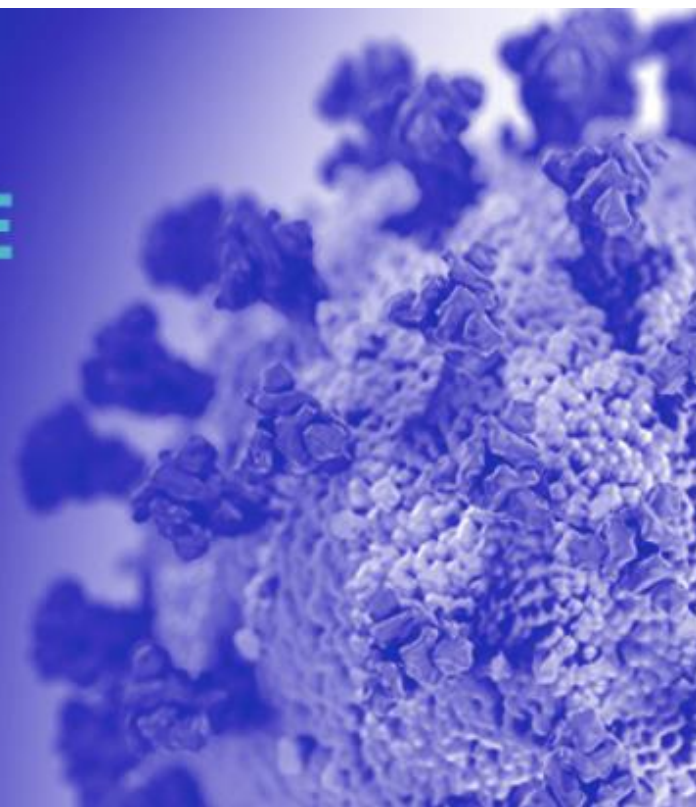# BSG

BIOTECH SUPPORT GROUP
Sample Prep that Matters

INVESTIGATE THE IMMUNE RESPONSE TO COVID-19 WITH OUR PRODUCTS

Re-imagining proteomics for developing precision medicine biomarkers of the innate immune response in SARS-CoV-2
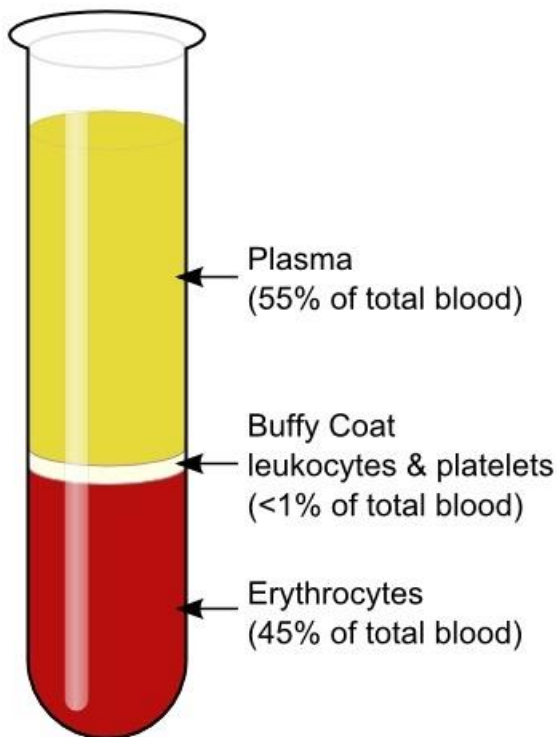
First published February 22, 2021

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WHO WE ARE

Located near Princeton, NJ, we supply research products used in proteomic analysis. Through their evaluation, we develop methods and biomarkers *central to understanding how individuals are uniquely predisposed to chronic inflammatory disease – especially cancer; how individuals uniquely adapt to the presence of infectious or non-infectious inflammatory conditions anywhere in the body; and how individuals uniquely respond to medical intervention.*

Plasma
(55% of total blood)

Buffy Coat
leukocytes & platelets
(<1% of total blood)

Erythrocytes
(45% of total blood)

>Plasma/serum proteins and other circulating factors directly regulate complex processes such as aging, the development of chronic diseases, and severe acute disease (i.e., SARS).

>Activated leukocytes and platelets release granulocytic cargo proteins in response to local inflammatory stimuli, generating a protease storm, that if not resolved alters steady state homeostasis contributing to both acute and chronic disease. The protease storm and its regulation can be monitored using our products and methods, **described in detail in this whitepaper.**

>Erythrocytes carry more than just oxygen and are now under investigations for many chronic inflammatory conditions including Malaria and Parkinson's Disease.

BSG's products and methods can help proteomic investigators explore all these blood compartments.

Albumin & IgG Removal Kits

Unique surface chemistries
depletes Albumin 90-95%
Species agnostic
Retains function and bio-activity

Hemoglobin Removal Kits

Unique surface chemistries
depletes Hemoglobin 90-95%
Species agnostic
Retains function and bio-activity

