

ProThera Biologics, Inc.:

A Novel Immunomodulator and Biomarker for Life-Threatening Diseases

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ABSTRACT

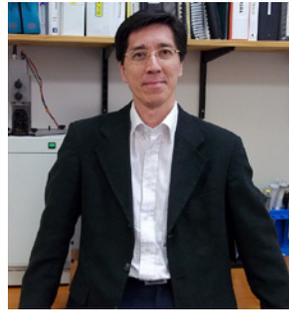
ProThera Biologics is a development stage bio-therapeutics company in East Providence, Rhode Island. The company was founded in 2002 to focus on the critical role and commercial potential of Inter-alpha Inhibitor Proteins (IAIP) for treating acute life-threatening inflammatory diseases. The discovery research originated in the basic research laboratories of the co-founders, Yow-Pin Lim, MD, PhD, and Douglas C. Hixson, PhD, at Rhode Island Hospital, a Lifespan partner. The company is backed by the Slater Technology Fund and has received research grants from the Rhode Island State Science and Technology Council (RI STAC) as well as continuous funding from the National Institutes of Health (NIH), with several Phase I and II Small Business Innovation Research (SBIR) grants over the past 10 years.

ProThera has developed a novel process to purify Inter-alpha Inhibitor Proteins from source material, and has conducted groundbreaking research into the usage of IAIP to fight systemic inflammation.

KEYWORDS: inter-alpha inhibitor proteins, sepsis, septic shock, anthrax

INTER-ALPHA INHIBITOR PROTEINS: ENDOGENOUS PROTECTIVE MOLECULES

Sepsis and Systemic Inflammatory Response Syndrome (SIRS) both refer to a severe physiochemical reaction that may occur following exposure to an infectious agent (e.g. bacterial, viral, fungal) or injury such as burn, or trauma. This systemic hyper-reaction typically includes an excessive production of cytokines, known as a "cytokine storm," and destructive proteases, with disturbances in metabolic, oxygenation, coagulation, and vascular functions leading to multi-organ dysfunction.¹ Consistent findings in the complex pathophysiology of systemic inflammation/sepsis has shown that SIRS activates excess systemic protease activity, neutrophil proteases, proteases of the complement and coagulation systems and matrix metalloproteinases.² An array of endogenous protease inhibitors has evolved to prevent excess activation of proteases and to limit the potential injurious actions of protease activation on endothelial and epithelial tissues. Many of these protease inhibitors, such



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as antithrombin, alpha-1 antitrypsin, C1 esterase inhibitor, tissue inhibitor of metalloproteinases, and secretory leukocyte protease inhibitors are rapidly consumed in acute inflammatory states, often leading to a failure to appropriately regulate protease activity.

One endogenous plasma protease inhibitor family that has recently received increased attention is Inter-alpha Inhibitor Proteins (IAIP). IAIP are a family of trypsin-type protease inhibitors composed of a unique combination of polypeptide subunits (light and heavy chains) covalently linked by a chondroitin sulfate chain.³ Two major forms of IAIP are found circulating in human plasma:

Inter-alpha-Inhibitor (*IaI*), a larger molecule with 250 kDa composed of two heavy chains (H1 & H2) and a single light chain (L).

Pre-alpha-Inhibitor (*PaI*), a smaller molecule with 125 kDa composed of one heavy (H3) and one light chain (L).

The light chain (also termed 'bikunin'=*bi-kunitz*=inhibitor with two Kunitz domains) is known to have broad inhibitory activity against plasma serine proteases such as trypsin, elastase, plasmin, cathepsin G and furin.^{4,5} Upon cleavage, the light chain, or bikunin, is released from the IAIP complex and rapidly excreted in urine. The high level of IAIP normally circulating in plasma, coupled with the fact that persons lacking IAIP have never been reported, suggests that these proteins have an essential physiological role. Relevant in this regard are our previous studies demonstrating that plasma IAIP level in infants are independent of gestational age and that even premature infants born at 24 weeks have similar levels to those found in adults.⁶

Although the full extent of the physiological functions of IAIP remains to be established, numerous studies have implicated that IAIP, or the lack thereof, play a significant role in systemic inflammation. Findings from ProThera and other laboratories have shown that IAIP comprise a crucial component of the body's protective defenses critical to modulating host response to pathological insults.

