Annual Report: The first three years of the Interleukin Foundation

The Foundation was registered in the State of Colorado as a non-profit corporation on October 22, 2009. On May 10, 2011, Internal Revenue Service of the United States of America granted the Foundation the status of a Public Charity. From 2011 to December 2013, the Foundation has been active in its primary goal of supporting both fundamental research as well as clinical applications of cytokine-mediated diseases. Support for the various projects are in the form of grants (limited to 25,000 USD one time) with specific goals and long-term benefit. The Foundation also funds smaller projects in order to generate data for grant applications from national funding agencies such as National Institutes of Health (NIH) in the USA and European and Israeli funding agencies and institutions. The by-laws of the Foundation state that grants are evaluated regardless of country of origin. Small travel grants are given by the Foundation in order to support young investigators to attend scientific meetings on cytokines and immunology in order to expand their knowledge and foster collaborations.

Below is a summary of each grant funded since the initiation of the Foundation. The name of the Principle Investigator (PI) or responsible scientist (recipient) are listed as well as the institutions.

Major Grants

Grant title: Molecular mechanisms of histone deacetylase inhibitor (HDACi)-mediated pancreatic β-cell protection. Professor Thomas Mandrup-Poulsen, MD, PhD, Principle Investigator. University of Copenhagen (Denmark).

Background. Diabetes continues to be difficult to treat, whether Type 1 or Type 2 diabetes, for complications of heart diseases, loss of vision and kidney function. If we are to reduce the burden of diabetes for an increasing number of persons, we need inexpensive, orally active drugs that protect against the loss of the insulin-producing cell in the pancreas. In animal models of diabetes, small molecules called histone deacetylase inhibitors (HDACi) have been shown to protect the insulin producing beta cells. Therefore studies by the PI hold promise to develop HDACi as novel therapies to treat either Type 1 or Type 2 diabetes.

The Study. The overall aim of the project was to clarify the effects of IL-1 and HDACi on NFκB acetylation and activity in pancreatic beta-cells and to identify how HDACi modifies basal and IL-1-induced miRNA expression. Also how HDACi modifies basal and IL-1-induced protein acetylation. In insulin-producing β-cells the HDAC inhibitor givinostat did not inhibit expression of the anti-inflammatory genes *SOCS1-3* or *sirtuin-1* but reduced levels of IL-1β + IFNγ induced pro-inflammatory *Il1a*, *Il1b, Tnfα, Fas, Cxcl2* and reduced cytokine-induced ERK-phosphorylation. Further, nuclear factor NFκB genomic *iNos* promoter binding was reduced by 50% and NFκB-dependent mRNA expression was blocked associated with NFκB subunit p65 hyperacetylation. These data are now published in *Proc Natl Acad Sci USA*.

The investigators also conducted a PCR-based miRNA array and identified a number of miRNAs regulated by HDAC inhibition. Bioinformatic analysis is ongoing to select the highest ranked miRNA candidates for functional validation. The complete insulin-producing cell acetylome has been mapped after exposure to inflammatory cytokines in the presence or absence of HDAC inhibitor. Bioinformatic analysis is ongoing to enable selection of top-ranked candidates for further validation.

Follow-up. These data are now published in *Proceedings of the National Academy of Sciences USA*. These data provide the basis for pre-clinical studies, which are required for clinical studies.

Title: *"A double-blind, randomized, placebo-controlled clinical trial of the safety and efficacy of anakinra in patients with hydradenitis suppurativa".* Professor Evangelos J. Giamarellos-Bourboulis, MD, PhD, ATTIKON University Hospital, Athens, Greece

Background information. Hydradenitis suppurativa (often called acne inversa) is a chronic inflammatory disease of the skin in the axilla and groin areas affecting at 1% of the population of the United States. It is a disfiguring and painful disease due to inflammation around the hair follicles. Despite the presence of bacteria in the lesions, antibiotics do not work to reduce the scarring, faul odor and pain of the disease. There are anecdotal reports that Interleukin-1 (IL1) blockade with anakinra may reduce the disease. Therefore, the Foundation was pleased to provide funds to carry-out a controlled study to block in hydradenitis suppurativa by the Hellenic Institute in Athens, Greece

