 37°C for 30 minutes. FMLP, or IL-8, or RANTES, or 4-β-phorbol 12-myristate 13-acetate (PMA), or chemotaxis buffer was added to give a final volume of 250 µl and the reaction mixtures were incubated for a further 30 minutes at 37°C.

Cells were precipitated by centrifugation (12,000 × *g* for 2 min) and the extinction of 200 µl portions of the supernatants was measured at 550 nm in a 96-well microplate reader. Cytochrome *c* reduction was calculated from the increase in extinction relative to a control sample to which SOD (30 U ml^{-1}) was added immediately before the stimulus. Results are expressed as nanomoles of cytochrome *c* reduced per 10^6 cells in 30 min, based on a molar extinction coefficient for ferrocytochrome *c* of 21.1×10^3 M^{-1} cm^{-1} [26].

2.4 Statistical Analysis

Statistical analysis of data was performed using the Statistical Package for social Science (SPSS) for the t-test, and the prism program. Descriptive data were given as mean ± standard deviation (SD). For the differences in the results, the P value was used to determine statistical significance. A difference with a P-value < 0.05 was considered statistically significant.

3 Results

3.1. Effect of PI3Kγ inhibitor II on IL-8-induced superoxide anion generation in human peripheral neutrophils

The optimal concentration of IL-8 that induced the highest superoxide anion generation from human peripheral blood neutrophils was 100 nM (Figure 1). IL-8 (100 nM) induced production of $O_2^{-\cdot}$ by human neutrophils that was higher than basal production (control).

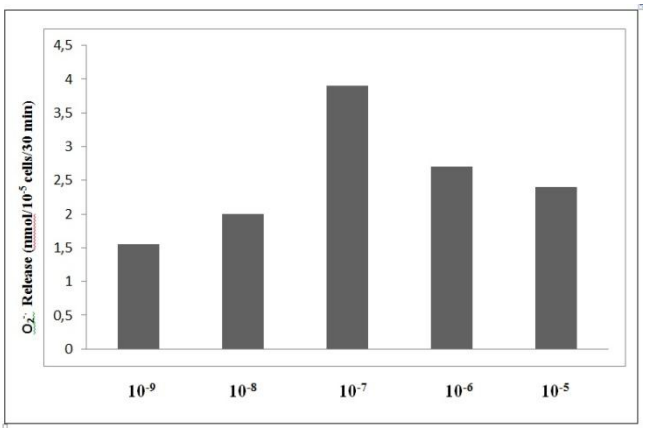

Figure 1 Concentration-response bar of IL-8 that induced superoxide anion generation from human peripheral blood neutrophils. The bar graph shows the effect of chemokine IL-8 on neutrophil superoxide radical generation. Neutrophil superoxide radical generation increases significantly ($P < 0.05$) when cells from normal control subjects (n = 10) are incubated with IL-8 for 30 minutes.

IL-8 (100 nM)-induced $O_2^{-\cdot}$ genaration was inhibited by selective PI3Kγ inhibitor II at a concentration of 100 nM (Figure 2). Basal production of superoxide anion showed no significant inhibition by the selective PI3Kγ inhibitor II.

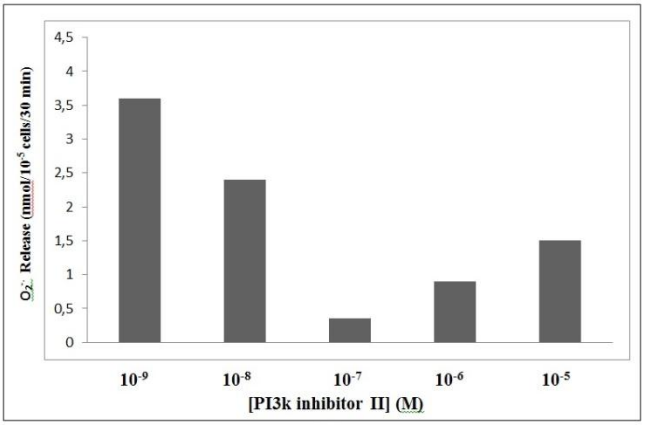

Figure 2 The effect of phosphoinositide 3-kinase gamma inhibitor II on respiratory burst in IL-8 stimulated human peripheral blood neutrophils. Neutrophils were preincubated with inhibitor for 30 minutes prior to addition of 100 nM IL-8. Data are shown as mean ± SEM from ten experiments conducted in triplicate. $^{**}P < 0.01$ compared to inhibitor-free control by the ANOVA and t-test.

