



2006 Research Grant Program Winning Abstract

Intracellular Signaling Networks in Inflammation: A Comparison of Three Diseases of Childhood Using BD Phosflow™ Technology

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Hypothesis: Systemic juvenile idiopathic arthritis (SJIA) is one of the subtypes of juvenile idiopathic arthritis (JIA), is characterized by a combination of systemic inflammatory features and arthritis, and is the most severe form of JIA. The JIA US prevalence is between 16 and 113/100.000. At onset, SJIA may be difficult to distinguish from Kawasaki Disease (KD), another childhood inflammatory disease. However, their disease courses are quite distinct. KD is self-limiting and the critical complication is coronary artery aneurysm, which can be prevented by early administration of intravenous immunoglobulin. SJIA has a variable clinical course: approximately half of the patients experience a monocyclic course, and half show progression to chronic arthritis. When chronic arthritis is prominent, SJIA may resemble another subtype of JIA, polyarticular (poly)JIA. All three diagnoses (SJIA, KD, polyJIA) are currently made on clinical grounds.

We hypothesize that, despite the commonality of inflammation in all three diseases, the signaling pathways in circulating mononuclear cells (PBMC) will have disease-specific features. We predict that these disease-specific features will shed light on the cellular processes involved in disease pathogenesis and progression, and may provide diagnostic markers. Additionally, our findings may have broader implications and applications for other arthritis and vasculitis syndromes.

Previous studies and preliminary data: We compared gene expression in paired PBMC samples from JIA patients at flare and quiescence and found distinct molecular patterns associated with SJIA and polyJIA at flare. The cell population(s) responsible for each transcriptional signature remains to be determined. However, our data strongly suggest activation of monocytes and reduced cytolytic function in SJIA flare and Th1 activation in polyJIA flare. Using FACS analyses, we have observed that monocytes are expanded during SJIA flare, but the pro-inflammatory subset CD14^{lo}/CD16^{hi} is expanded, even at quiescence. These studies have led us to focus on monocytes (CD33⁺) and T cells (CD3⁺) and to hypothesize that characterization of activation pathways in these cells will reveal disease-specific features.

We have begun to characterize disease-associated signaling networks in SJIA by analysis of signal transduction pathways in response to cytokines and other stimuli using BD Phosflow. We examined cells from 8 SJIA patients (4 in quiescence, 2 in remission and 2 in flare) and 8 healthy controls. Using cytokines IFN-gamma, IL-10, IL-6 and IL-7 (all BD), GM-CSF and PMA and ionomycin as stimuli, we measured levels of pStat1, pStat3, pStat5 and pERK (BD antibodies). We found that monocytes from SJIA patients showed reduced phosphorylation of downstream targets of IFN-gamma (pStat1) and GM-CSF (pStat5) [both at $p < 0.03$], and of IL-10 (pStat3) ($p = 0.053$). These changes were independent of disease status and medication use and were not due to a global deficit in monocyte responsiveness, as levels of response to IL-6 (pStat3), IL-7 (pStat5) and



PMA+ionomycin (pERK) were comparable between SJIA and control monocytes. Basal levels of all intracellular phosphoproteins analyzed were similar between SJIA and control cells. The phosphorylation response of T cells from SJIA subjects was comparable to control T cells, except for diminished STAT-1 ($p < 0.01$) phosphorylation in response to IFN-gamma. These preliminary results suggest selective desensitization of signaling pathways, in SJIA monocytes. Intriguingly, the affected pathways are those thought to counter-regulate TNF and IL-1-driven inflammation.

Experimental approach: We propose to compare levels of phosphoproteins in PBMC samples from age- and gender-matched patients with SJIA, KD and pJIA to identify diagnostic and prognostic phosphoprotein signatures. We will measure baseline and induced levels of a panel of phosphoproteins in monocytes and T cells, using surface markers to further distinguish subsets of these cells (CD16+ monocytes, CD4, CD8 T cells, Tregs, NKTs). Serial samples from individual patients will be analyzed, allowing comparison of profiles across disease states (flare, quiescence on medication, remission off medication). Thus, we may elucidate immune dysfunctions that are (1) intrinsically related to susceptibility to these diseases (patients at remission), (2) disease-specific patterns (flare in one disease) and (3) patterns associated with immune cell activation that are shared by inflammatory responses in all three diseases.

Data analysis: Cells will be analyzed on a FACSCalibur or LSRII instrument. Data will be analyzed using FlowJo (TreeStar) and, for induced levels, expressed as fold change upon stimulation (fold change = median fluorescence intensity (MFI) stimulated/MFI unstimulated). Data will be log₂ transformed and analyzed by standard t tests. Additionally Spotfire data visualization software will be used to display analyses and to perform hierarchical clustering. Pathway maps will be generated according to the data.

Patient population and power: Subjects for study are recruited at Stanford University, UC San Francisco and UC San Diego. We intend to study serial samples from 40 subjects from each disease. We have already cryopreserved samples from 33 SJIA, 17 PolyJIA, 40 KD patients and 35 controls, and observed comparable phospho-responses in fresh and frozen cells. Using a sample size of 40 patients and 40 controls will yield 80% power to detect effect sizes of 0.64 and higher at the $\alpha = 0.05$ level (two-sided test).

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