Christopher R. Lucas
Ph.D. Candidate

“Prohibitins and the Cytoplasmic Domain of CD86 Cooperate to Mediate CD86 Signaling in B Lymphocytes”

Wednesday, November 28th, 2012
Institute for Behavioral Medicine Research Building
460 Med. Ctr. Dr., Rm. 109
9:00 am
VITA

August 2, 1984 .......................... Born – Columbus, OH

June, 2007 ............................... B.S., Biochemistry
                                      The Ohio State University

2007-present ............................ Graduate Research Associate, The Ohio State University

2011-2012 ............................... Pelotonia Graduate Fellow for Cancer Research, The Ohio State University

COMMITTEE MEMBERS

Professor Virginia M. Sanders, Advisor

Professor John Byrd

Professor Michael Freitas

Professor Jeanette Marketon

Professor Susheela Tridandapani

- American Association of Immunologists Trainee Travel Award to Annual Scientific Meeting. (San Francisco, CA, May, 2011)

- Finalist, Hayes Annual Graduate Research Forum, The Ohio State University. (Columbus, OH, March, 2011)

- Vice President, Integrated Biomedical Science Student Organization, The Ohio State University. (Columbus, OH, 2007-2008)

FUTURE PLANS

Upon graduation, I plan to pursue a Bioengineering Post-Doctoral position in the laboratory of Dr. Carlos Castro in the Department of Mechanical and Aerospace Engineering at The Ohio State University. We hope to apply my present knowledge in a unique B cell signaling mechanism to engineer a scaffolding system to present CD86 blocking ligands to limit the CD86-induced proliferation, thus providing a novel treatment strategy for Chronic Lymphocytic Leukemia. I ultimately aspire to be an independent scientist.
ABSTRACT

The goal of this dissertation was to determine the proximal molecular mechanism by which CD86 engagement signals directly to the B cell to increase the level of IgG1 antibody produced. Previous reports showed that CD86 engagement signals directly to a CD40L/IL-4 primed B cell to increase the level of IgG1 produced in vitro and in vivo, without affecting the level of class switch recombination (CSR). The CD86-induced increase in IgG1 production occurs due to the activation of a unique intracellular signaling network that increases the rate of IgG1 transcription via an Oct-2- and NF-κB-dependent increase in the activity of the 3′-Igh enhancer. The CD86-dependent NF-κB activation is regulated via two proximal signaling networks that induce IκBα phosphorylation, subsequent degradation, and release of NF-κB (p50/p65), and subsequent NF-κB (p65) phosphorylation. Although it was understood how CD86 engagement increased the level of IgG1 produced, the proximal molecular mechanism to one of the most proximal CD86-induced signaling intermediates, PLCγ2, remained unknown. Prior reports showed that PLCγ2 was recruited to phosphorylated tyrosine residues, which are absent within the cytoplasmic domain of CD86. Therefore, the hypothesis tested in this dissertation was that the CD86 cytoplasmic domain associated directly with a protein/protein complex capable of undergoing tyrosine phosphorylation to activate PLCγ2 and was required for the CD86-dependent increase in IgG1 production by a B cell. In order to test our hypothesis we designed the following specific aims: 1) To determine if a functional signaling protein(s) associated directly with CD86 to mediate the CD86-induced increase in PLCγ2 activation and IgG1 and 2) To evaluate if the CD86 cytoplasmic domain was required for the CD86-induced increase in IgG1.