3.2. Effect of PI3Kγ inhibitor II on RANTES-induced superoxide anion generation in human peripheral neutrophils

RANTES (100 nM) induced production of $O_2^{-\cdot}$ by human neutrophils that was higher than basal

production (control), as shown in Figure 3.

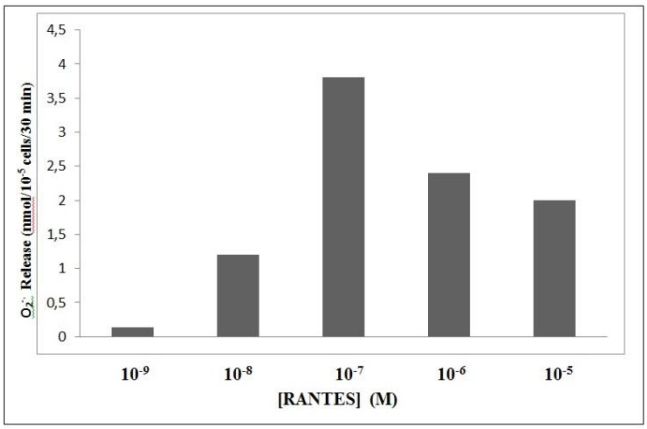

Figure 3 Concentration-response bar of RANTES (n = 10) that induced superoxide anion generation from human peripheral blood neutrophils. Data are mean ± SEM **P < 0.01 by repeated-measures.

RANTES (100 nM)-induced $O_2^{\cdot-}$ genaration was partially inhibited by selective PI3Kγ inhibitor II at a concentration of 10 μM. RANTES-induced $O_2^{\cdot-}$ genaration caused modest inhibition by selective PI3Kγ inhibitor II at a concentration of 10 μM (Figure 4). Basal production of superoxide anion showed no significant inhibition by the selective PI3Kγ inhibitor II.

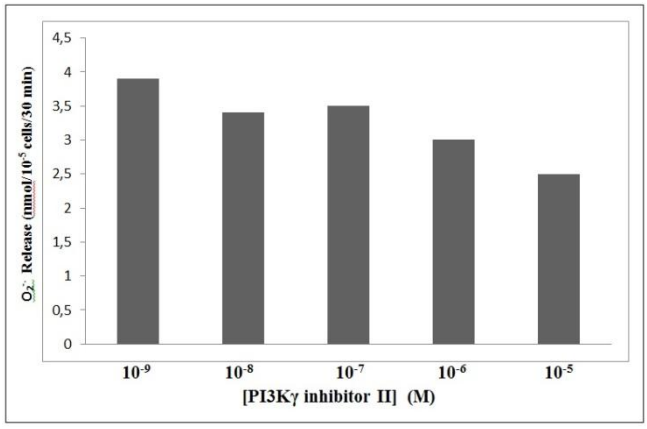

Figure 4 The effect of phosphoinositide 3-kinase gamma inhibitor II on respiratory burst in RANTES stimulated human peripheral blood neutrophils. Neutrophils were preincubated with inhibitor for 30 minutes prior to addition of 100 nM RANTES. Data are shown as mean ± SEM from ten experiments conducted in triplicate. $^{**}P < 0.01$ compared to inhibitor-free control by ANOVA and t-test.

3.3. Effect of PI3Kγ inhibitor II on fMLP-induced superoxide anion generation in human peripheral neutrophils

fMLP (100 nM) induced production of $O_2^{\cdot-}$ by human neutrophils that was higher than basal production (control) (Figure 5).

