1. FINAL PUBLISHABLE SUMMARY REPORT

Inflammation is an innate biological response to injury or infection. It is a key part of any immune response and it is needed to eliminate harmful microorganisms and heal damaged tissue. The inflammatory process is influenced by components (cells and chemicals) in the immune and vascular systems that work together to produce an inflammatory response of a reasonable magnitude and duration. Changes in the body that allow inflammation to persist longer than necessary are especially problematic as this can give rise to a multitude of chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, psoriasis, and asthma. These inflammatory disorders afflict millions of people worldwide and lead to poor quality of life, economic loss and premature death.

Oxidants are produced from a number of different sources within cells. An important group of cellular oxidants, called 'reactive oxygen species (ROS)' are produced in all cells as a normal part of cellular respiration ('breathing'). In immune cells called phagocytes, huge quantities of ROS (called 'phoxROS') are also made to attack and kill harmful microorganisms. Through that process, phoxROS can also damage healthy tissue. In this context, phoxROS have given oxidants 'a bad name' in the mainstream and scientific literature as harmful molecules that cause tissue injury and inflammatory diseases. People with an inherited condition called Chronic Granulomatous Disease (CGD) are incapable of producing phoxROS. As would be expected, these people are susceptible to life-threatening infections. We also might expect that these people are less prone to inflammatory diseases, when in reality they frequently suffer from a range of inflammatory diseases.

This project was intended to advance our understanding of the role of ROS in inflammation, and in particular, reconcile laboratory data suggesting that phoxROS promote inflammation with the clinical observation that CGD patients, without phoxROS, have severe inflammatory disease.

For this project, we acquired two populations of immortalized human cells ('cell lines') that were identical in all respects except that one population was, through genetic manipulation, incapable of making phoxROS. Methods were introduced into the lab for maintaining these cells and inducing their maturation into two different types of immune system phagocytes. In accordance with previous work of ours on cells from CGD patients (Bylund *et al* 2007 *Eur J Immunol*), we found that the CGD cell line made more inflammatory mediators, called pro-inflammatory cytokines, than normal cells. As the cells were grown under sterile conditions, it offered strong evidence against the long-standing notion that inflammatory disorders in CGD are caused by an inflammatory response to persistent infections. Moreover, it showed that the absence of phoxROS causes CGD cells to continuously make pro-inflammatory cytokines without appropriate cues to do so (Brown *et al* 2008 *Clin Immunol*).

In response to emerging evidence in the scientific literature that ROS inside the cell are needed to make pro-inflammatory cytokines, we implemented new techniques into the lab to look at the presence of ROS, other than phoxROS, in CGD cells. As a result we found that the mitochondria in CGD phagocytes make more ROS (mtROS) than normal cells. This was observed in the cell line and in cells we acquired from a person with CGD. Signs of a cellular response to too much mtROS were evident by changes in gene expression in phagocytes from CGD patients and by the presence of modified proteins on the surface of the CGD cell line (Sundqvist *et al* 2011 *Eur J Clin Invest*). In addition, we found that ROS that are excreted external to the cell, while not needed to make pro-inflammatory cytokines, are required for particular phagocytes to dispose of dying immune cells; a critical step for resolving inflammation (Brown *et al* 2009, *J Innate Immunity*). So both the diminished ability of CGD phagocytes to remove dying cells from

afflicted tissues as well as their heightened ability to produce cytokines without appropriate cues result from changes in ROS and likely contribute to persistent inflammation and the development of inflammatory disease in CGD.

Findings from this project significantly advance our understanding of ROS in inflammation and in particular, the presence of inflammatory disorders in CGD. Our findings of elevated mtROS in CGD phagocytes (Brown *et al* manuscript under revision *J Immunol*) are in line with two high-profile publications this year (Zhou *et al, Nature* 2011, 469:221 and Bulua *et al, J Exp Med* 2011, 208:519) that also implicated mtROS as intracellular oxidants responsible for pro-inflammatory cytokine production and, in one report, the underlying cause of chronic inflammation in a rare autoinflammatory disease. To extend our findings in CGD to other human inflammatory diseases, we have begun analyzing different types of intracellular oxidants in children with various inflammatory conditions. This work is in collaboration with clinicians at Queen Silvia Children's hospital in Gothenburg, SWE (Brown *et al* 2010 *BMC Pediatrics* and Sundqvist *et al* 2011 manuscript in preparation) and at the Child and Family Research Institute in Vancouver, CAN.

In the past few years, a new light has been cast on ROS as molecules, that when properly controlled, play beneficial roles in the normal inflammatory process - a sharp contrast to previous indications that ROS drive unwanted inflammation by toxicity-induced tissue damage. In addition, our work and that of others this year suggest that mitochondria may be a novel target for new anti-inflammatory drugs. To communicate these changes in the field of oxidants to the broader scientific, clinical, and pharmaceutical communities, we published two reviews that speculate on the role of intracellular oxidants in human health & disease (Bylund et al, 2010 *Free Radic Biol Med* and Björkman *et al* 2008 *Arthritis & Rheumatism*). We are also guest editors for a Special Issue devoted to 'ROS in Inflammation' that will be published in 2012 (Reactive Oxygen Species and Inflammation: An Increasingly Connected and Complex Relationship, *Mediators of Inflammation*).

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