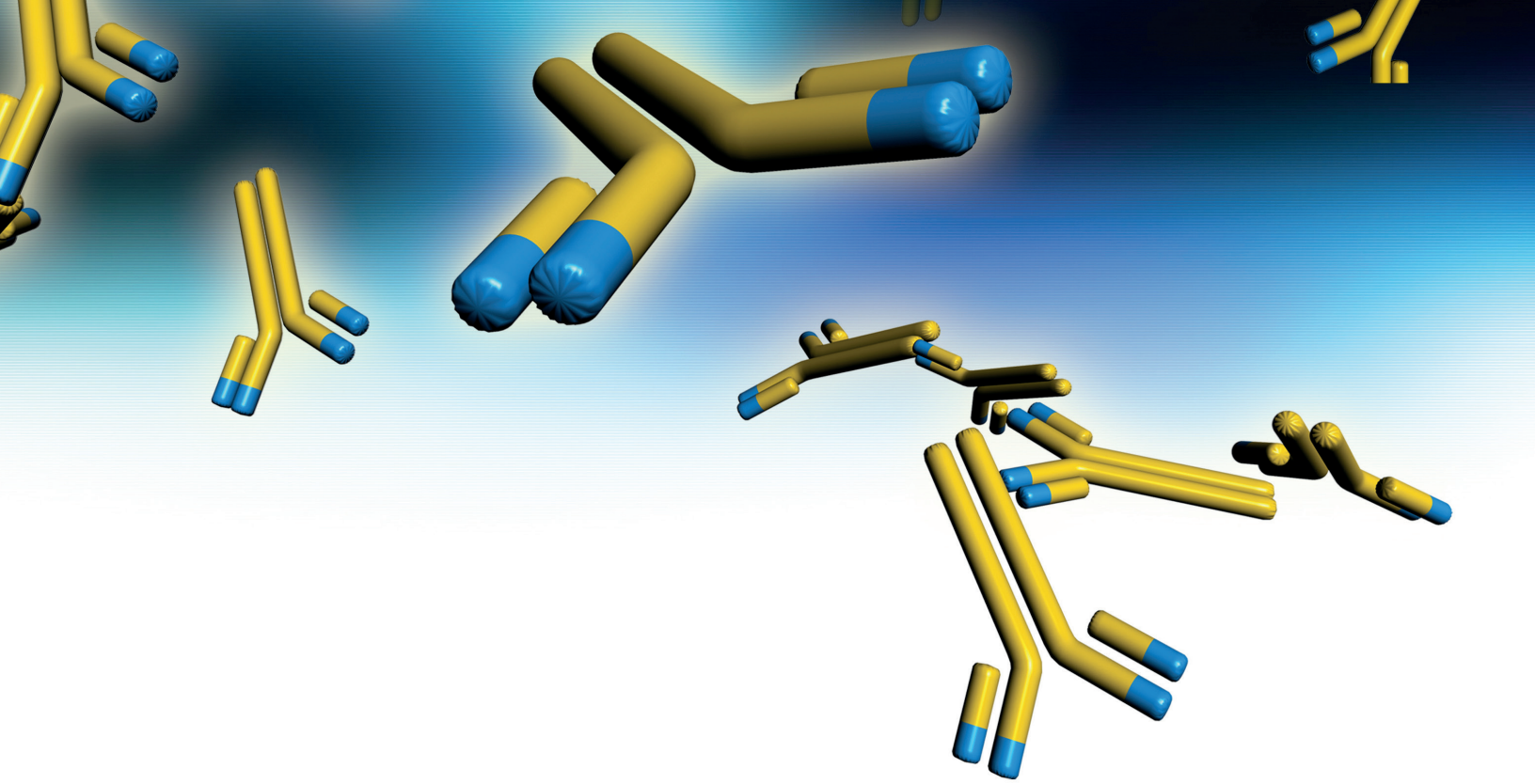

Inflammasomes: A Promising Therapeutic and Diagnostic Target

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What are inflammasomes?

Inflammasomes, discovered in 2002 by the late Dr. Jürg Tschopp and colleagues, are important signaling complexes that play a critical role in innate immunity, the body's first line of defense against foreign infectious organisms and cell damage. They also potentiate the adaptive immune response.