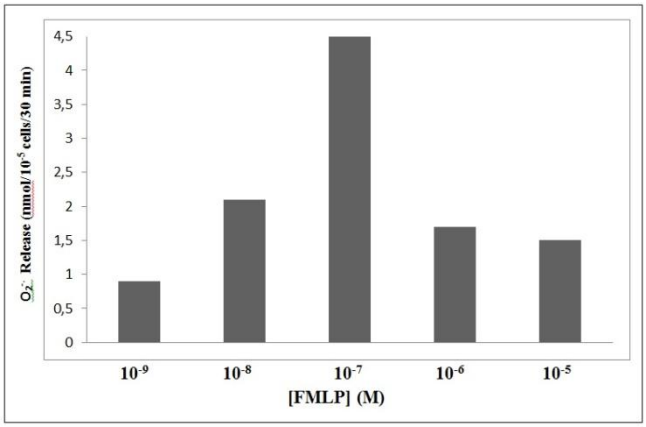

Figure 5 Concentration-response bar of fMLP (n = 10) that induced superoxide anion generation from human peripheral blood neutrophils. Data are mean ± SEM **P < 0.01 by repeated-measures.

fMLP-induced $O_2^{\cdot-}$ genaration was inhibited in a concentration-dependent mannar by the selective PI3Kγ inhibitor II at a concentration of 10 μM and higher (Figure 6). Basal production of superoxide anion showed no significant inhibition by the selective PI3Kγ inhibitor II.

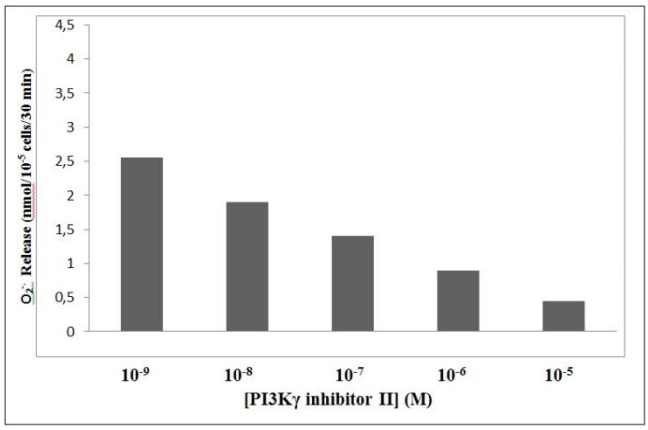

Figure 6 The effect of phosphoinositide 3-kinase gamma inhibitor II on respiratory burst in fMLP stimulated human peripheral blood neutrophils. Neutrophils were preincubated with inhibitor for 30 minutes prior to addition of 100 nM fMLP. Data are shown as mean ± SEM from ten experiments conducted in triplicate. $^{**}P < 0.01$ compared to inhibitor-free control by ANOVA and t-test.

4 Discussion

Neutrophils are important effector cells during inflammatory responses. Further, their many functions, controlled by cell surface receptors and intracellular signaling pathways, provide multiple opportunities for modulating many vital responses. The capacity of neutrophils to produce ROS, such as superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) during the respiratory burst is essential for their bactericidal activity (2). ROS production can be stimulated by chemotactic factors, such as the bacterial peptide N-formyl-L-methionyl-L-lucyl-L-phenylalanine (fMLP) [27]. It is also becoming apparent that ROS may regulate the neutrophil lifespan, modify the extracellular matrix through which the neutrophils migrate, and modulate the function of other cells participating in the inflammatory response [28].

Two important intracellular signaling pathways have been found to be of particular importance in the recruitment, activation and survival of human neutrophils: phosphoinositide 3-kinase (PI3K) and p38 mitogen-activated protein kinas (p38 MAPK). The physiological processes that regulate these activation events in neutrophils are largely unknown. PI3K-dependence varies with stimulus, cell type and response. The effect of PI3Kγ II inhibitor on human neutrophils is expected to depend on the activity of one or more PLC isoforms that are shown to be involved in ROS production [29].