Lipid Removal & Clarification

Extensively cited
Replaces hazardous hydrocarbons
Diverse samples

Immune Response
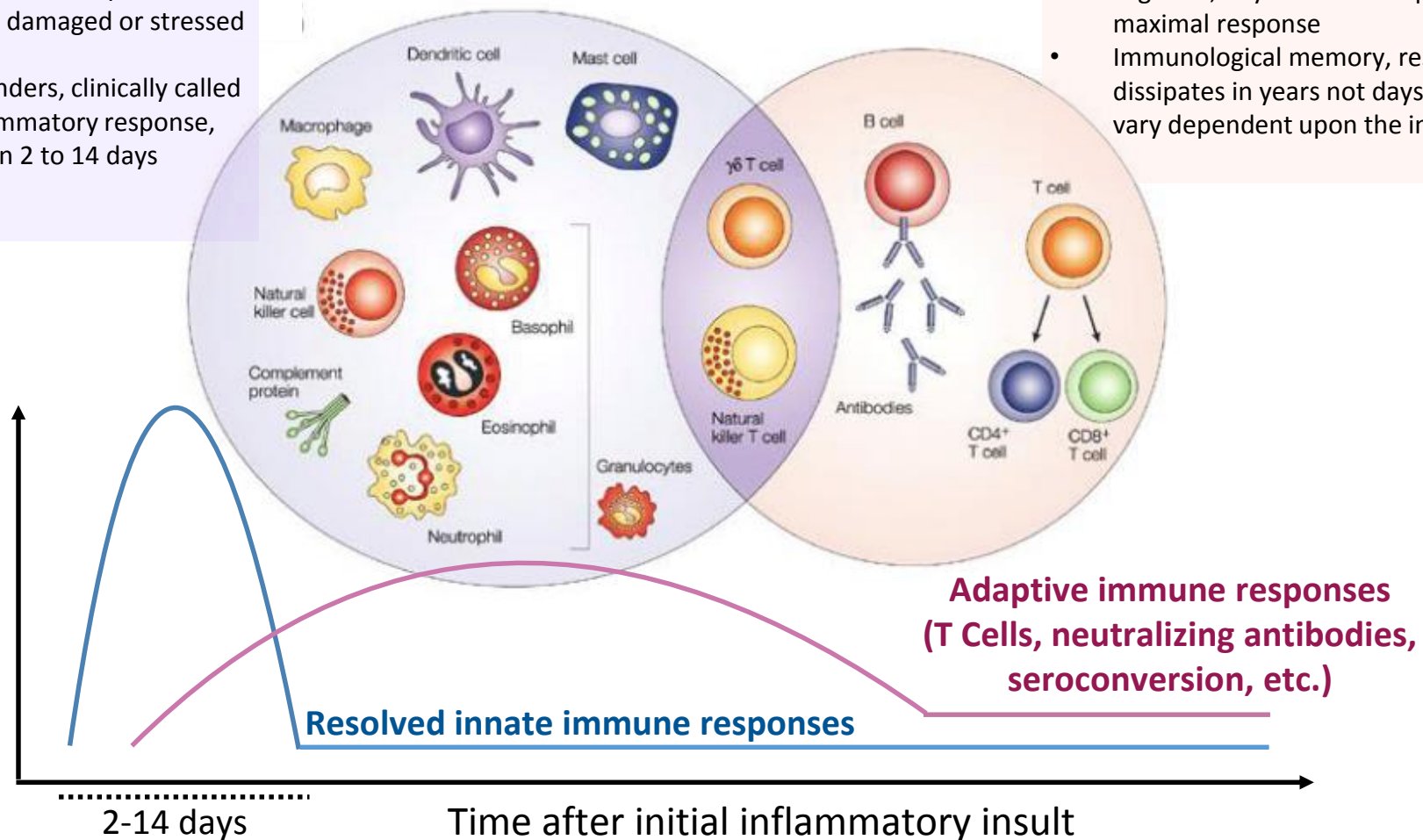# Innate vs. Adaptive & the Unmet Need
for Proteomic Information

"The innate and adaptive immune system work in concert to respond to this virus, and when this rhythm is disrupted, it can lead to unhampered viral spread and hyperinflammation...There is an unmet need for models that allow for collection of spatiotemporal information on how the virus spreads and how the body reacts to it." Cel. Mol. Bioeng. 13, 259–284 (2020).

Innate
- Non-Specific alarm system to pathogens, damaged or stressed cells
- First responders, clinically called acute inflammatory response, dissipates in 2 to 14 days

Adaptive
- Lag time, days between exposure and maximal response
- Immunological memory, response dissipates in years not days, and can vary dependent upon the initial insult
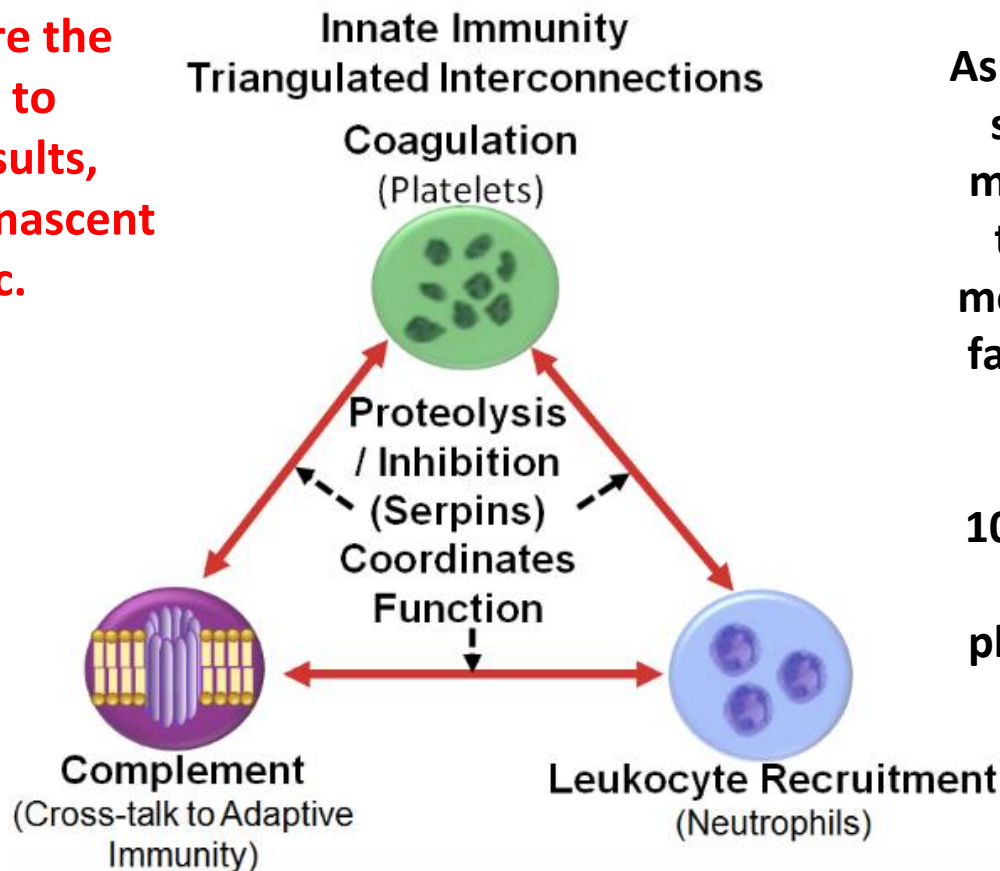

Adaptive immune responses (T Cells, neutralizing antibodies, seroconversion, etc.)

Resolved innate immune responses

2-14 days

Time after initial inflammatory insult

Unleashing the Power of Proteomics To Better Understand the Innate Immune Response to Infectious and Non-infectious Inflammatory Stimuli

"We identified molecular changes in the sera of COVID-19 patients compared to other groups implicating dysregulation of..., platelet degranulation and complement system pathways". Cell 2020.

"Significant evidence indicates that a dysregulated innate immune response contributes to the clinical presentation of patients with severe COVID-19 infections." J Exp Med (2020) 217 (6): e20200678.