The Study. The trial is being conducted in two departments of ATTIKON University Hospital of Athens, Greece i.e. 4th Department of Internal Medicine and 2nd Department of Dermatology and Venereology. The trial was issued EudraCT number 2011-005145-12 and it was approved by the National Ethics Committee on 19th January 2012 (approval 100/11) and by the National Organization of Medicines of Greece on 1st February 2012 (approval 89111). The trial was registered in www.clinicaltrial.gov (identifier NCT 01558375). The goal of this study is to enroll 20 patients. The first patient was enrolled on February 16th 2012. 30 patients were

screened for eligibility and 19 patients were enrolled. It is anticipated that the last patient will be enrolled by mid-February 2014 and data available summer of 2014.

Follow-up. Once this trial is completed, we anticipate that anakinra will be tested in larger trials and eventually this treatment will be used by those who suffer from hydradenitis suppurativa. The Foundation's funding of the this controlled pilot trial serves as an example of the work of the fundamental work of the Foundation, that is, to improve the health of persons who bear the burden of disease by supporting basic and clinical research on cytokines.

Title: *Blocking IL32 with alpha1antitrypsin in patients with severe grade 3-4 Graft versus Host Disease*. A. Mario Marcondes, MD, PhD, Principle Investigator, Fred Hutchinson Cancer Center, Seattle, WA.

Background information. Graft versus Host Disease (GvHD) is a life-threatening complication of bone marrow transplantation. Bone marrow transplantation is necessary in order to treat patients with leukemias and lymphoma. The bone marrow from a donor is used to replace the bone marrow in the patients with leukemia following the destruction of the patients own bone marrow by high doses of chemotherapy and radiation. However, the bone marrow of the donor (graft) will often "attack" the patient's own tissues (host). GvHD can be a fatal disease. Because animal studies revealed that alpha1antitrypsin, a natural protein in our circulation, protected against GvHD, the Foundation approved the request to support the studies at the Fred Huchinson Cancer Center, one of the top centers for bone marrow transplantation in the world.

The Study. The funds were used to purchased alpha1antitrypsin and to treat three patients with severe GvHD. The purchase was necessary because alpha1antitrypsin use for GvHD is experimental, despite the data from the

animal studies. The first case was a patient with GvHD having intestinal inflammation and producing 5 quarts of water stools per day associated with abdominal cramps, dehydration, fevers and electrolyte imbalances. This patient was being treated with high doses of immunosuppressives and steroids yet was not responding. After the third infusion, the patient improved markedly and was discharged home within one week of the treatment. A second patient had the liver form of the GvHD which is assocaited with toxic condition due to a failure of the liver to function. This patient was also being treated with high doses of immunosuppressives and steroids yet was not responding. Alpha1antitrypsin treatment was started and within 3 days the liver enzymes revealed tat the liver was again functioning normally. A third patient with the liver form of GvHD was also successfully treated.

Follow-up. Because of these results with alpha1antitrypsin in treating these patients, Fred Hutchinson applied to the NIH for funds to carry-out a larger trial. This trial has started and the first two patients have been treated successfully with alpha1antitrypsin. The Foundation's funding of the original pilot trial serves as an example of the work of the fundamental work of the Foundation, that is, to improve the health of persons who bear the burden of disease by supporting basic and clinical research on cytokines.

Project Title: *Specific Inhibition of Spi-1/PU.1 for proIL-1β Blockade in Human Monocytes*. Professor Philip E. Auron, PhD, Duquesne University, Pittsburgh, PA.