Decreased IAIP levels in life-threatening diseases

In healthy individuals, the amount of circulating IAIP in blood is relatively high (between 300-600 mg/L). However, IAIP levels rapidly decrease during systemic inflammation and sepsis.⁷ In more severe cases, IAIP may be depleted up to 90% of the baseline value found in plasma of septic patients. To date, IAIP levels have been measured in over 2,000 patients serving as study participants in systemic inflammation following bacterial and viral infections.⁷⁻¹¹ The decreased levels of IAIP correlate strongly with the progression of the disease. As the diseases progress to more advanced and life-threatening levels, IAIP levels drop precipitously, suggesting IAIP's clinical utility as a prognostic and therapeutic (a diagnostic test that helps the clinician to make the right therapeutic decision for the right patient) marker in assisting clinicians in monitoring disease progression and making informed treatment decisions.

Moreover, we recently studied the receiver operating characteristics of IAIP levels in 573 high-risk infants with suspected sepsis and demonstrated that IAIP level is a reliable biomarker with high sensitivity and specificity (89.5% and 99%) and a high positive and negative predictive value (85% and 98%) for neonatal sepsis.⁸ Studies have also demonstrated that the IAIP level is significantly reduced in neonates with necrotizing enterocolitis (NEC), a devastating acute inflammatory condition of the gastrointestinal tract commonly found in premature infants with birth weights of less than 1500 grams. IAIP blood levels in infants with the confirmed NEC (Bell stage 2 and 3) was found significantly lower compared to the matched age control infants with non-specific gastrointestinal disorders.¹¹

Thus, IAIP have proven to be a reliable diagnostic marker for neonatal sepsis and systemic inflammatory conditions like NEC. Since these acute conditions present a very serious threat to neonates, there is an urgent need to obtain confirmation as soon as possible. ProThera is currently conducting an NIH-funded project to develop a rapid point-of-care IAIP test that can be used to identify sepsis and necrotizing enterocolitis in high-risk infants with a simple, user-friendly and portable device suitable for use in the NICU setting.

BENEFICIAL EFFECTS OF IAIP THERAPY IN SYSTEMIC INFLAMMATION

As IAIP are found abundantly in human plasma and can be extracted and purified in high yield by a scalable production process similar to other blood derived products such as albumin and immunoglobulin (IVIg), a replacement IAIP therapy to reverse the decrease in systemic levels in pathological conditions is feasible.

Using a rat model of polymicrobial sepsis (Cecal Ligation and Puncture), we demonstrated dramatic improvements following treatment with human plasma derived IAIP administered 1 hour after sepsis induction as evidenced by recovery of hemodynamic stability, decreased organ inju-

ry, increased survival (2-3 fold) and arrested progression of sepsis.¹² Similar beneficial effects were achieved even if the IAIP administration was delayed 10 hours after the sepsis challenge, although at this point, the disease had progressed to a severe stage with at least one organ dysfunction.¹³

Similarly, studies in the neonatal model of endotoxin-induced systemic inflammation revealed that administration of IAIP significantly improves survival of the experimental animals.¹⁴ The therapeutic effects of IAIP were also demonstrated in live organism models of sepsis using *E. coli* and Group B hemolytic *Streptococcus* (GBS), two of the most relevant pathogens in sepsis in human infants. Moreover, IAIP was found to attenuate the marked increase in pro-inflammatory cytokine TNF-alpha and to augment anti-inflammatory cytokine IL-10 in the septic animals.¹⁴