Innate Immunity - a triangulated interconnected system of : Coagulation, Complement and Leukocyte pathways; regulated through a complex serine protease system

These pathways are the first responders to environmental insults, wounds, infections, nascent neoplasms, etc.
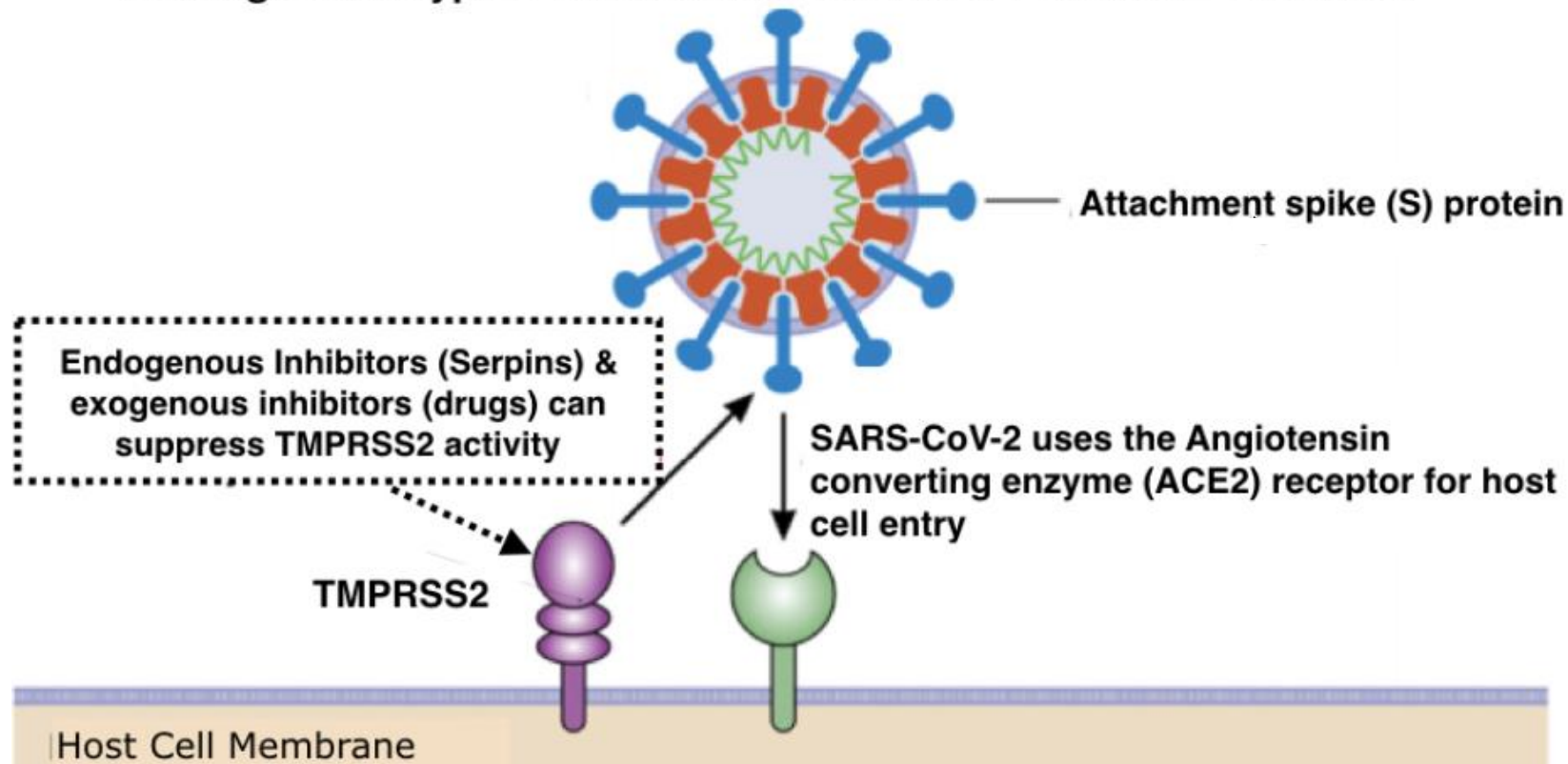

Innate Immunity
Triangulated Interconnections

Coagulation
(Platelets)

Proteolysis / Inhibition (Serpins) Coordinates Function

Complement
(Cross-talk to Adaptive Immunity)

Leukocyte Recruitment
(Neutrophils)

As proteolysis is irreversible, all species of life have evolved molecular regulatory systems to control aberrancies. The most distinguished is a protein family of regulators known as SERPINs.

10 inhibitory SERPINs account for 5-10% protein mass in plasma, and provide a central control function for innate immunity

Transmembrane serine proteases also play a major role in SARS-CoV-2 host infectivity

"Our results demonstrate that endogenous lung- and plasma-derived α1-antitrypsin (Serpin A1) inhibits SARS-CoV-2 infection in cell lines and fully differentiated airway epithelium cultures. …α1-AT may inhibit TMPRSS2-mediated priming of the Spike protein, thereby preventing engagement of the ACE2 receptor and subsequent fusion." bioRxiv preprint entitled *Alpha-1 antitrypsin inhibits SARS-CoV-2 infection*

The spike (S) protein of SARS-CoV-2 is activated or primed by proteolytic cleavage from Type II Transmembrane Serine Protease - TMPRSS2
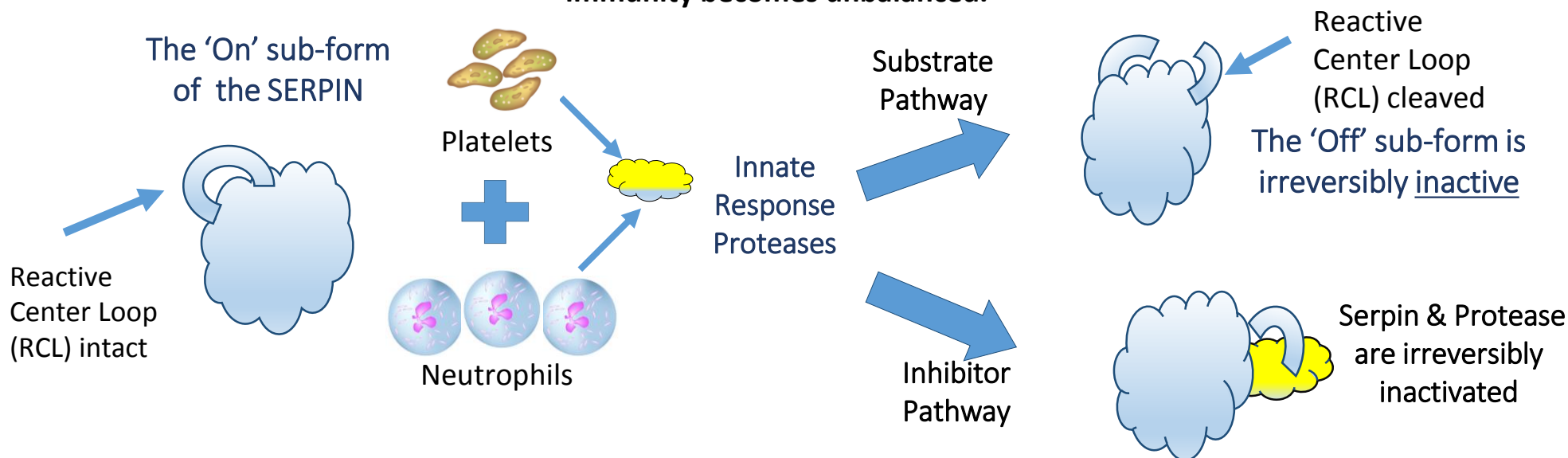
Attachment spike (S) protein

Endogenous Inhibitors (Serpins) & exogenous inhibitors (drugs) can suppress TMPRSS2 activity

SARS-CoV-2 uses the Angiotensin converting enzyme (ACE2) receptor for host cell entry

TMPRSS2

Host Cell Membrane

Clinical trials have started for Camostat mesilate, an orally administered serine protease inhibitor to suppress TMPRSS2 activity.