Inflammasomes are multiprotein complexes comprised of three basic proteins:

- Sensor molecule
- Adaptor ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain or CARD)
- Pro-caspase-1

In the presence of harmful pathogens or cell damage, the protein components listed above assemble into an inflammasome. Each of the inflammasome components has a unique role.

SENSOR MOLECULES

The role of the sensor molecule is to detect the presence of harmful pathogens or danger signals from dead or dying cells.

There are multiple sensor molecules, including the NOD-like receptors, NLRP1, NLRP2, NLRP3, NLRC4, the AIM2 receptor, and Pyrin, each of which respond to different pathogens or internal danger signals, and form a unique inflammasome. Each inflammasome is named by its associated sensor molecule as reflected in Figure 1.

PRO-CASPASE-1

Pro-caspase-1 is the inactive form of caspase-1. Caspase-1 converts inactive pro-IL-1β and pro-IL-18 to their active forms, triggering an immune response.

ADAPTOR ASC

Adaptor ASC is associated with each of the most common inflammasomes, as depicted in Figure 1. Its role is to recruit pro-caspase-1 into the inflammasome. ASC is involved in functioning of NLRP2, NLRP3, AIM2, and Pyrin inflammasomes, and it enhances the activity of NLRP1 and NLRC4 inflammasomes.

Common Inflammasomes

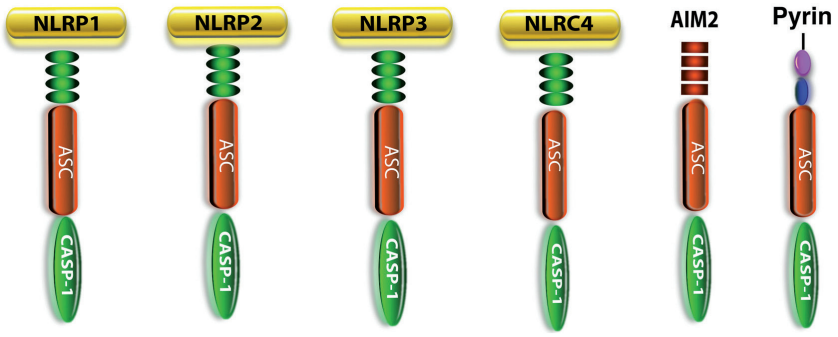


Figure 1

What is the role of inflammasomes in immunity?

Inflammasomes initiate an immune response to pathogens or internal danger signals. When triggered, an intracellular sensor molecule (e.g. NLRP3) recruits ASC, which in turn recruits pro-caspase-1 to form the associated inflammasome (Figure 2).

The inflammasome is the organizing center that recruits additional ASC to form a large filamentous signaling platform, known as an ASC Speck. ASC Specks provide a scaffold for pro-caspase-1 recruitment, which triggers conversion of pro-caspase-1 to active caspase-1, which in turn converts the cytokine pro-IL-1β to its active form, initiating the inflammatory response. ASC Specks are then released outside the cell to create a signaling platform that continues to activate pro-IL-1β, intensifying and propagating the inflammatory response (Figure 3).

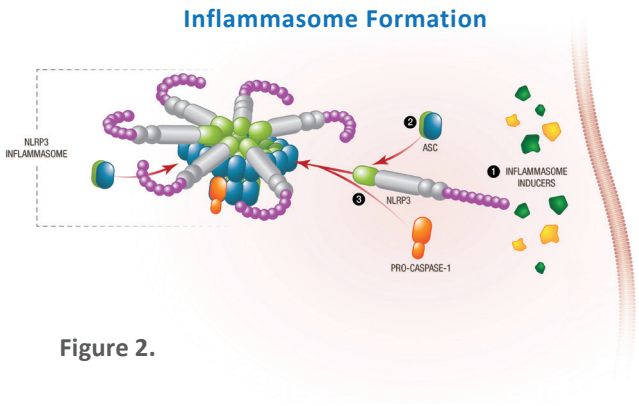


Figure 2.