Background Information. Because IL1β is a major mediator of inflammation and because antibodies to IL1β sich as canakinumab are now approved for the treatment of refractory gout, sustemic onset juvenile idiopathic arthritis and Cryopyrin Associated Periodic Syndromes, understanding the control of IL1β production plays an imporatnt role treating disease. The supposition is that knowledge of this mechanism can be useful in designing

future anti-inflammatory therapeutics that could prove more effective, since they would target transcription prior to protein expression and manifestation of symptoms.

The Study. The studies are aimed at understanding the molecular mechanism associated with induction of transcription for the IL1 beta gene which codes for the precursor of human interleukin 1beta (proIL-1β) in activated human monocytes. The data have demonstrated that the rapid induction of the human IL1B gene in response to Toll-like receptor (TLR) activation, in contrast to the numerous other rapidly induced genes (such as TNF, coding for tumor necrosis factor α), does not depend upon pre-recruited RNA polymerase II (Pol II), but depends upon the constitutive binding of the Spi-1/PU.1 (Spi1) transcription factor to the transcription start site. Spi1, which was reported to be essential for IL1B gene induction, directly recruits TATA binding protein (TBP) in response to TLR4 signaling, supporting TLR-dependent Pol II recruitment. Ectopic expression of Spi1, along with its binding partner (IRF8) and the TLR4 surrogate, TRAF6, supports ILIB gene activation in a non-myeloid cell line, and that TBP and Pol II recruitment requires the amino terminal sequence of Spi1. In addition, the data are evidence for long-range genomic interaction between Spi-1 binding at the IL1B gene promoter and both NF-ϰB and C/EBPβ binding at far-upstream TLR-dependent enhancer sequences, as well as a greater dependence upon the metabolic state of the cell for IL1B gene expression.

Follow-up. These data, which have important implications for intervening in specific IL-1 vs. TNF expression, have been published in the journal PLoS ONE (Adamik, J., Wang, K.Z.Q., Unlu, S., Su, A-J. J., Tannahill, G.M., Galson, D.L., O'Neill, L.A., and Auron, P.E. Distinct Mechanisms for Endotoxin Induction and Tolerance Regulate the IL-1β and TNFα Genes. PLoS ONE 8(8): e70622 (2013)). The observed greater dependence upon the metabolic state of the cell for IL1B gene expression, as compared to that of TNF, resulted in our further investigation of this phenomenon. This resulted in an extensive collaborative study with Luke A.J. O'Neill at

Trinity College, Dublin, Ireland, that not only provided a detailed metabolomic analysis of the regulation of the human and mouse human IL1B genes, but determined that Warburg metabolism and the generation of excessive amounts of intra-cellular succinate resulted in a macrophage pseudohypoxic state. This pseudohypoxia resulted in stabilization of hypoxia inducing factor 1α (HIF-1α), which targeted the mouse il1b and human IL1B genes, generating the sustained expression that likely mediates the incomplete TLR tolerance reported in the above described PLoS ONE paper. A complete report of this work, which could have important implications for understanding the role of metabolism in determining macrophage polarization and its role in metastasis, has now been published in the prestigious journal Nature (Tannahill, G.M., Curtis, A.M., Adamik, J., Palsson-McDermott, E.M., Frezza, C., Goel, G., McGettrick, A.F., Bernard, N.J., Zheng, L., Kelly, B., Gardet, A., Clish, C., Tong, Z., Foley, N.H., Jany, S.S., Corr, S.C., Walmsley, S., Beasley, F.C., Cummins, E., Nizet, V., Whyte, M., Taylor, C.T., Masters, S.L., Lin, H., Gottlieb, E., Kelly, V., Auron, P.E., Xavier, R.J., O'Neill, L.A. Succinate is a danger signal that induces IL-1β via HIF-1α. Nature 496:238-242 (2013)). The Interleukin 1 Foundation is acknowledged for support in all three of the publications describing the above work**.**

Project Title: *Interleukin-37 suppresses contact hypersensitity dermatitis.* Mayumi Fujita, PI. Professor of Dermatology, University of Colorado, Aurora, Colorado

The Study. Contact hypersensitivity dermatitis is a chronic skin disease that has an allergic basis. The disease often leads to scars and has considerable occupational consequences. Cytokines such as IL1α are thought to play a role. Although steroid creams are used, chronic use results in remodeling of the skin. Newer therapies as well as a better understanding of IL37 were the goals of the study. The study has now been completed and the data generated by the Foundation funding is being used to obtain NIH funding.