Why this protease imbalance matters!

"...an array of immune response-virus interactions and consequent pathologies exist. Unraveling such interactions occurring in COVID-19 will be a crucial challenge to better address more effective therapeutic strategies. First, we do not have information on the innate responses to SARS-CoV-2. Delayed or weak antibody responses associate with severe outcomes." Journal of Allergy and Clinical Immunology 146.1 (2020): 18-22.

By monitoring SERPIN sub-forms, it will be possible to characterize and monitor the innate immune response to all chronic diseases as well as those predisposed to severe infections!

- **90% of metastatic patients exhibit coagulation abnormalities**
- **Coagulation and Complement are interconnected cascades functionally activated by proteolysis**
- **Functionally active Serpins maintain proteolytic homeostasis providing central control to innate immunity**
- **Progressive loss of functionally active Serpins results in dysregulation of innate immunity pathways, and subsequent unresolved proteolysis can lead to severe acute disease, example - thrombosis in Covid-19**

Inflammatory Proteases

ACTIVE Serpin Protease Inhibitors
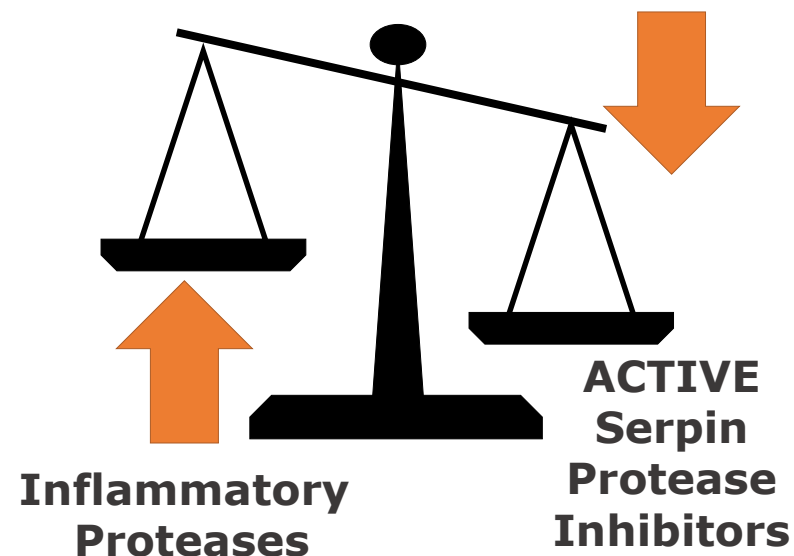
Until now, it has <u>not been possible</u> to measure the relative functional abundancies of Serpins for differential comparison proteomic analysis. Our new proteomic counting method of SERPIN function can now report the balance between 'On' and 'Off' sub-forms, and consequent dysregulation of proteolysis

New insights are gained for therapeutic modulation and precision medicine

The tug-of-war within the innate response

"Overall, we need to look closer at the role of the complement system, the recruited innate immune cells and their combined role in pathogenesis, viral clearance and the eventual resolution of the infection. *Frontiers in immunology* 11 (2020): 1979.

Activation

Complement

Regulation

Platelets
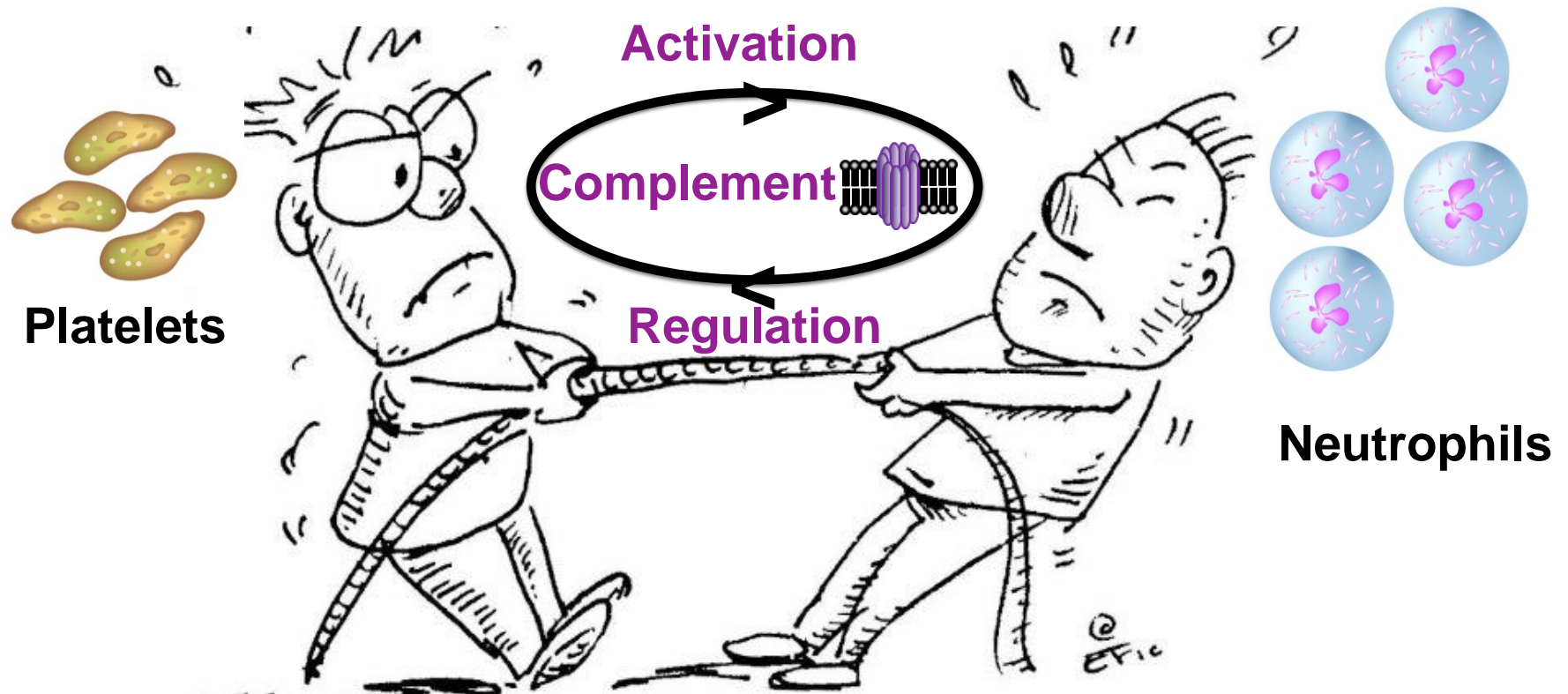
Neutrophils

Many investigations report Complement-mediated interactions between Neutrophils and Platelets, suggesting a tug-of-war between platelets and neutrophils for the localized control and regulation of Complement activation. Any dysfunction in this regulation can lead to a lag or dysfunction in the adaptive (i.e., T Cell, seroconversion) response.