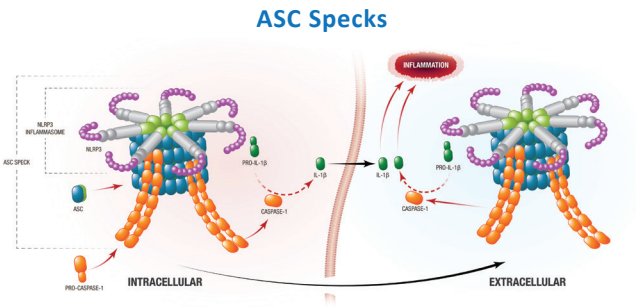


Figure 3.

What is the role of inflammasomes in inflammatory diseases?

Inflammasome-triggering of the innate immune response is essential to protect healthy people against pathogens. However, overactivation of inflammasomes can lead to chronic inflammation underlying a variety of inflammatory conditions and diseases, which include:

- Autoimmune diseases like multiple sclerosis, systemic lupus erythematosus, lupus nephritis, rheumatoid arthritis and colitis
- Metabolic diseases like diabetes, atherosclerosis, non-alcoholic fatty liver disease and gout
- Neurodegenerative diseases like Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis
- Injuries such as spinal cord injury, traumatic brain injury and stroke

Numerous inflammatory disorders are linked to more than one inflammasome

The NLRP3 inflammasome, which is activated by a variety of ligands, is the most studied inflammasome associated with inflammatory diseases. However, there is now evidence that more than one inflammasome is involved in the pathogenesis of certain inflammatory diseases, not just the NLPR3 inflammasome. For example, there is experimental evidence that the NLRP6, NLRC4 and AIM2 inflammasomes contribute to non-alcoholic steatohepatitis (NASH) pathology in addition to the NLRP3 inflammasome (Table 1). As such, inhibition of multiple inflammasomes is likely required to achieve a therapeutic effect.

DISEASE/CONDITION	INFLAMMASOMES IMPLICATED	REFERENCES
Multiple Sclerosis	AIM2, NLRP1, NLRP2, NLRP3, NLRC4	Huang et al., 2004; Soulika et al., 2009; Maver et al., 2017; Freeman et la., 2017; Noroozi et al, 2017; Soares JL et al, 2019
NASH	AIM2, NLRP3, NLRP6, NLRC4	Xiao and Tipoe, 2016; Mridha et al., 2017; Thomas, 2017; Ohashi et al, Hepatology, 2019
Lupus Nephritis	AIM2, NLRP3	Choubey and Panchanathan, Clin.Immun.176:42-48, 2017; Cytokine, 2019; Fu et al, Arthr. And Rheum., 2019
Diabetic Nephropathy	AIM2, NLRP3	Anders and Muruvue, 2011; Hutton et al., 2013
CNS Injury	AIM2, NLRP1, NLRP2, NLRP3	de Rivero Vaccari et al., 2008, 2009, 2012; Abulafia, 2009; Liu et al., 2013; Bartolotti et al., 2018
Alzheimer's Disease	AIM2, NLRP1, NLRP3	Ahmed et al., 2017; Venegas et al., 2017; White et al., 2017; Wu et al., 2017LeBlanc, 2018; Lang et al., 2018
Parkinson's Disease	NLRP1, NLRP3	Lenart et al., 2016; Mao et al., 2017; Sarkar et al., 2017; vonHerrmann et al., 2019
Rheumatoid Arthritis	AIM2, NLRP1, NLRP3, NLRP6	Goh et al, 2017; Grandemange et al., 2017; Li et al., 2019; Addobbatti et al., 2018; Lin and Luo, 2016; Sode et al., 2015; Wang et al., 2014
Inflammatory Bowel Disease	AIM2, NLRP1, NLRP3, NLRP6, NLRC4	Vanhove et al., 2015; Ratsimandresey et al., 2017; Lazaridis et al., 2017; Kanneganti et al., 2017; Normand et al., 2011; Levy et al., 2015; Seregin et al., 2017; Tye et al., 2018; Williams et al., 2018; Opipari and Franchi, 2015

Table 1.

IC 100, a novel approach for the treatment of inflammatory diseases

IC 100 is a biologic/monoclonal antibody. IC 100 binds to and inhibits the ASC protein, thus preventing assembly of the multiprotein inflammasome required for initiation of the inflammatory response (Figure 4).

IC 100 also binds to and inhibits ASC in ASC specks, both intracellulary and extracellularly. This inhibits propagation of the large filamentous signaling platform, preventing perpetuation of inflammation associated with chronic inflammatory diseases (Figure 5).

