CITATION NOTE: MEASURING LPS-INDUCED PKC ACTIVITY IN U937 CELLS

ROMAN, J. *ET AL.* (2004) LIPOPOLYSACCHARIDE INDUCES EXPRESSION OF FIBRONECTIN $\alpha_5\beta_1$-INTEGRIN RECEPTORS IN HUMAN MONOCYTIC CELLS IN A PROTEIN KINASE C-DEPENDENT FASHION. *AM. J. PHYSIOL. LUNG CELL MOL. PHYSIOL* **287**, L239–49.

REVIEW BY MICHELE ARDUENGO, PH.D., PROMEGA CORPORATION

In this article the authors use the Kinase-Glo® Luminescent Kinase Assay to assess PKC activity after LPS treatment in U937 cells. They demonstrate that LPS induces α5-integrin expression and show that PKC is activated in response to LPS.

Introduction

Infection with Gram negative bacteria can lead to an excessive inflammatory response by the host that results in tissue destruction and endotoxic shock. The inflammatory response, including the recruitment and migration of immune cells to the site of the infection and the release of cytokines, is mediated by lipopolysaccharide (LPS), a component of the outer membrane of Gram negative bacteria. The Lipid A domain of LPS (also called endotoxin) mediates the severe systemic inflammatory response.

In this study the authors set out to determine if LPS influences expression of matrix-binding integrin receptors on immune cells, enabling migration of immune cells to the site of infection. They tested the effect of LPS-treatment on expression of the $\alpha_5\beta_1$-integrin (a fibronectin receptor) on U937 cells, a monocytic cell line (ATCC CRL 159.3), and freshly isolated peripheral blood mononuclear cells (PBMCs).

LPS Treatment Upregulates α_5-Integrin Expression

The authors first demonstrate increased adhesion of U937 cells and PBMCs to fibronectin-coated surfaces upon treatment with LPS. This adhesion was blocked by antibodies against the α_5 and β_1 proteins. Using real-time PCR, the authors demonstrate an increase in α_5-integrin mRNA in both U937 cells and PBMCs in a time- and dose-dependent manner after treatment with LPS.

To determine whether this increase in mRNA was due to an increase in transcription of the α_5 gene, the authors transiently transfected U937 cells with a genetic construct in which the human α5-gene promoter was fused to a luciferase reporter. Luciferase activity was detected using the Luciferase Assay Reagent[a,b,c] (Cat.# E1483) and normalized to a β-galactosidase transfection control. Luciferase activity was induced by treatment with LPS, with maximal induction at 5µg/ml LPS for 20 hours. The Lipid A component of LPS alone (endotoxin) also induces α_5 gene expression. Furthermore this transcription is inhibited if the cells are pretreated with anti-CD14 antibody. CD14 receptors on immune cells are known to mediate some of the effects of LPS.

PKC Signaling Is Involved in LPS-Induced Responses

The next question that the authors of this study asked was whether or not the integrin transcription was dependent on protein kinase C (PKC). The authors measured PKC activity in response to treatment of U937 cells using the Kinase-Glo® Luminescent Kinase Assay[a,d] (Cat.# V6711). U937 cells were cultured with or without 5µg/ml LPS for 4 hours. The cells were harvested, washed and resuspended in kinase reaction buffer (40mM Tris [pH 7.5], 20mM $MgCl_2$, 0.1mg/ml BSA) and sonicated. Samples were diluted in PKC reaction buffer (20mM Tris [pH7.5], 10mM $MgCl_2$, 0.1mg/ml BSA, 250µM EGTA, 400µM $CaCl_2$, 0.32mg/ml phosphatidylserine, 0.032mg/ml diacylglycerol) with 10µM ATP and 100µM neurogranin$_{23-43}$) for 90 minutes at room temperature. The Kinase-Glo® Reagent (50µl) was added, and the samples were incubated at room temperature for 10 minutes. Results were recorded as inverse relative light units. The authors show a statistically significant increase in PKC activity in U937 cells treated with LPS versus untreated control cells.

In summary the authors demonstrate that LPS induces the expression of $\alpha_5\beta_1$-integrin, which mediates adhesion of U937 cells to a fibronectin-coated matrix. Regulation of $\alpha_5\beta_1$-expression is at the level of transcription and appears to be mediated by a CD14-dependent PKC signaling pathway. ■

Ordering Information

Product	Size	Cat.#
Kinase-Glo® Luminescent Kinase Assay	10ml	V6711
	10 × 10ml	V6712
	100ml	V6713
	10 × 100ml	V6714
Luciferase Assay Reagent	1,000 assays	E1483
Also Available: Neurogranin$_{(28-43)}$ (PKC) Peptide Substrate	1mg	V5611

[a]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673

[b]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents.

[c]Certain applications of this product may require licenses from others.

[d]U.S. Pat. No. 6,602,677, Australian Pat. No. 754312 and other patents pending.