Measurements of SERPIN function can help stratify high risk patient populations

"The diagnostic criteria for hyperinflammation are incompletely defined, especially in the context of COVID-19. Associations between elevated inflammatory markers, escalation of respiratory support, and survival in people with COVID-19 indicate the existence of a high-risk inflammatory phenotype."
COVID-19-associated hyperinflammation and escalation of patient care: a retrospective longitudinal cohort study. The Lancet Rheumatology 2.10 (2020): e594-e602.

Activation
Complement
Regulation
Platelets   Neutrophils

Adaptive immune responses
(T Cells, neutralizing antibodies, seroconversion, etc.)

Resolved innate immune responses

Normal/Healthy Serpin proteomic profile
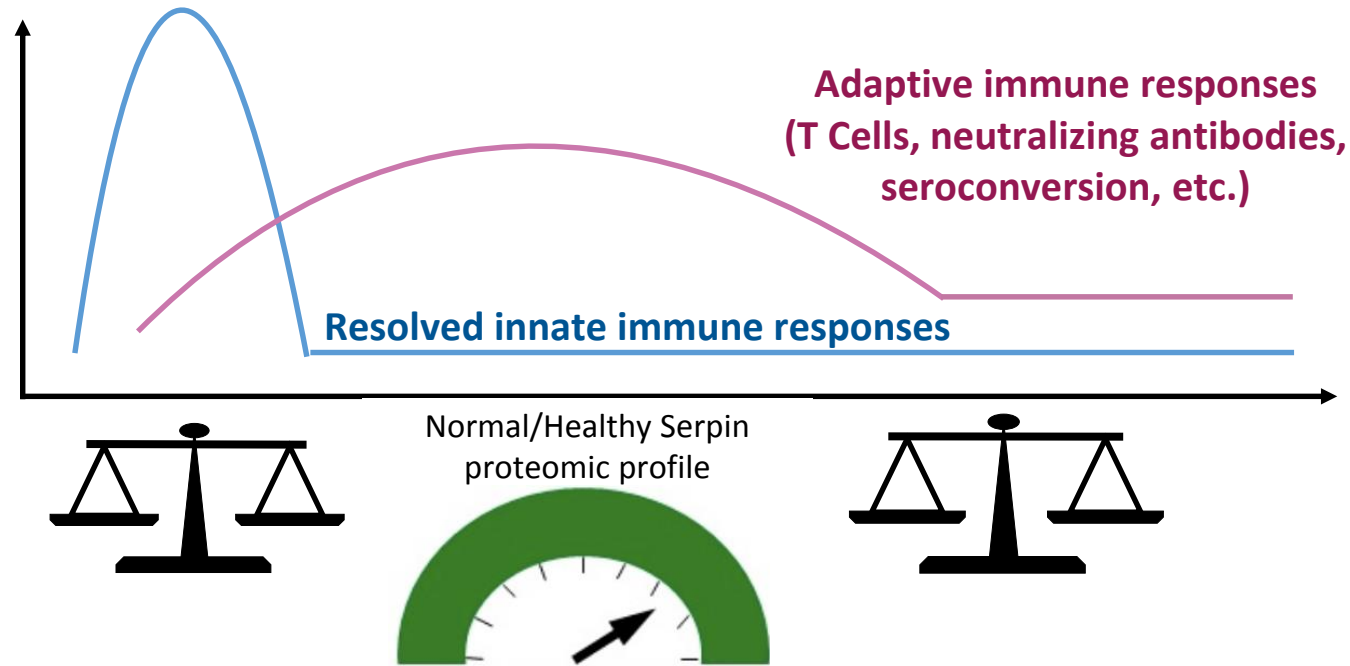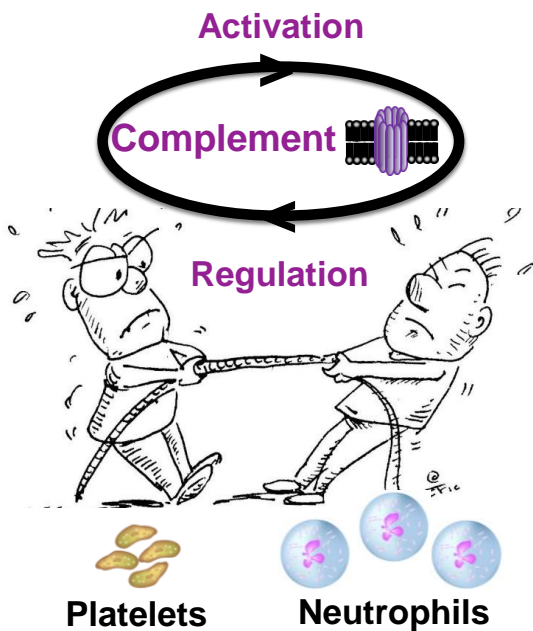
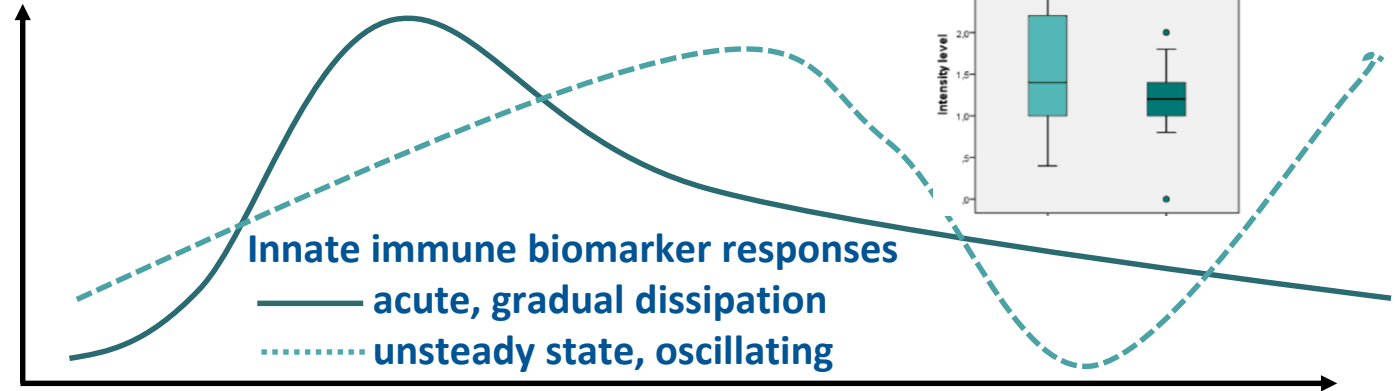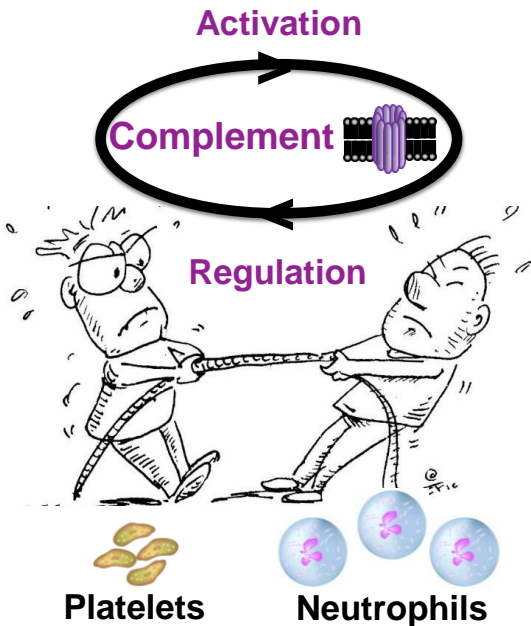Reservoir of ACTIVE Serpins is sufficient for short term regulation