IC 100 inhibits inflammasome formation, blocking initiation of the immune response

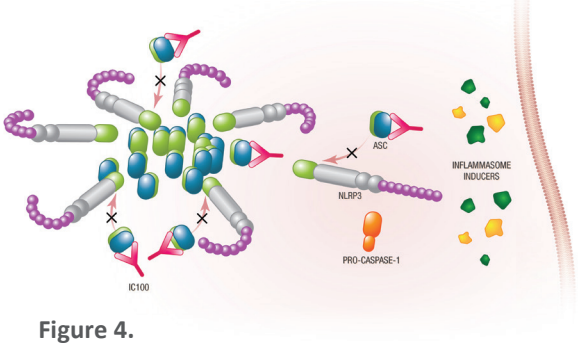


Figure 4.

IC 100 inhibits ASC in ASC specks, preventing perpetuation of inflammation

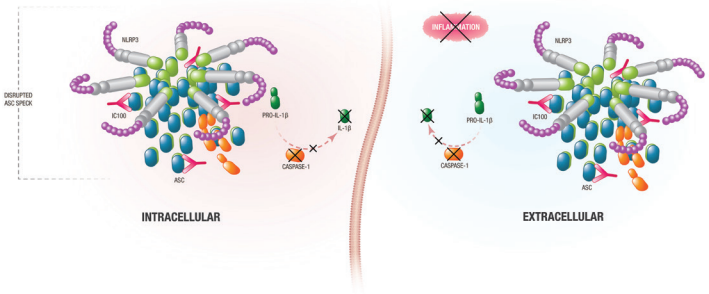


Figure 5.

What is the evidence that IC 100 Acts Intracellularly?

There is both in vitro and in vivo data demonstrating that IC 100 penetrates cells. Intracellular uptake of IC 100 appears to be increased during inflammasome activation.

FLUORESCENCE-LABELED IC 100 DEMONSTRATES INTRACELLULAR UPTAKE INTO THP-1 CELLS

In experiment 1, IC 100 was labeled with fluorescence and then incubated with unstimulated THP-1 cells (human monocytic cell line).

IC 100 was taken up by unstimulated THP-1 cells and incorporated into ASC specks (Figure 6).

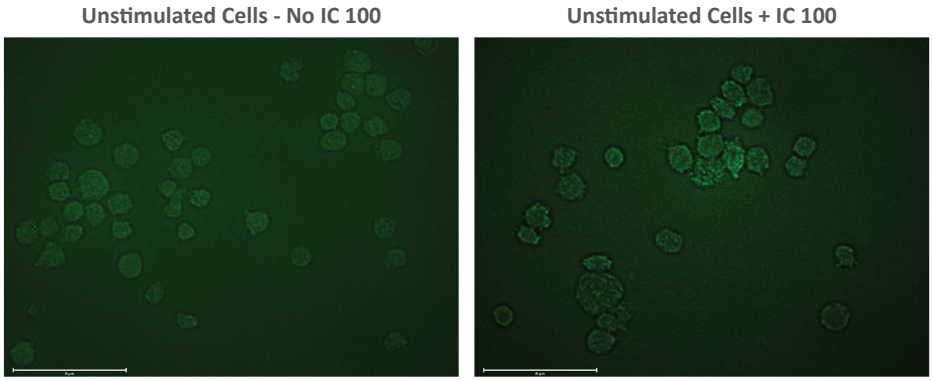


Figure 6.

In experiment 2, IC 100 was labeled with fluorescence and then incubated with THP-1 cells activated by inflammasome inducers, LPS and Nigericin. Results demonstrate that uptake of IC 100 into intracellular ASC specks was significantly increased in inflammasome activated THP-1 cells (Figure 7).