During an infection, many chemoattractants are released from various locations including the vascular endothelium, interstitial cells (macrophages and mast cells), and the infectious agent itself. These multiple sources of chemoattractants result in a very complex environment of conflicting chemoattractant gradients that neutrophils must navigate through in order to reach the site of infection. One can envision numerous situations where neutrophils would be faced with having to make decisions between multiple gradients of different chemokines. In fact, one could argue that this would occur anytime a neutrophil encounters intermediary chemokines (e.g., IL-8 and RANTES) on the surface of endothelium, adheres, and now must migrate away from this site and toward a tissue source of end target chemoattractant (e.g., fMLP). Therefore, that these pathways would function in a hierarchical manner with end terminal pathways dominating, and that an active inhibition by the end target signaling pathway of the intermediary chemoattractant-induced intracellular signaling is an important mechanism in the neutrophil decision-making process [30].

Sasaki's research in mice lacking PI3Kγ demonstrated that this isoform is important to produce PtdIns(3,4,5)P3 and activate protein kinase B (PKB) in neutrophils exposed to the chemoattractants IL-8, fMLP and C5a, which activate G protein-coupled receptors (GPCRs). The absence of these responses led to a lack of $O_2^{\cdot-}$ generation in response to stimuli [5, 6]. Another study [31] showed that, PI3Kgamma-deficient neutrophils exhibited severe defects in migration and respiratory burst in response to heterotrimeric GTP-binding protein (G protein)-coupled receptor (GPCR) agonists and chemotactic agents such as formyl peptides such as fMLP. PI3Kgamma links GPCR stimulation to the formation of phosphatidylinositol 3,4,5-triphosphate and the activation of protein kinase B. Thus, PI3Kgamma regulates thymocyte development, T cell activation, neutrophil migration, and the oxidative burst [31]. Stimulation of neutrophils with a variety of stimuli can result in the activation of phospholipase C, D or A_2 (PLC, PLD, PLA_2) with the resultant hydrolysis of plasma membrane phospholipids and the formation of important second messenger molecules. In the neutrophil, the activities of these phospholipases have been implicated in the processes of both stimulating and maintaining oxidase activation. The products of phospholipase activation may have a possible role in reactive oxidant production by the neutrophil NADPH oxidase [32]. This study aimed to investigate the role of a selective inhibitor of PI3K (PI3Kγ inhibitor II) on the superoxide anion production induced by IL-8, or RANTES, or fMLP in human neutrophils.

In the present study IL-8, RANTES, and fMLP were demonstrated to be activators of the respiratory burst in human neutrophils (Figures 1, 3, and 5). These stimulated responses were capable of being inhibited by the selective inhibitor of PI3K (PI3Kγ inhibitor II). The optimal concentration that induced the respiratory burst in human neutrophils was 100 nM for RANTES, IL-8 and fMLP.

fMLP-induced $O_2^{\cdot-}$ genaration was inhibited in a concentration-dependent mannar by the selective PI3Kγ inhibitor II at a concentration of 10 μM and higher (Figure 6). But basal production of superoxide anion showed no significant inhibition by the selective

PI3Kγ inhibitor II. The fMLP-induced interaction between PI3Kγ and fMLP receptor, and the translocation of proteins to the plasma membrane may contribute to the stimulation of protein kinase C/protein kinase B (PKC/PKB) activity. PI3Kγ enzymatic activity is an essential mediator of fMLP-dependent stimulation of superoxide generation in neutrophils. The present investigations suggest the involvement of PI3Kγ protein kinase activity in the control of NADPH oxidase activity, which is in accordance with previous findings [33] that provides evidence for a signaling path in the form: fMLP → fMLP receptor → PI3Kγ protein kinase → p47phox → NADPH-oxidase, which induced superoxide anion production.