In a normal/healthy population, a balanced resolution of the innate response leads to a productive handoff to the adaptive response. With a sufficient reservoir of Serpin activity, the protease storm upon viral exposure is regulated, and remains balanced throughout the course of disease. Such pre-disposition would indicate a low-risk sub-population.

Measurements of SERPIN function can help stratify high risk patient populations

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"In a retrospective observational study of 11,116 patients… with suspected SARS-CoV-2, we found that history of macular degeneration (a proxy for complement activation disorders) and history of coagulation disorders… are risk factors for morbidity and mortality in SARS-CoV-2-infected patients…these data suggest that hyperactive complement and coagulative states predispose individuals to adverse outcomes associated with SARS-CoV-2 infection…" Nature Medicine (Aug. 2020).

Activation

Complement

Regulation

Platelets   **Neutrophils**

Innate immune biomarker responses
—— acute, gradual dissipation
······· unsteady state, oscillating

High risk Serpin proteomic profile

Reservoir of ACTIVE Serpins is sufficient for short term regulation

Reservoir of ACTIVE Serpins is tenuous for regulation of protease storm

However, such textbook resolution is not always the case. Patient sub-populations with risk factors supporting chronic exhaustion of Serpin activities, may influence the response. These may come from heredity, lifestyle or other underlying inflammatory conditions. In such cases, the adaptive response can be delayed or dysfunctional and these patients are at higher risk for severe disease.

Measurements of SERPIN function can help stratify high risk patient populations

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"… studies suggest that at least a subset of severe COVID-19 infection involves a catastrophic, complement-mediated thrombotic microvascular injury syndrome with sustained activation… of complement." Translational Research 2020.

Activation

Complement

Regulation

Platelets   Neutrophils

Innate immune biomarker responses
— acute, gradual dissipation
········· unsteady state, oscillating

Reservoir of ACTIVE Serpins is tenuous for regulation of protease storm

Severe disease Serpin proteomic profile

Reservoir of ACTIVE Serpins is insufficient for regulation of protease storm
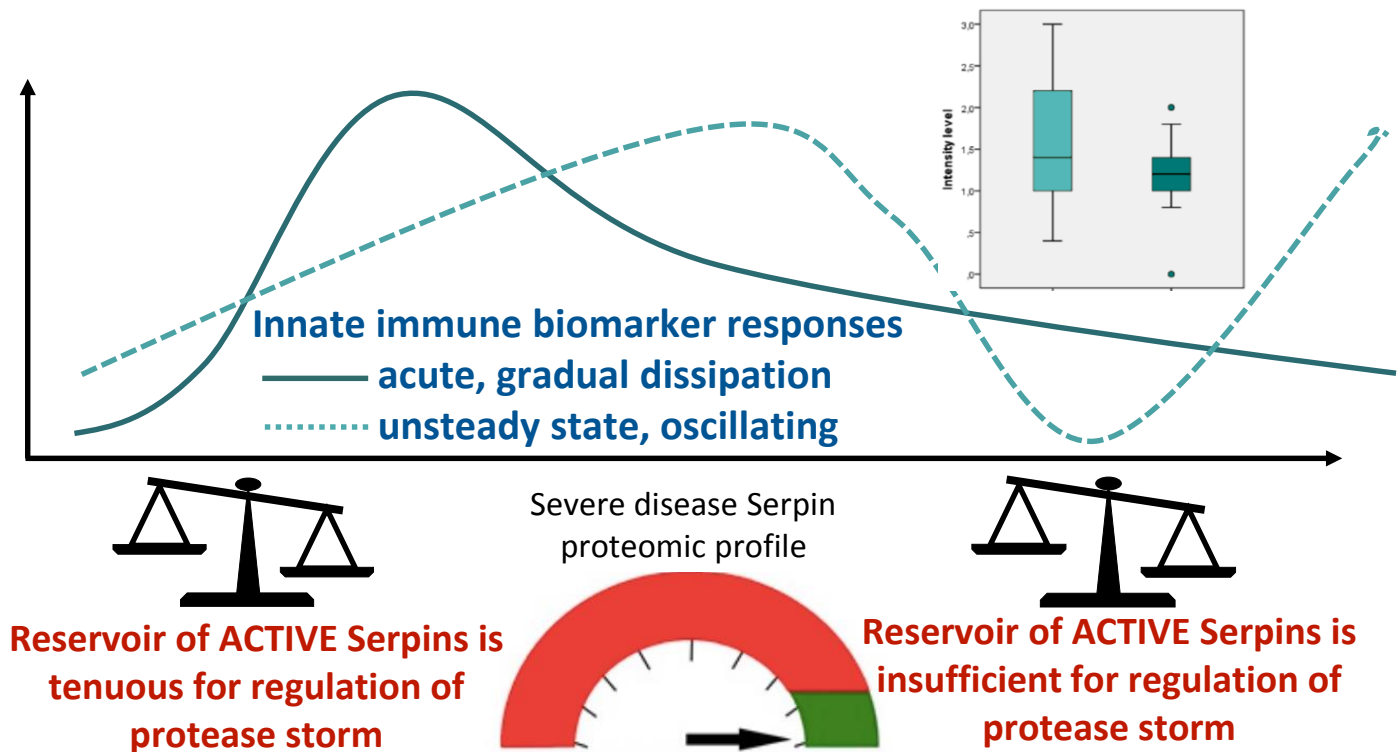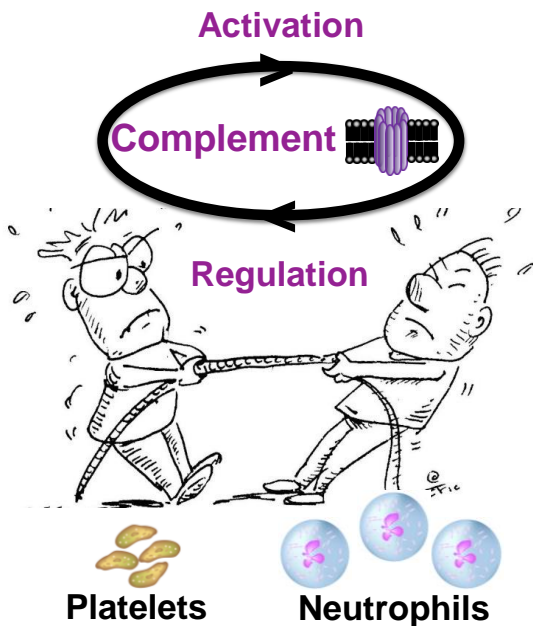
When there is <u>insufficient</u> reservoir of Serpin activities present at time of exposure, the adaptive response may be paralyzed, and severe disease can be anticipated. Because of the range in scope and kinetics of innate responses in this tug-of-war scenario, inflammatory biomarkers may oscillate over time.