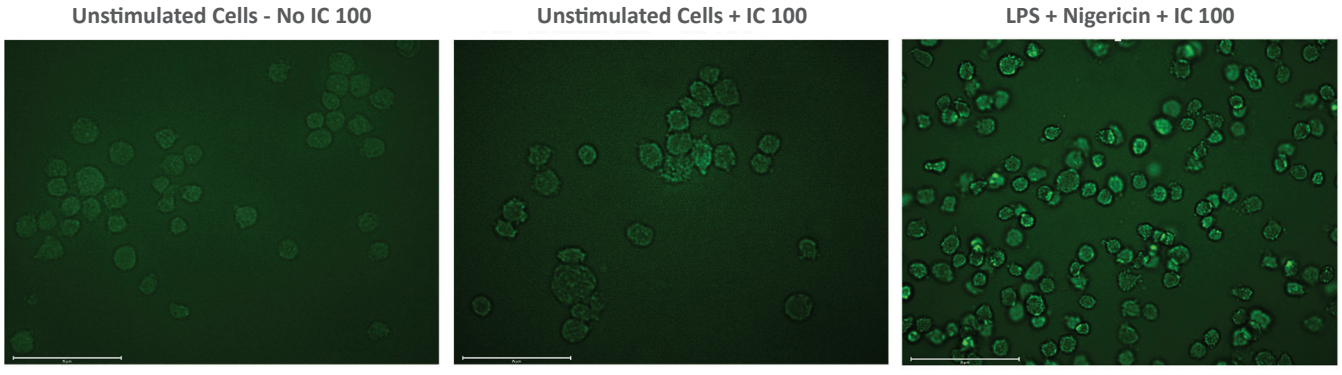


Figure 7.

These data clearly demonstrate intracellular uptake of IC 100.

IC 100 INCUBATED WITH STIMULATED THP-1 CELLS INHIBITED INTRACELLULAR INFLAMMASOME ACTIVATION

IC 100 was incubated with THP-1 cells activated by inflammasome inducers, LPS and ATP.

Intracellular inflammasome activation and release of IL-1β into the medium were inhibited (Figure 8). To achieve these results, IC 100 had to be taken up by the cell.

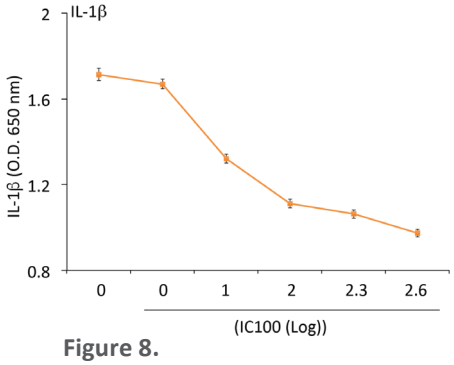


Figure 8.

ANTI-ASC WAS TAKEN UP BY SPINAL CORD NEURONS IN-VIVO

Rats were subjected to moderate cervical spinal cord injury (SCI). At 20 min after SCI, 50 µg of anti-ASC labeled with fluorescein dye (FITC) was injected intraperitoneally and intravenously. Control injured rats received FITC dye alone.

RESULTS.

At 6 hours after SCI, cervical motor neurons in the ventral horn demonstrated uptake of the anti-ASC (left panel), whereas there was no fluorescence seen in the control group (middle panel). The right panel shows spinal cord sections immunostained with anti-ASC followed by appropriate secondary antibody for comparison (Figure 9).

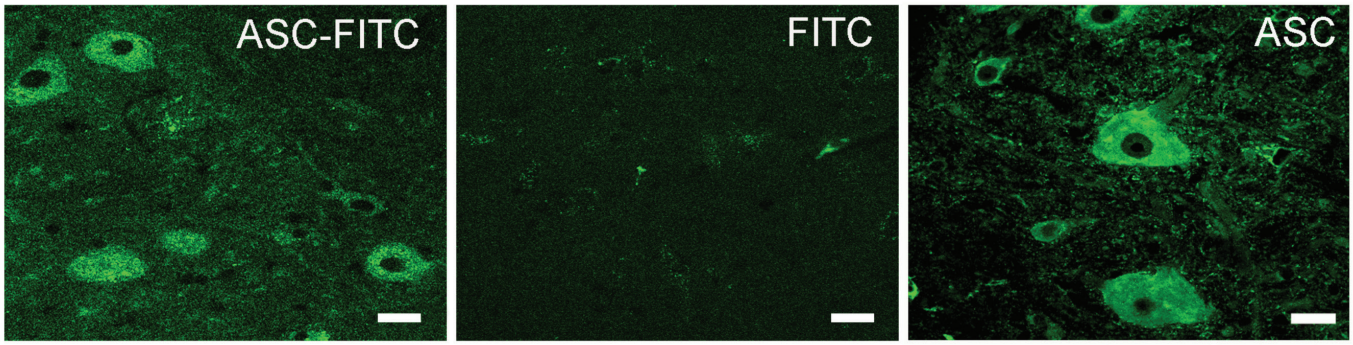


Figure 9.