Using a proteomics-based identification approach, we show for the very first time that the tyrosine-containing transmembrane adaptor proteins, prohibitin-1 (Phb1) and prohibitin-2 (Phb2), bind
to CD86. The expression of Phb1/2 and association with CD86 increased primarily after priming with CD40. The CD86-induced increase in Oct-2 and IgG1 was less when either Phb1/2 expression was reduced by shRNA or the cytoplasmic domain of CD86 was truncated or mutated at serine/threonine PKC-phosphorylation sites, which did not affect Phb1/2 binding to CD86. In addition, we show that an intact CD86 cytoplasmic domain is necessary for an optimal IgG1 response against a T-dependent antigen in vivo. Furthermore, we also show that Phb1/2 and the CD86 cytoplasmic domain are required for the CD86-induced phosphorylation and subsequent degradation of IκBα, which we previously reported leads to NF-κB p50/p65 activation; whereas, only Phb1/2 was required for the CD86-induced phosphorylation of PLCγ2 and PKCα/βII, which we have previously reported leads to NF-κB (p65) phosphorylation and subsequent nuclear translocation. Thus, our findings suggest that Phb1/2 and the CD86 cytoplasmic domain cooperate to mediate CD86 signaling in a B cell through differential phosphorylation of distal signaling intermediates required to increase IgG1. The significance of this dissertation is that it is the first to identify the proximal CD86-induced intracellular signaling mechanism in a B cell that regulates the level of IgG1 produced. Knowledge gained from this work will provide novel therapeutic targets to either elevate or suppress the level of IgG1 produced.

We have applied this knowledge of CD86 signaling to the B cell malignancy, Chronic Lymphocytic Leukemia (CLL), and found that CD86 engagement on the surface of MEC1 B-CLL cells increased the number of viable cells and induced activation of the pro-growth/survival factor NF-κB. In addition, CD86 associated directly with prohibitin-1 (PHB1) in B-CLL cells. Furthermore, PHB1 depletion via shRNA inhibited MEC1 B-CLL proliferation. Thus, a novel CD86-PHB1 signaling complex may be involved in mediating a signal in B-CLL cells directly to promote a proliferative response, thus providing a potential therapeutic target.

**RECENT ABSTRACTS AND PRESENTATION**

- **Lucas, C.R.,** Cordero-Nieves, H.M., Erbe, R.S., McAlees, J.W., Bhatia, S., Hodes, R.J., Campbell, K.S., and Sanders, V.M. Prohibitins and the Cytoplasmic Domain of CD86 Cooperate to Mediate CD86 Signaling in B Lymphocytes. American Association of Immunologists Annual Scientific Meeting (Boston, MA, May, 2012)

- **Lucas, C.R.,** and Sanders, V.M. A Novel Role for CD86 Signaling/the CD86 Signaling Complex in Chronic Lymphocytic Leukemia. The Ohio State University Comprehensive Cancer Center (OSUCCC) Annual Scientific Meeting (Columbus, OH, March, 2012)

- **Lucas, C.R.,** Cordero-Nieves, H.M., Erbe, R.S., McAlees, J.W., Bhatia, S., Hodes, R.J., Campbell, K.S., and Sanders, V.M. Prohibitins and the Cytoplasmic Domain of CD86 are necessary to Mediate CD86 Signaling in B Lymphocytes. Psychoneuroimmunology Research Society Annual Scientific Meeting. Hilton Indian Lakes Resort. (Chicago, IL, June, 2011)

- **Lucas, C.R.,** Cordero-Nieves, H.M., Erbe, R.S., McAlees, J.W., Bhatia, S., Hodes, R.J., Campbell, K.S., and Sanders, V.M. Prohibitins and the Cytoplasmic Domain of CD86 are required to Mediate CD86 Signaling in B Lymphocytes. American Association of Immunologists Annual Scientific Meeting. Moscone Conference Center. (San Francisco, CA, May, 2011)

- **Lucas, C.R.,** Cordero-Nieves, H.M., Erbe, R.S., McAlees, J.W., Bhatia, S., Hodes, R.J., Campbell, K.S., and Sanders, V.M. Prohibitins and the Cytoplasmic Domain of CD86 are necessary to Mediate CD86 Signaling in B Lymphocytes. Integrated Biomedical Science Graduate Program Annual Retreat. The Ohio State University. (Columbus, OH, December, 2011)

- **Lucas, C.R.,** Cordero-Nieves, H.M., Erbe, R.S., McAlees, J.W., Bhatia, S., Hodes, R.J., Campbell, K.S., and Sanders, V.M. Prohibitins and the Cytoplasmic Domain of CD86 are necessary to Mediate CD86 Signaling in B Lymphocytes. Hayes Annual