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"Inflammatory Biomarkers Distinguish Mild from Progressive COVID-19. CRP Levels Correlate with Physiological Measures of Disease Severity and Respiratory Failure". *Inflammatory biomarker trends predict respiratory decline in COVID-19 patients.* Cell Reports Medicine 1.8 (2020): 100144.

"Although limited by its observational nature…, our findings suggest that systemic anticoagulation may be associated with improved outcomes among patients hospitalized with COVID-19." Journal of the American College of Cardiology.

Oscillations of Complement activity are observed clinically, especially in more severe cases.

C-Reactive Protein (CRP) is an acute-phase plasma protein that is used clinically as a biomarker for activation of Complement and the innate immune response.

Understanding and characterizing the innate immune response to viral infections will help provide insight into:
- why some individuals are pre-disposed to more severe complications than others,
- how immune response may be modulated with selection and timing of existing treatments (anticoagulant, anti-inflammatory),
- guidance and strategies for new drugs and vaccines.
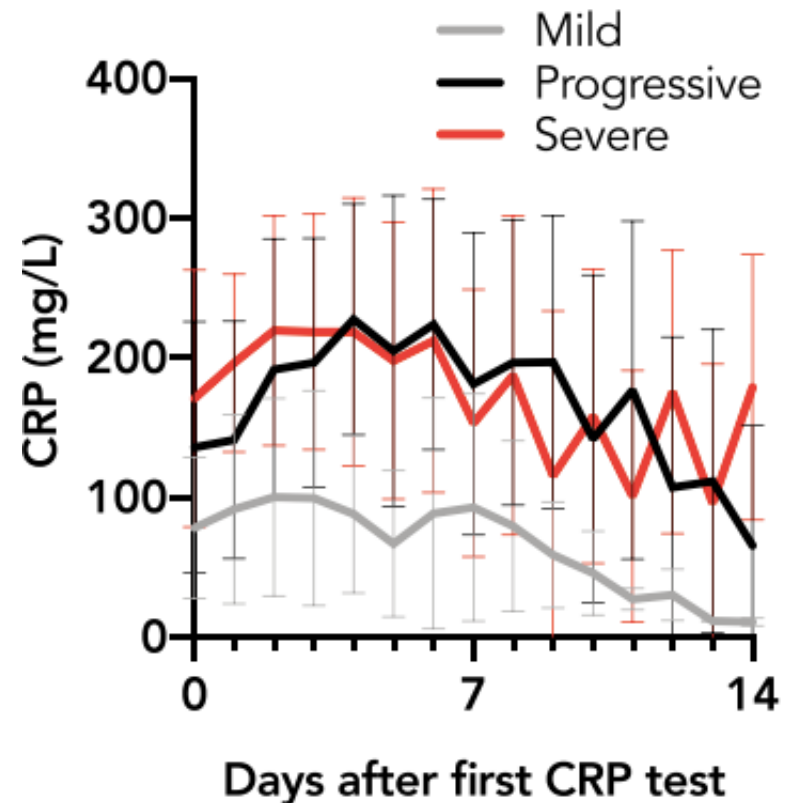
From: *Inflammatory biomarker trends predict respiratory decline in COVID-19 patients.* Cell Reports Medicine 1.8 (2020): 100144.

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"Persistent inflammation is perhaps a common denominator in the genesis of nearly all age-associated health problems or cancer. Future challenging opportunities for diagnosis, prevention, and/or therapy of chronic illnesses will require an integrated understanding and identification of developmental phases of inflammation-induced immune dysfunction". Cell Biochem Biophys. 2009;55(2):55-79.

"The cellular and molecular profiles of immune activation in chronic inflammatory disorders overlap considerably with those patterns observed in effective, self-limited host responses to microbial pathogens and involve similar initiating mechanisms. *Cell* 124.4 (2006): 823-835.

Similarly, CRP/Complement oscillations are observed in cancer patients

From: Coventry, Brendon J., et al. "CRP identifies homeostatic immune oscillations in cancer patients: a potential treatment targeting tool?." Journal of translational medicine 7.1 (2009): 1-8.

"We have used frequent serial L-CRP across three clinical laboratories in two countries and for different advanced cancers, and have demonstrated similar, repeatable observations of cyclical variation in CRP levels in these patients. We hypothesise that these L-CRP oscillations are part of a homeostatic immune response to advanced malignancy and have some preliminary data linking the timing of therapy to treatment success."
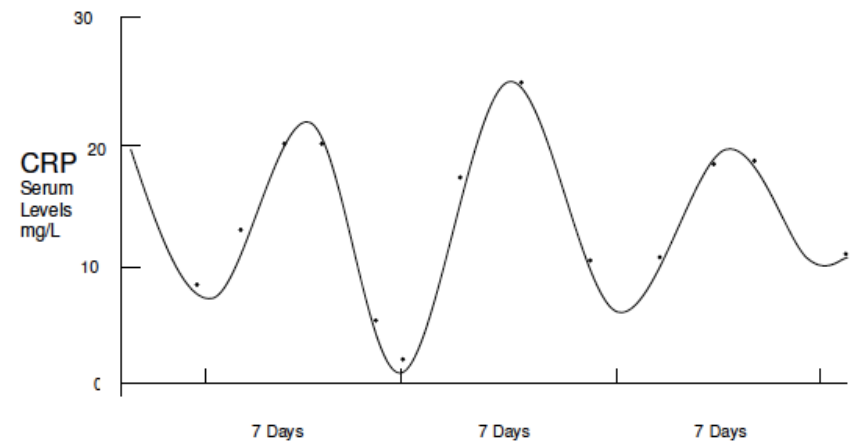
Figure I
CRP cycle in a patient with advanced melanoma. Representative oscillation in L-CRP serum levels (y-axis; 0-30 mg/L) vs time in days (x-axis; bars show 7 days duration) in a patient with advanced melanoma, as also observed in other patients with advanced melanoma (Adelaide). From the serial CRP data-points a 'standard CRP curve' was mathematically derived.

Here's how BSG can help!

