

## **Astaxanthin (ASTX): A safe, natural, and multi-faceted anti-inflammatory for maintaining lung health and possibly minimizing SARS-CoV-2 effects**

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### **Summary**

*The recent emergence of the SARS-CoV-2 viral pandemic has underscored the importance of maintaining pulmonary health as well as the need to minimize SARS-CoV-2 induced chronic inflammation and the resulting pathologic levels of inflammatory cytokines. SARS-CoV-2 infection results in COVID-19 syndrome that includes coughing, sneezing, fever, pneumonia, and potentially death. Current treatments for viral infection and the ensuing chronic inflammation include anti-viral medications as well as steroidal and non-steroidal anti-inflammatories (NSAIDS); however, safety and efficacy limitations restrict anti-inflammatory treatment regimens for many patients. Coronaviruses have been shown to induce lung damage by increasing inflammatory signaling pathways and cytokine production leading to elevated immune cell infiltration and macrophagic polarization shifts (M2 to M1). Astaxanthin (ASTX), a safe, orally bioavailable, naturally occurring molecule with strong anti-inflammatory and antioxidant activity, has been shown to (i) significantly attenuate pathological elevation of critical inflammatory cell signaling pathways (NF- $\kappa$ B), (ii) decrease the resulting elevated proinflammatory cytokine levels, (iii) reduce immune cell infiltration of the lung, and (iv) positively influence macrophage polarization in humans and animal models of disease.*

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### **SARS-CoV-2 and Inflammation**

The SARS-CoV-2 virus (Severe Acute Respiratory Syndrome Coronavirus 2) recently emerged in China and has rapidly spread worldwide leading the World Health Organization to classify it as a pandemic. SARS-CoV-2 is closely related (70% genetically identical) to the SARS-CoV-1 virus that was responsible for a total of 774 deaths in 17 different countries in 2002-2003. Both viruses belong to the Coronaviridae “crown” family of enveloped, single-stranded positive-sense RNA genome viruses and both pandemics resulted from zoonotic transmission (animal reservoirs to human hosts).

SARS-CoV-2 induced syndrome, referred to as Coronavirus Disease 2019 (COVID-19), consists of coughing, sneezing, fever, pneumonia and potentially pulmonary failure/death. The currently available pharmaceutical treatments are very limited and include anti-virals (remdesivir, ritonavir, lopinavir). Due to the complexity of anti-inflammation strategies to reduce virus-associated pathology versus attenuation of immune-mediated viral clearance, utilization of steroids and NSAIDSs (ibuprofen) are questionable. Potential treatments under development include prophylactic

vaccines, chloroquine, angiotensin II receptor type I inhibitors (losartan, candesartan) and various targeted anti-inflammatories including Actemra (tocilizumab, IL-6 inhibitor).

While it is too early to extensively discover the exact or unique pathomechanisms involved in SARS-CoV-2 infection/disease, observations of COVID-19 patient physiology as well as genetic similarities between the two viruses, support the assumption that SARS-CoV-2 molecular mechanisms will be similar to those of SARS-CoV-1. Both viruses infect the lungs and gastrointestinal tract and appear to utilize a membrane-incorporated “spike” glycoprotein interaction with human angiotensin-converting enzyme 2 (ACE2) for cell attachment/entry.<sup>1</sup> Pathophysiologically, SARS-CoV-1 infection results in upregulation of NF- $\kappa$ B inflammatory signaling pathway, increased proinflammatory cytokine levels (TNF- $\alpha$ , IL-6, COX-2, etc.), enhanced immune cell infiltration into the lung (due in part to increased levels of Monocyte Chemoattractant Protein-1 (MCP-1)), and a heightened level of macrophagic polarity shift from the healing M2 phenotype towards the proinflammatory M1 phenotype.<sup>2-5</sup> Combined,

these inflammatory mechanisms lead to acute lung injury, pneumonia, and ultimately pulmonary failure. Indeed, several studies have underscored the capacity for SARS-CoV-1 viral N and Spike proteins to specifically upregulate NF- $\kappa$ B gene expression.<sup>2-3</sup> Additionally, mice infected with a genetically modified SARS-CoV-1 virus lacking the ability to upregulate NF- $\kappa$ B demonstrate increased survival, decreased immune cell infiltration in lung and reduced inflammatory cytokine levels (TNF- $\alpha$ , IL-6).<sup>6</sup> Application of NF- $\kappa$ B inhibitors in this mouse model also increased survival following unmodified SARS-CoV-1 infection and reduced inflammation and lung pathology.<sup>6</sup>

SARS-CoV-1 activation of the NF- $\kappa$ B pathway results in elevated proinflammatory cytokines levels (TNF- $\alpha$ , IL-6, MCP-1, etc.).<sup>2-5</sup> Additionally, pathogen-associated molecular patterns activate the “inflammasome” further upregulating inflammatory cytokines levels (IL-1 $\beta$ , IL-18).<sup>7</sup> SARS-CoV-1 proteins have also been shown to upregulate cyclooxygenase-2 (COX-2).<sup>8-9</sup> Importantly, prolonged elevation of these cytokines leads to acute lung injury.