Why target ASC to treat inflammasome-related diseases and conditions?

Inflammasomes show promise as a therapeutic target for inflammatory diseases and conditions based on growing evidence of their role in the pathogenesis of these conditions. Inflammasomes have a role not only in initiation and perpetuation of inflammation, but they also potentiate the adaptive immune response. Of the three components of the inflammasome (sensor molecules, adaptor ASC, and pro-caspase/caspase-1), ASC has potential to be the most effective target for the following reasons:

- Sensor molecules (e.g. NLRP3), are involved only in intracellular inflammasome formation, whereas ASC is critical for inflammasome formation and activation, as well as perpetuation of the immune response.

  Intracellularly, ASC polymerizes into long ASC filaments (specks) that are crucial for inflammasome activation. Extracellularly, ASC Specks create a signaling platform that continues to recruit pro-caspase-1 and activate pro-inflammatory cytokines, intensifying and perpetuating the inflammatory response. This causes prolonged and more damaging inflammation.

- Sensor molecules (e.g. NLRP3) are each associated with only one inflammasome, whereas the adaptor ASC is associated multiple inflammasomes. Targeting more than one inflammasome is likely critical to achieve a meaningful therapeutic effect in a broad range of inflammatory conditions, as numerous chronic inflammatory diseases are associated with activation of more than one inflammasome (Table 1).

- Targeting caspase-1 may be inadequate to achieve a therapeutic effect since it is associated with only one of the two inflammasome pathways, the canonical pathway (the non-canonical pathway is associated with caspase-4 and 5). In the absence of capsase 1, caspase 4 and 5 can still activate cytokines. Additionally, safety may be a concern with caspase-1 inhibitors, as Pralnacasan, a caspase-1 inhibitor, demonstrated liver toxicity in clinical trials for rheumatoid arthritis, requiring suspension of the trial.

- Targeting IL-1β may be too late in the pathway for a therapeutic effect, as active inflammasomes will continue to produce IL-1β.

Can ASC inhibition result in unsafe immunosuppression?

Although IC 100 attenuates the innate immune response, it does not result in complete immunosuppression based on preclinical studies. In an EAE mouse model of multiple sclerosis, IC 100 modestly reduced the numbers of inflammatory T cell populations and antigen presenting cells, but the number of cells was not completely suppressed (Figure 10).

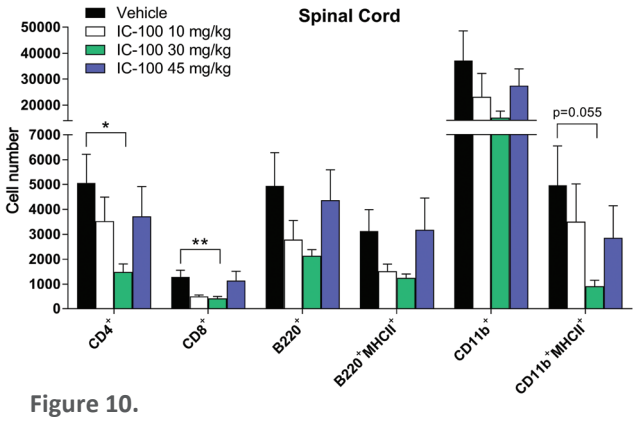


Figure 10.

