Report of The Interleukin Foundation December 31, 2017.

The Interleukin Foundation continues to support fundamental research projects focused on cytokines with emphasis for the development of novel therapeutics. The Foundation provides funds for specific grants, travel grants and salary support grants. Below are grant recipients and a short summary of the research.

Grant Title: "An atomic description of Interleukin-37 and its anti-inflammatory activities".
Principle Investigator: Elan Z. Eisenmesser, Professor of Biochemistry and Molecular Biology, University of Colorado Denver.

Scientific Summary: To determine the X-ray crystal structure of the IL-37 dimer: Crystals have already been obtained and we anticipate studying these at the ALS synchrotron. In order to produce an IL-37 monomer, multiple point mutations of the IL-37 dimer interface will be constructed and assessed for both monomeric behavior (S75 size exclusion) and stability (CD thermal melts). We will assess the anti-inflammatory IL-37 activities of wild-type and monomeric IL-37 constructs using several cell lines. Currently, a luciferase-GFP NFkB reporter cell line is being constructed and will be used to compare IL-37 activity. Additionally, the ability of recombinant forms of IL-37 to suppress IL-6 and IL-8 effects on mouse macrophages (J-cells) and human THP-1 cells will be assessed by ELISAs will be performed.

Our goals were to first identify the dimer interface of IL-37 and then mutate this in order to probe the ramifications on activity. To this end, we found crystallization conditions for IL-37, which diffracted to 3.4 angstroms and revealed the dimer interface. During this time, another X-ray crystal structure of IL-37 was published that indicated the same interface as we have found and allowed us to produce two monomeric versions of IL-37. It is both these dimeric and monomeric forms that are now currently undergoing biological interrogation (proposed goal 3 above).

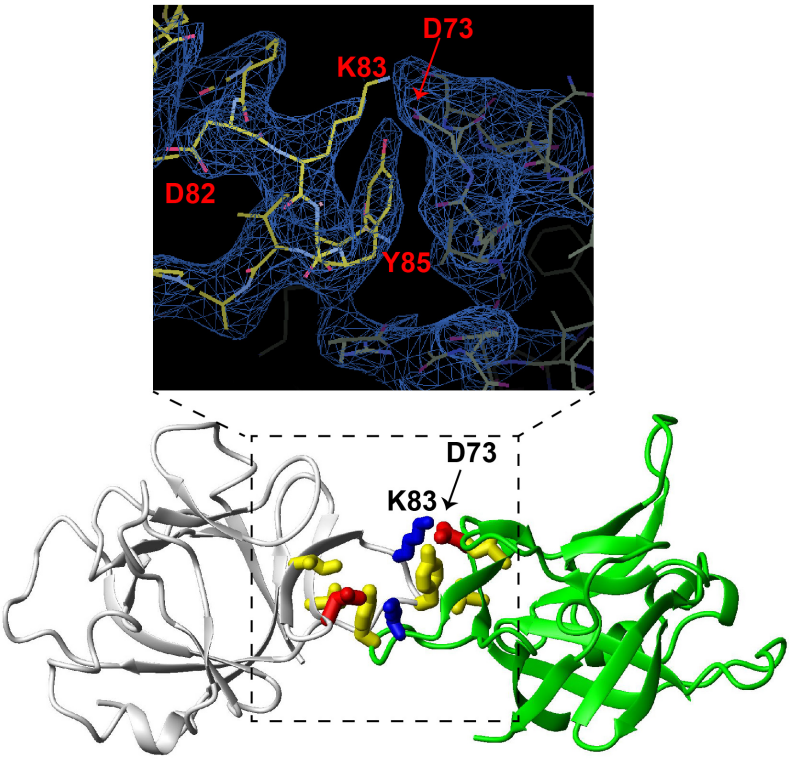

Figure: X-ray crystal density of the IL-37 interface is shown (top) along with critical dimer interactions that include D73/K83.

Lay Summary: IL-37 is a member of the IL-1 family of cytokines. An increasing number of studies indicate that the anti-inflammatory properties of IL-37 reduce disease severity is several models of human diseases in mice. Therefore, recombinant IL-37 is a potential therapeutic to treat humans with various inflammatory diseases. Professor Eisenmesser, who will produce forms of IL-37 with greater potency than natural IL-37, is undertaking the above studies. The basis for having greater potency to suppress inflammation enables for greater efficacy for clinical development of IL-37 as a therapy.

Grant Title: "Studies on alpha-1-antitrypsin to treat fungal infections."
Principle Investigator: Mark Gresnigt, PhD, Radboud University Medical Center, Nijmegen, The Netherlands.
Co-Investigator: Martin Jaeger, PhD., Radboud University Medical Center, Nijmegen, The Netherlands.

Scientific Summary. These studies are based on the well-known susceptibility of patients receiving immunosuppressive drugs for Graft-versus-Host Diseases (GvHD) to succumb to lethal infections with *Candida albicans*. However, mice transgenic for human alpha-1-antitrypsin are resistant to such lethal infections. Recently, we have observed that patients treated with alpha-1-antitrypsin for GvHD survive and do not succumb to infections with fungi. The laboratory studies are designed to examine the effects of alpha-1-antitrypsin on the growth of *Candida albicans* in mice infected with the live yeast. The goal of the studies is to define the mechanism by which alpha-1-antitrypsin protects the host from the lethal infection with *Candida albicans*, including possible mechanisms such as phagocytosis, killing and the role of autophagy.

