

**THE PI3K KINASE INHIBITOR, LY294002, DECREASES RANTES-
INDUCED PERITONEAL RECRUITMENT OF NEUTROPHILS
IN MICE**

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Recruitment of pro-inflammatory cells is a critical process for the development and progression of a variety of autoimmune/inflammatory diseases. The directed movement of leukocytes toward a site of inflammation is predominately mediated by chemoattractants, also called chemokines that bind to G-protein coupled receptors that in turn activate a complex network of intracellular signaling cascades. Extravasation of neutrophils from blood vessels requires adhesion to vascular endothelial cells and subsequent migration into the tissue. These events are mediated by a diverse array of molecules including members of the CC and CXC chemokine families. CXC chemokines containing the glutamic acid-leucine-arginine motif, such as IL8, induce neutrophil chemotaxis and activation, whereas CC chemokines (i.e. RANTES, etc.) are able to activate monocytes and eosinophils. However, mouse neutrophils express preferentially CCR1, which is activated by CC chemokines like RANTES. Consequently, CCR1 receptors may play a more important role in mouse than in human neutrophils to compensate the apparent absence of CXCR1 receptors on mouse neutrophils [1].

Recently, it has been shown that RANTES injected in mouse peritoneum is able to induce recruitment of a variety of inflammatory cells [2]. To investigate which leukocyte sub-population could be more affected by RANTES stimulation, we developed an *in vivo* model of peritoneal cavity chemotaxis using r-human RANTES as stimulus. Briefly, 8-12 week old, female Balb/C mice were injected intraperitoneally with saline or 0.05-1.5 mg/kg of r-hRANTES. Four hours after r-hRANTES injection, mice were sacrificed and peritoneal cavity was washed three times with ice-cold PBS. The recruited cells were then automatically counted with a Beckman Coulter® A^cT 5diff™. At least 6 mice per experimental group were used in all experiments.

The intraperitoneal administration of r-hRANTES resulted in a dose-dependent increase of total leukocyte cells recruited into the peritoneum, measured 4 hours after injection. The mainly affected leukocyte subpopulation was neutrophils.

Chemoattractant receptors transmit their signal to heterotrimeric G protein complexes. Upon stimulation, the G protein complex dissociates and subsequently recruits various signaling components such as nucleotide exchange factors, phospholipid lipases and lipid kinase phosphoinositide-3'OH-kinase isoforms, like PI3K. Recent advances using genetic approaches and

pharmacological tools revealed a central role of the phosphoinositide 3-kinase class IB isoform, PI3K γ , in chemokine-induced recruitment of leukocytes. Mutant mice lacking PI3K γ , as well as pharmacological inhibition of its enzymatic activity, revealed that leukocytes are unable to produce 3'-phosphorylated phosphoinositides when stimulated with fMLP, C5a, IL8 and other chemoattractants. Consequently, chemokines failed to stimulate the phosphoinositides-dependent kinase cascades (e.g. PKB/AKT) in isolated cells that lack PI3K γ expression. In PI3K γ mutant mice recruitment of neutrophils, monocytes and macrophages in response to chemokines *in vitro* and *in vivo* was significantly reduced, while the response to pleiotropic inflammatory stimuli such as carrageenan was unaffected [3].

To investigate the involvement of PI3K γ in peritoneal cavity chemotaxis, we injected 0.5 mg/kg of r-hRANTES in the peritoneum of wild-type (WT) and PI3K γ deficient mice (KO). PI3K γ KO mice showed an impaired recruitment of total recruited cells. In fact, the total number of cells in WT mice treated with saline or RANTES was 1.4 ± 0.2 and $4.8 \pm 0.4 \times 10^6$ cells ml⁻¹, while in KO mice it was $1.7 \pm 0.1 \times 10^6$ and $2.7 \pm 0.2 \times 10^6$ ($p < 0.0005$ vs WT controls), respectively. The total number of neutrophils in WT mice treated with saline or RANTES was 0.005 ± 0.3 and $0.2 \pm 22.1 \times 10^6$ cells ml⁻¹, while in KO mice it was $0.003 \pm 0.05 \times 10^6$ and $0.08 \pm 4.2 \times 10^6$ ($p < 0.0005$ vs WT controls), respectively.

An additional aim of the study was to investigate the ability of a specific non-selective PI3K inhibitor, LY294002, to interact with the recruitment of neutrophils induced by a suboptimal dose of r-hRANTES (0.5 mg/kg *i.p.*) which was selected in the initial dose-response study. LY294002 was administered at 30, 100 and 300 mg/kg *p.o.*, 30 minutes before r-hRANTES. Dexamethasone at 1 mg/kg given *s.c.* was used as reference. While in the vehicle-treated group the recruited neutrophils were $0.2 \pm 0.02 \times 10^6$ cells ml⁻¹, in the group receiving RANTES the neutrophils were increased to $0.7 \pm 0.05 \times 10^6$ cells ml⁻¹. Administration of LY294002 at 30 mg/kg ($0.6 \pm 0.05 \times 10^6$ cells ml⁻¹), 100 mg/kg ($0.4 \pm 0.1 \times 10^6$ cells ml⁻¹, $p < 0.05$ vs RANTES-treated group) and 300 mg/kg ($0.3 \pm 0.02 \times 10^6$ cells ml⁻¹, $p < 0.001$) resulted in a marked dose-dependent inhibitory effect which was comparable to that obtained with dexamethasone at 1 mg/kg ($0.2 \pm 0.03 \times 10^6$ cells ml⁻¹, $p < 0.001$).

The present findings suggest that neutrophils are the main leukocyte subpopulation recruited by r-hRANTES in mice 4 hours following the challenge. PI3K γ KO mice showed an impaired recruitment of neutrophils in response to r-hRANTES. Further, RANTES-induced neutrophil migration is blocked by a non-selective PI3K inhibitor, indicating that PI3K may play an important role in controlling neutrophil chemotaxis involved in early steps of inflammation. Further investigations with selective PI3K inhibitors are required to understand the role of PI3K isoforms in controlling leukocyte traffickings involved in many inflammatory and autoimmune diseases.

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