Expected Benefits of Targeting ASC with IC 100

- IC 100 inhibits ASC and ASC in specks, attenuating intracellular initiation of the immune response and extracellular perpetuation of inflammation. This is expected to result in a meaningful therapeutic effect in chronic inflammatory diseases.
- IC 100 inhibits multiple inflammasome complexes, which is expected to achieve a more meaningful therapeutic effect than targeting just one. Numerous chronic inflammatory diseases are associated with activation of more than one inflammasome (Table 1).
- IC 100 has lower immunogenicity (9%) than many available biologics, which is expected to translate into less potential for acquired drug resistance and drug discontinuation due to side effects.
- IC 100 has strong preclinical support demonstrating:
  - Delayed onset, reduced disease severity, and improved functional outcomes in an EAE mouse model of multiple sclerosis
  - Effective reduction of inflammation and improved outcomes, including prevention of inflammatory cell death and reduced tissue destruction in animal models of spinal cord injury, brain injury, and acute lung injury

ASC as a Biomarker

Biomarkers are biologic measures that can be used to determine the presence or progress of a disease, and the effects of treatment. The ideal biomarker is readily quantifiable with high specificity and sensitivity, elevated in the presence of disease, and correlates with disease outcomes.

ASC has demonstrated promise as a biomarker for various inflammatory diseases.

ASC LEVELS ARE ELEVATED IN VARIOUS INFLAMMATORY DISEASES

Patients with multiple sclerosis, stroke, brain injury, depression and mild cognitive impairment demonstrate higher levels of serum ASC than healthy individuals, as demonstrated in Table 2.

DISEASE/ CONDITION	ASC (PG/ML)
Multiple Sclerosis	> 352.4
Depression	> 273.7
Stroke	> 404.8
TBI	> 275
Mild Cognitive Impairment (MCI)	> 264.9
Healthy	< 195.3

Table 2.

ASC DEMONSTRATES A HIGH DEGREE OF SENSITIVITY AND SPECIFICITY IN MS

When comparing serum levels of ASC, caspase-1, IL-18, and IL-1beta as potential biomarkers for multiple sclerosis (MS), ASC demonstrated the most promise, based on a sensitivity and specificity of >80%, and a clearly defined cut off point based on an area under the curve >0.9 (Figure 11 and Table 3).

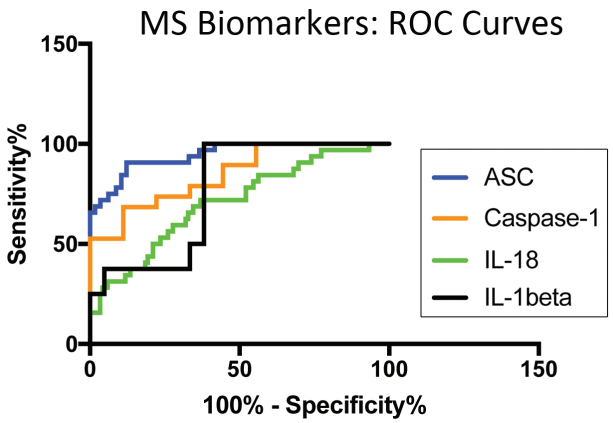


Figure 11.

BIOMARKER	CUT-OFF POINT (PG/ML)	SENSITIVITY	SPECIFICITY
Caspase-1	>1.302	89%	56%
ASC	>352.4	84%	90%

Table 3.

ASC LEVELS CORRELATE WITH MS DISEASE SEVERITY

Serum samples from 32 patients with MS segmented into two groups (mild or moderate disease severity) demonstrated that ASC levels were higher in the serum of patients with moderate versus mild disease (Figure 12).

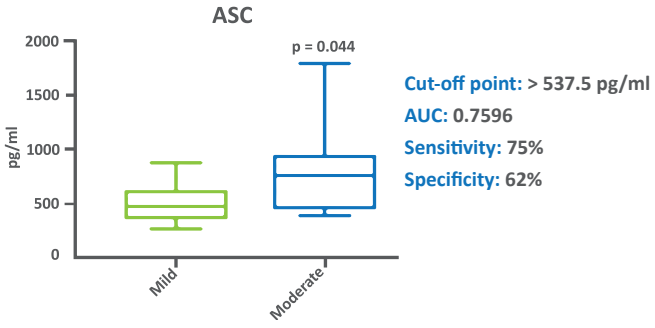


Figure 12.

ASC LEVELS CORRELATE WITH DISEASE OUTCOMES IN BRAIN INJURED PATIENTS

In brain injured patients, levels of ASC proteins within the first 5 days after injury were predictive of outcomes 5 months after trauma. Patients with high levels of ASC proteins in tissue fluids within the first 5 days did not show much improvement when compared to patients with low levels of these proteins (Figure 13).