These results suggest that administration of IAIP exerts potent immunomodulatory activity that leads to a significant beneficial effect in adult and neonatal sepsis models. At least three distinct mechanisms in the inflammatory pathways contribute to the protective effects of IAIP: 1) inhibition of the serine proteases (elastase, cathepsin G, and several others)⁴; 2) modulation of pro-inflammatory cytokines (TNF-alpha)^{12,14} and 3) blockage of excess complement activation (C5a).¹⁵

IAIP IN BIODEFENSE APPLICATIONS

Some scientific progress has been made in developing countermeasures for various biothreat pathogens in the post-9/11 era. However, it is becoming clear that the "one bug-one drug" approach such as vaccines or antiserum specific against a single agent is not a practical or sustainable approach.

The life-threatening consequences following exposure to biothreat pathogens do not typically arise directly from the causative agent, but from dysregulated host response leading to lethal systemic inflammation. As potent immunomodulators, IAIP can serve as a "first line of defense" in providing crucial protection against deadly acute systemic inflammation triggered by biothreat pathogens. In addition, as a broad-spectrum serine protease inhibitor, IAIP has a capability to block furin,⁵ a cell membrane-associated endogenous serine protease that plays a critical role in the anthrax pathogenesis and several viral diseases.¹⁶ As ProThera's IAIP therapy is independent from the causative agent (bacterial/viral or toxin), it can be applied immediately following exposure without risk of overdosing or misdiagnosis. Subsequently, once the causative agent is identified and confirmed, a more specific and targeted therapy with antibiotics or antiviral drugs to eliminate the pathogens can be initiated.

ProThera's current focus is targeted on Anthrax intoxication and infection, where the company has generated robust and promising data in the experimental animals,^{5,17} and is conducting confirmatory studies in large animals including non-human primates (baboons). As the biodefense focus has shifted from the "single agent specific" countermeasures

to more “universal” defenses that address multiple pathogens, IAIP not only can potentially serve as an effective broad-spectrum therapy against biothreat pathogens (CDC Category A, B and C) but also against naturally emerging pathogens. To this end, early investigations have also indicated encouraging protective effects of IAIP in viral diseases (Influenza A and Dengue virus infection).

IAIP IN OTHER ACUTE INFLAMMATORY DISEASES

While ProThera is mainly focusing on the development of IAIP as novel therapeutic proteins in systemic inflammation/sepsis and Anthrax biodefense, recent investigations of IAIP effects in hypoxic and ischemic brain injury have revealed exciting results as well. In collaborations with Barbara Stonestreet, MD, at Women & Infants Hospital and Steve Threlkeld, PhD, at Rhode Island College, ProThera has been able to demonstrate the beneficial effects of IAIP in hypoxic and ischemic brain injury models in fetal sheep and neonatal/adult rats.

IAIP have been detected in neurons, astrocytes, and meningeal cells of the brain and, may function as endogenous neuroprotective molecules.¹⁸ Moreover, studies have observed significant decreases of IAIP in brain tissues of experimental animals following ischemia-reperfusion injury. IAIP might serve as a novel agent to prevent/attenuate brain damage in infants at risk for mental/developmental disorders such as cerebral palsy and in adults following acute ischemic stroke.

SUMMARY

ProThera Biologics develops novel products that are based on its proprietary technology to produce and treat using Inter-alpha Inhibitor Proteins. The key to ProThera’s strategy is a single biological product to be developed for the treatment of widespread pathological conditions caused by dysregulated inflammatory response such as sepsis/septic shock, Anthrax infection/intoxication, necrotizing enterocolitis and acute ischemic stroke. ProThera offers a rational targeted solution to treat deadly diseases by combining both the predictive test and the effective replacement therapy of natural occurring Inter-alpha inhibitors. The successful development of an efficient and scalable manufacturing process combined with the implementation of viral inactivation steps will ensure a safe and effective IAIP product for testing in humans.

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