Lay Summary. Alpha-1-antitrypsin is a natural occurring protein that is in the circulation of humans. Persons with a genetic deficiency of alpha-1-antitrypsin develop chronic lung and liver diseases. In these persons alpha-1-antitrypsin, isolated from the circulation of healthy subjects, is given as a supplement to replace their alpha-1-antitrypsin deficiency state. However, new studies show that persons without alpha-1-antitrypsin deficiency also benefit from treatments with alpha-1-antitrypsin. Thus, it is possible that alpha-1-antitrypsin as a treatment for patients receiving immunosuppressive drugs can be life-saving. Indeed, two clinical studies now report that patients being treated with immunosuppressive drugs for severe, life-threatening GvHD and who are treated with alpha-1-antitrypsin survive and do not develop fungal infections.

Grant Title: "Mechanism of Action of OT1177".
Principle Investigator: Carlo Marchetti, PhD.
University of Colorado Denver, Aurora, CO 80045

Scientific Study. These studies are designed to investigate the mechanism of action of OLT1177, a small synthetic molecule. The structure of OLT1177 is similar to others that fall into the class of NLRP3 inhibitors (NLRP3, Nucleotide-binding domain and Leucine-rich Repeat Pyrin containing 3). The primarily biochemical property of NLRP3 inhibitors is to prevent the activation of the IL-1β inflammasome that converts inactive pro-caspase-1 to active caspase-1. Active caspase-1, in turn, catabolizes the inactive IL-1β precursor to active IL-1β and allows for secretion. The studies are to investigate the mechanism by which OLT1177 is able to prevent the activation of NLRP3. For example, does OLT1177 bind to and inhibit ATPase? Does OLT1177 bind to a component of NLRP3, such as the NACHT domain? Does OLT1177 inhibit the phosphorylation of a critical kinase that is triggers activation of the oligomerization?

Lay Summary. Several NLRP3 inhibitors are in the development stage for use in humans to treat IL-1β-mediated diseases. OLT1177 is the most advanced since this small, orally active synthetic compound has been shown safe in humans during a Phase I trial. Knowing the mechanism of action of OLT1177 is essential to the clinical development for use in humans with IL-1β-mediated inflammatory disease. Demonstration of efficacy of OLT1177 would be substantial advance in medicine, as it would replace the present use of parenteral therapies such as anakinra, canakinumab, rilonacept.

Grant Title: "Effects of an antibody to the IL-1 Receptor 3 (IL-1R3)".
Prinicple Investigator: Jesper Hoejen, MD
Aarhus University, Aarhus, Denmark

Scientific Summary. IL-1R3 is the co receptor for IL-1α, IL-1β, IL-33 and IL-36α, IL-36β and IL-36γ. The studies examine in vitro and in vivo models of inflammation and the ability of IL-1R3 blockade to reduce the severity of the disease models. Initial studies will examine the in vitro properties of a monoclonal antibody to human IL-1R3 as well as the in vivo model of gout in mice using specific anti-mouse IL-1R3 monoclonal antibody. In these studies, comparisons will be made with anakinra. For example, in human PBMC stimulated with heat-killed *Candida albicans* the production of IL-17 will be measured and the reduction in IL-17 by co-incubation with anakinra compared to the reduction in IL-17 by anti-human IL-1R3 determined. In the mouse model of monosodium urate (MSU) crystal-induced inflammation, a comparison will be made between a reduction in inflammation by anakinra and anti-mouse IL-1R3.

Lay Summary. Antibodies that target specific cytokines and specific receptors have been used to treat a broad spectrum of diseases from inflammation to cancer. In the case of monoclonal anti-human IL-1R3, there is a unique target since blocking this receptor has the potential to treat several diseases mediated by IL-1α, IL-1β, IL-33 and IL-36α, IL-36β and IL-36γ. The studies in mice will examine whether inhibition of the IL-1R3 cytokines is more effective than anakinra, which inhibits only IL-1α and IL-1β. These studies are the first to examine blocking IL-1R3 and as such are the first pre-clinical studies.

Grant Title: "Effect of anti-mouse IL-1R3 for the treatment of psoriasis."
Principle Investigator: Marie Louise Christensen, B.S., M.S.
Co-Investigator, Jesper Hoejen, MD
Arrhus University, Denmark

Scientific Summary. Application of the TLR7 ligand Imiquimod (IMQ) to the skin of mice has been shown to induce a psoriasis-like skin inflammation as well as systemic symptoms. The IMQ-induced psoriasis mouse model has many of the characteristics of psoriatic skin. The current treatment for humans with moderate to severe psoriasis is a monoclonal antibody that targets IL-17. Since anti-IL-1R3 also reduces IL-17, the goal of these studies is to treat mice with anti-mouse IL-1R3 and examine the effect on Imiquimod-induced disease in mice. The rationale for these studies is based on anti-IL-1R3 will reduce not only IL-1α and IL-1β but also IL-36. IL-36 also has a role in psoriasis. Persons with a mutation in the naturally occurring IL-36 Receptor antagonist develop a severe pustular psoriasis.

Lay Summary. New and safe methods are important for treating psoriasis. Although psoriasis is primarily a skin disease, psoriasis is a systemic disease. Antibodies to IL-1R3 have the promise to treat the skin manifestations of the disease but because IL-1R3 is also part of systemic inflammation, the promise of these studies is to provide the patient with greater reductions in the skin as well as total body inflammation.