BIOTECH SUPPORT GROUP
Sample Prep that Matters

Most proteins from the innate immunity proteome are highly observable in blood even with minimal LC-MS/MS acquisition time (= or < 1 hour). Also, most are in the sweet spot (>1 µg/ml) for targeted quantitative LC-MS/MS analysis, especially after enrichment with BSG's products and methods.

Proteomic productivity is not measured by Venn diagrams, but rather by quantitative differences between proteins in samples representing a challenge or disease state, vs. samples representing a normal or control state. However, while technical variance in LC-MS/MS analysis has improved, small biological variances remain hard to measure robustly.

Large variance proteins (>2x) therefore are the best choices for targeted analysis. So for targeted biomarker proteins, an optimal number of proteins in the 10-20 range should be more than adequate for innate immunity profiling. Aligned with that need, is also the need to measure protein concentrations across several logs in one analysis.

Here's how BSG can help!

In blood for example, the central Complement protein, C3 circulates at ≈1500 µg/ml, while Complement Factor D circulates at ≈3 µg/ml. To measure both in one analysis requires that the MS signal intensities at both ends of the spectrum be at least reasonably proportional to the real concentrations, which at times can be across > 4 log ion abundance signal. Thus low abundance proteins are foremost subject to signal to noise variance, making them barely detectable and often well beyond the range where signal intensity is proportional to concentration. The solution demands:

- Enrichment of biomarker proteins from low-abundance to mid-abundance to improve linearity between the measurable peptide ion signals and true protein abundances.

- BSG's enrichment products have proven to be robust, reproducible and quantitatively linear across >4x log of LC-MS/MS signal intensity data.

- Consumable products adaptable to high-throughput formats.

- BSG's On-bead (BASP™) digestion methods within AlbuVoid™ or HemoVoid™ simplified workflows, to help normalize digestion efficiencies for better protein quantification across multiple targets.

BSG has strived to address and service all these demands, to bring proteomics from discovery to the clinic.

Albumin & IgG Removal Kits

Unique surface chemistries
depletes Albumin 90-95%
Species agnostic
Retains function and bio-activity

Hemoglobin Removal Kits

Unique surface chemistries
depletes Hemoglobin 90-95%
Species agnostic
Retains function and bio-activity

Lipid Removal & Clarification

Extensively cited
Replaces hazardous hydrocarbons
Diverse samples

Here's how BSG can help!

BSG's products and methods can help proteomic investigators explore all blood compartments including all cellular components.

HemogloBind™ Cited in Comparison Proteomics Study of Covid-19 Patients in Peripheral Blood Derived Mononuclear Cells (PBMCs) – The Barometer of the Immune System

HemogloBind™ sample preparation technology provides deep and quantitative LC-MS proteome and phosphoproteome analysis. This study provides a comparison of SARS-CoV-2 positive ICU patients with age- and sex-matched SARS-CoV-2 negative ICU patients and healthy individuals, using peripheral blood derived mononuclear cells (PBMCs).

The citation is:

Kaneko, Tomonori, et al. "System-wide hematopoietic and immune signaling aberrations in COVID-19 revealed by deep proteome and phosphoproteome analysis." Research Square preprint (2021).

Erythrocytes do not have a nucleus, and being in continuous contact with complement proteins in plasma, have a different make-up of complement regulators than nucleated cells. Increased complement activation or decreased complement regulation may result in red cell dysfunction. This may explain some of the clinical symptoms seen in severe COVID-19 patients- low oxygen levels and neurological complications. Therefore, proteomic investigation of erythrocytes is warranted.

BSG's hemoglobin removal products have proven extremely efficient in coverage, detecting post-translational modifications, and quantifying the erythrocyte proteome. Click on our whitepaper to learn more.

Here's how BSG can help!

Patent Application Describes New Proteomic Methods to Monitor Protease Inhibitor Function During Covid-19 Infections

Notably, conventional immunoassays (i.e., ELISA) fail to report or represent in any way, the Serpin bifurcated functional pathways. However, without better measurements for Serpin functionality, unresolved protease activity and consequential predisposition to severe disease upon exposure to viral infection, cannot be properly evaluated.

The patent disclosure describes new methods of proteomic analysis that can monitor how the body's protease inhibitors are pre-disposed (or not), to suppress SARS-CoV-2 (Covid-19) viral entry. The US provisional patent application is entitled "METHODS TO MONITOR FUNCTIONAL SUBFORMS OF SERINE PROTEASE INHIBITORS FROM BIOFLUIDS DURING VIRAL INFECTIONS"; Inventors: M. Kuruc & D. Roy, filed on October 27, 2020.

With viral infections, there comes an increased level of serine protease activity, due in part to both an exuberant innate immune response to counter the infectious insult, and the added membrane protease - TTSP activity that derives from the viral load and propagation. As a result, current clinical practice has no ability to predict and monitor the protease storm triggered by SARS-CoV-2 (Covid-19) and similar infections, and with that, any possible severity, thrombosis and other complications.

The purpose and scope of this patent application is to: >develop clinically actionable blood (and other accessible biofluids) biomarkers for viral infections that have a cell entry mechanism which utilize Type II Transmembrane Serine Proteases (TTSPs) as priming components, through proteomic measurement of functional subforms of the major inhibitory proteins that regulate protease storms - the Serpins, and >utilize one or more proteomic patterns from these Serpin subforms as biomarkers for precision medicine to clinically manage viral infections.

The BSG Advantages

Cost Effective & Efficient

Sample prep methods essential for expanding proteomic biomarkers into routine healthcare

Consumable Research Products

Supporting the expanding installation of LC-MS instruments & computational infrastructure

Serves All Proteomic Analytical Platforms

Mass Spectrometry (LC-MS/MS, MALDI), Immunoassays, ELISAs, Western blots, 1 & 2 DE, Enzyme & Functional Assays

Species Agnostic

Not derived from immuno-affinity, all products work for all species

Knowledgebase of 1000+ Serum Proteins

Supports targeted & quantitative protein markers from serum/plasma

Other Resources:

- Poster presented at The Serpins2019 Conference, entitled "Loss of Functional Alpha-1-Antitrypsin and Heparin Cofactor II in Inflammation and Cancer". Authors were: Ingrid M. Verhamme, Vanderbilt University Medical Center; Swapan Roy, Sowmya Avadhani, Matthew Kuruc, Biotech Support Group LLC.

- Whitepaper entitled "Stroma Liquid Biopsy™ - Blood-based biomarkers to monitor stromal conditioning in cancer." Published February, 2019.

- Chapter from Book: Functional Proteomics – Methods and Protocols, publisher Springer 2018. "Methods to Monitor the Functional Subproteomes of SERPIN Protease Inhibitors".

Review Article: Open Access

New Strategies to Categorize Blood for Proteomic Biomarker Discovery

Matthew Kuruc[1*], Haiyan Zheng[2], Amenah Soherwardy[2], Sowmya Avadhani[1], Devjit Roy[4], Ingrid M Verhamme[3] and Swapan Roy[1]

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E-BOOK

Categorization of Blood Based Biomarkers

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