PI3Kγ II inhibitor can cause an immediate potentiating effect on ROS production from isolated human neutrophils, but the maximal effect elicited by IL-8 at concentration of 100 nM occurs after 30 minutes of exposure to PI3Kγ II inhibitor (Figure 2). Basal production of superoxide anion showed no significant inhibition by the selective PI3Kγ inhibitor II. Neutrophils express two CXC chemokine receptors, CXCR1 and CXCR2, which are seven-transmembrane-domain G-protein coupled receptors. IL-8 or CXCL8 is the main ligand for CXCR1. Activation via CXCR1 and CXCR2 promotes neutrophil chemotaxis into sites of inflammation and induces neutrophil degranulation with the release of enzymes such as human neutrophil elastase and proteinase-3. CXCR1 is involved in production of superoxide via NADPH [34].

In this research, we propose that IL-8 induces the activation of PI3Kγ, which may activate one or more isoforms of PLC that in turn may stimulate the phosphorylation and activation of Akt (PKB), leading to activation and recruitment of other downstream molecules that mediate NADPH oxidase activation. This study agrees with the study by Li and coworkers [6] that showed PLC has an important role in the IL-8–induced respiratory burst in human neutrophils that is inhibited byPI3Kγ inhibitor II.

Neutrophils are important effector cells during inflammatory responses. Furthermore, their many functions, controlled by cell surface receptors and intracellular signalling pathways, provide multiple opportunities for modulating unwanted responses [6]. The major RANTES-binding receptors in human neutrophils are CCR1, CCR3, and CCR5. Several biological effects of RANTES have been suggested to occur in an aggregation-dependent manner. For example, in a recently described study [35], aggregated but not disaggregated RANTES was shown to activate human neutrophils with a substantial increase in CD11b expression. Thus, aggregation of RANTES in vivo may also be responsible for its neutrophil chemoattractant properties. Because resting neutrophils were shown to express CCR1 [36], it is possible that this basal level of CCR1 expression is sufficient for responsiveness to aggregated RANTES [25].

Results obtained show that the optimal concentration of RANTES-induced superoxide production from human peripheral blood neutrophils was 10^{-7} μM and the optimal concentration of PI3Kγ II inhibitors that can inhibit $O_2^{-\cdot}$ generation was 10^{-5} μM.

In conclusion, RANTES induced the respiratory burst in human peripheral blood neutrophils that was inhibited partially by PI3Kγ inhibitor II. The results of this study suggest that a possible alternative signaling pathway, such as p38 MAPK (which activates lipid body formation that is mediated by CCR3 receptor), in addition to the PI3K pathway, which stimulates NADPH oxidase activation that in turn induces superoxide anion production in human neutrophils that were activated by RANTES. In addition, there is the possibility of another mechanism involving PI3Kγ implied by the sensitivity of CCR3-dependent action and PI3Kγ involvement in RANTES signaling through the activation of more than one isoform of PI3K or, on the other hand, CCR3 activation may recruit additional signaling pathways which interfere with PI3K-dependent responses.

Also the results of this study are comparable with Banderia-Melo's study, which showed that chemokine-induced lipid body formation was mediated by CCR3 receptor G protein-linked downstream signaling involving activation of PI3K and p38 MAPK [37]. We addressed a possible cross talk between the PI3K and p38 MAPK pathways. Thus, we demonstrated that the common response to the different stimulus might involve different signaling pathways. The results of this study which showed that there are important roles of PI3Kγ as well their downstream effector targets (PKB/Akt, PLC) in mediating chemokine CC (e.g. RANTES) and CXC (e.g. IL-8) stimulated cell functions. These results are novel in human neutrophils, and may be important in identifying targets for novel therapeutics for the treatment of many inflammatory

and allergic diseases.

For future work there are several important questions remaining to be investigated, including the explanation of the basis for the specificity of the signal on the basis of subcellular localization, and the intensity or duration of the production of PI(3,4,5)P_3 in the initiation and regulation of the functional responsiveness of human neutrophils. Future research studies should focus on the PI3K-dependence and p38 MAPK-dependence that shows heterogeneity with stimulus, response, species and cell type. Additional experiments need to be conducted by using siRNA to silence the PI3Kγ gene in human peripheral neutrophils before using the respiratory assay that is induced by different chemoattractants such as RANTES, IL-8 and fMLP.

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