Studies on SARS-CoV-1 infection have shown an important shift in macrophage polarization from the healing M2 phenotype (produces IL-4 and IL-10) to the proinflammatory M1 phenotype (produces inflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$ ).<sup>5</sup> In addition, both increased NF- $\kappa$ B inflammatory signaling and increased M1 macrophages elevate MCP-1 levels contributing to increased infiltration of immune cells (mast cells, neutrophils, macrophages, etc.) into the lungs further exacerbating the proinflammatory environment and ultimately leading to lung complications.

### **Astaxanthin helps to maintain lung health and may minimize SARS-CoV-2 effects**

Astaxanthin (ASTX) is a safe, orally bioavailable, naturally occurring molecule with strong antioxidant and anti-inflammatory activity.<sup>10,11</sup> Following oral administration and intestinal uptake, ASTX is delivered initially to the liver via chylomicrons and subsequently distributed systemically to tissues throughout the body integrating within cellular and mitochondrial

membranes. ASTX completely traverses the lipid bilayer component of cell membranes, facilitating its anti-oxidant functions. Mitochondrial ASTX localization allows for the unique regulation of redox stress at the source. Astaxanthin has been shown in humans to significantly lower important inflammatory and metabolic disease measures including tumor necrosis factor-alpha (TNF- $\alpha$ ), low density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), and triglycerides, while significantly raising adiponectin and high density lipoprotein cholesterol (HDL-C) levels.<sup>10-13</sup> ASTX has also positively affected markers of oxidative stress in obese humans including significantly lowering isoprostanes and malondialdehyde (MDA) levels and significantly increasing total antioxidant capacity and superoxide dismutase (SOD).<sup>14,15</sup>

Importantly to lung inflammation and SARS-CoV-2 infection, ASTX has been shown to significantly decrease NF- $\kappa$ B pathway signaling both *in vitro* and *in vivo*.<sup>16-19</sup> Reduction of NF- $\kappa$ B signaling leads to decreased levels of the inflammatory cytokines seen elevated in SARS-CoV-1 infections. Indeed, ASTX has been shown to, at very low doses, reduce TNF- $\alpha$  in humans.<sup>12</sup> In rodents, ASTX reduces TNF- $\alpha$  equivalent to a corticosteroid, the gold standard of anti-inflammatory compounds, with no evidence of immunosuppressive effects.<sup>16</sup> ASTX also significantly decreased other important mediators of inflammation in animal models including IL-6, IL-1 $\beta$ , COX-2, CRP, PGE-2, iNOS, and nitric oxide (NO).<sup>16-19</sup>

ASTX has also been shown to reduce inflammatory cell infiltration (neutrophils, eosinophils, macrophages) in a lung model of inflammation.<sup>20</sup> In fact, the reduction response measured with ASTX was superior to Ibuprofen treatment. This is further supported by observations of MCP-1 reduction following ASTX treatment that will in turn lead to inflammatory cell infiltration attenuation.<sup>19,21</sup> In addition to reduced macrophagic infiltration, ASTX has been shown to reduce the numbers of M1 macrophages displaying M1 phenotype in favor of M2 phenotype.<sup>22-23</sup> In addition to inhibition of NF- $\kappa$ B pathway activation, reduction in the M1/M2 macrophage phenotype ratio is important in decreasing levels of inflammatory cytokines.

## Astaxanthin Safety

ASTX has a long history of dietary use in humans and animals and is Generally Recognized as Safe (“GRAS”) as a food substance according to FDA regulations. No significant side effects have been reported in published ASTX human studies with more than 1,800 subjects; and ASTX has undergone extensive toxicity testing with no clinically meaningful issues even at extremely high doses.<sup>24-25</sup> Commonly used anti-inflammatory drugs such as aspirin, ibuprofen, naproxen, COX-2 inhibitors, corticosteroids, and various biologics have risks of side effects associated with chronic use, including gastrointestinal bleeding, heart attacks, strokes, and severe infections, whereas based on its exceptional safety profile, ASTX is well suited for chronic administration.

## Conclusion

In summary, ASTX has the potential, as a safe and strong anti-inflammatory molecule, to maintain healthy lung function as well as the potential to decrease SARS-CoV-2 associated pulmonary pathophysiology by (i) reducing NF- $\kappa$ B pathway activation, (ii) decreasing inflammatory cytokine production, (iii) attenuating immune cell infiltration, and (iv) shifting macrophage population phenotype (M1 to M2).

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