Follow-up. IL-1 family member IL37 functions to suppress innate inflammation in models of colitis, LPS-induced shock and ischemic myocardial injury. In the present study, we examined a role for IL37 in the adaptive immune response, namely the development of skin contact hypersensitivity (CHS) to specific antigen. We generated a strain of transgenic mice expressing human IL37 (IL37Tg). Not expressed in the resting state, IL37 was induced in the skin of IL37Tg mice 6 hours following application of the hapten antigen 2,4-dinitrofluorobenzene. Mice were challenged by local application of antigen five days later and we observed a marked reduction in ear swelling in IL37Tg mice compared to wild-type (WT) mice at 48 hours (-60%; $p<0.001$). Although the distribution, phagocytic activity and migration of skin dendritic cells (DCs) to regional lymph nodes in IL37Tg mice were comparable to those from sensitized WT mice, LPS induction of a costimulatory molecule, CD40, was reduced (-51%; $p<0.01$) in IL-37-DCs. Also, LPS-induced secretion of IL1b, IL6 and IL12 was significantly suppressed (-30%, -43% and -36% with $p<0.01$, $p<0.05$ and $p<0.01$, respectively), whereas the secretion of IL10 was enhanced (1.37-fold; $p<0.001$) in IL37-DCs compared to WT-DCs. Consistent with these data, IL37-DCs exhibited reduced ability to stimulate naïve T cells ($p<0.001$) and induced 86% more T regulatory cells ($p<0.001$) in vitro. Lastly, when sensitized DCs were adoptively transferred to WT mice, CHS responses to antigen challenge were impaired in mice receiving IL37-DCs compared to those receiving WT-DCs (-57%; $p<0.01$ at 48 hours after the challenge). Histological analysis revealed decreased CD8+ T cells (-73%; $p<0.01$) and increased T regulatory cells (2.60-fold; $p<0.01$) in CHS skin in mice receiving sensitized IL37-DCs. These findings provide evidence that DCs expressing IL-37 are tolerogenic, thereby impairing activation of T effector responses and inducing T regulatory cells. IL-37 thus emerges as an inhibitor of adaptive immunity.

Project Title: Common and rare genetic variants in the interleukin-1 pathway - and their influence on infection and inflammation

Principal Investigators: Frank L. van de Veerdonk, Department of Medicine, Radboud University Medical Center, Nijmegen, The Netherlands and

Alexander Hoischen, Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

The Study. The interleukin-1 family of ligands, receptors and associated processing and signaling molecules has a fundamental role in infection and inflammation; genetic variations in the genes encoding the IL1 family members have been associated with infection, cardiovascular disease, and cancer. However, most studies have been restricted to the role of common SNP variants in the underlying genes. Our hypothesis is that only the comprehensive understanding of rare and common genetic variants in the interleukin-1 pathway will help the full understanding of the relevance to immune response in health and disease. Here we will explore all genetic variants in the IL1 pathway (ligands, receptors, signaling molecules, and IL1 regulating genes) in infectious (for example, invasive aspergillosis) and inflammatory diseases (for example sarcoidosis, atherosclerosis and gout) including 300 or more patients per group. We will apply a novel ultra-low cost method that enables a genetic screen for the complete coding sequence of the interleukin-1 pathway. This enables the comprehensive identification of all variants, *i.e.* SNVs, indels and CNVs, affecting the coding sequence of these genes. The study will uncover common genetic variants but also rare and private genetic mutations in this pathway. We believe this new understanding will ultimately reveal the basis for individual responses.