

Flow Cytometric Measurement of Signal Transduction Pathways in Lipopolysaccharide (LPS) Activated Human Peripheral Blood Monocytes

Abstract:

LPS is known to activate multiple signaling pathways in peripheral blood monocytes via the TLR4 receptor complex. Following LPS exposure in vitro, monocytes rapidly activate all three major MAP Kinase (ERK, p38, and SAP/JNK), in addition to PI3 Kinase and IKK/NFκB pathways. Flow cytometry provides a unique methodology to monitor these signaling pathways, allowing a coordinated measurement of the quantity of multiple phospho-epitopes in the context of cell surface plus other cellular markers. Used in conjunction with specific kinase inhibitors, flow cytometry provides insight into the contribution of different pathways to downstream signaling. We have developed antibody fluorophore conjugates that allow simultaneous monitoring of four different phospho-epitopes in conjunction with one or more cell surface specific (CD) receptors, and have used this approach to monitor the kinetics of stimulation and specific pathway inhibition in peripheral blood monocytes. In addition to providing a methodology to study signaling pathways and their interactions in monocytes, this approach provides a useful way for labs to establish signaling pathway analyses using flow cytometry, and to validate methodologies and reagents for flow cytometric analysis of these signaling pathways.

Dr. T. Vincent Shankey received his Ph.D. degree in Immunology and Medical Microbiology from the University of Florida School of Medicine (1977). He was a postdoctoral fellow at the University of Pennsylvania in Philadelphia from 1977 to 1981 where he worked on the genetics of Chronic Lymphocytic Leukemias, and on regulation of Immunoglobulin synthesis in CLL B-cells. He has been involved in research utilizing flow and image cytometry for over thirty years and worked in clinical flow cytometry for much of that time. Before joining the Advanced Technology Group at Beckman Coulter in 2001, he was the Director of Research for the Urology Department and Scientific Director of the Clinical Flow Cytometry laboratory at Loyola University Medical Center near Chicago, Illinois.

Research Interest: Signal transduction pathways represent the major communications networks that transmit signals from multiple cell surface receptors to the nucleus, activating specific gene targets. These pathways serve an important function in integrating signals from individual pathways, to regulate cell death, or survival, cell proliferation and cell differentiation. Flow cytometric techniques have been developed to measure specific signaling pathways in the context of cell surface immunophenotypic analysis (2,3). In adult Myeloid Leukemias we have used this strategy to study STAT5 phosphorylation in response to in vitro and in vivo treatment with the targeted agent Gleevec (1). In AML patients, we have studied the impact of several targeted agents on the MAPK and PI3K pathways, in addition to monitoring phosphorylation of the ribosomal S6 protein (4). In conjunction with cell surface receptor staining, we have developed a standardized assay methodology and a method to correlate ZAP-70 protein expression in CLL cells to internal positive (T-cell) and negative (B-cell) control populations (3). These approaches provide a unique insight into the biologic heterogeneity of human leukemias, and offer the potential to monitor individual patients'

responses to targeted agents, including the potential for “real time” drug dose monitoring (4).

Selected Publications:

1. Jacobberger JW, Sramkoski RM, Frisa PS, Ye PP, Gottlieb MA, Hedley DW, **Shankey TV**, Smith BL, Paniagua M, and Goolsby CL. Immunoreactivity of STAT5 phosphorylated on tyrosine 694 as a cell-based measure of Bcr/Abl kinase activity. *Cytometry* 54A;75-88, 2003.
2. Chow, S., Hedley D, Grom P, Magarri R, Jacobberger, and **T.V. Shankey**. A whole blood fixation and permeabilization protocol with red blood cell lysis for flow cytometry of phosphor-epitope expression in leukocyte subpopulations. *Cytometry* 67A;4-17, 2005.
3. **Shankey, T.V.**, M. Forman, P. Scibelli, J. Cobb, C.M. Smith, R. Mills, E. Bernal-Hoyos, M. Van Der Heiden, J. Popma, and M. Keeney. An optimized whole blood method for flow cytometric measurement of ZAP-70 protein expression in Chronic Lymphocytic Leukemia. *Cytometry* 70B:259-269, 2006.
4. Hedley D.W., S. Chow , C. Goolsby, and **T.V. Shankey**. Pharmacodynamic Monitoring of Molecular-Targeted Agents in the Peripheral Blood of Leukemic Patients using Flow Cytometry. *Toxicol Pathol.* 36:133-139, 2008.