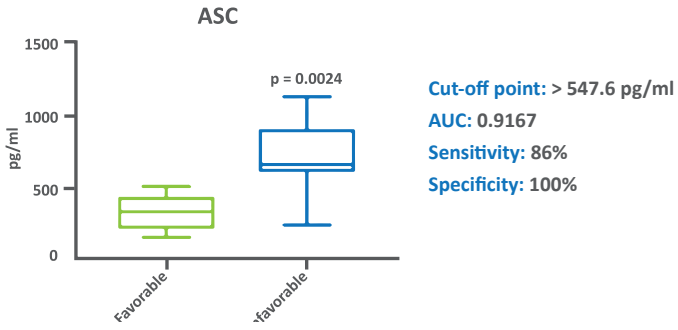
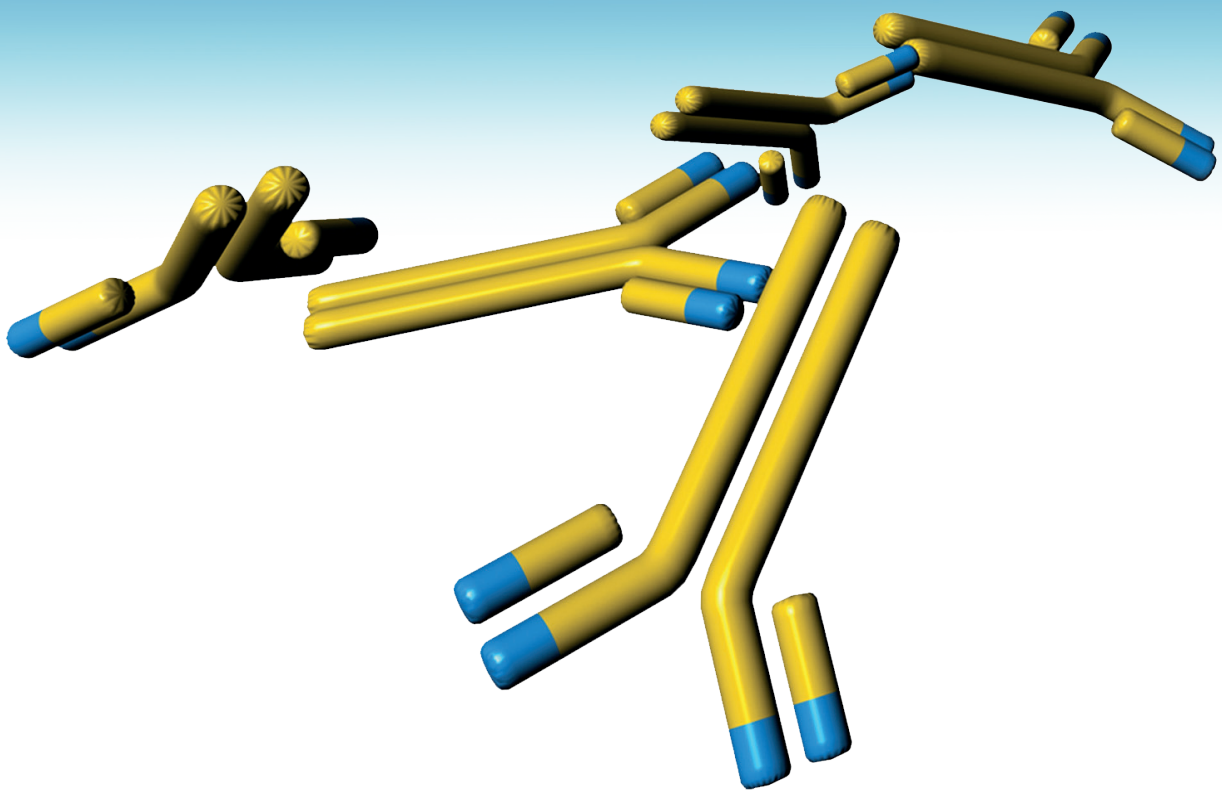


Figure 13.

Conclusion

With potential to block perpetuation of the intense inflammatory response contributing to immune-related diseases, inhibiting the ASC component of inflammasomes could transform treatment for millions of people suffering from a broad range of inflammatory diseases. Availability of an ASC biomarker would help target patients who would most benefit.



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