Strategies for Controlling Serum Amyloid A, a Key Mediator of the Impact of Systemic Inflammation on Cardiovascular Disease

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Abstract

Recent genetic epidemiology confirms a key role for interleukin-6 signaling in the increased cardiovascular risk associated with metabolic syndrome and systemic inflammation. There is good reason to suspect that IL-6’s pathogenic activity in this regard is largely indirect, mediated by hepatic induction of acute phase proteins. Although C-reactive protein (CRP) is a well-established cardiovascular risk factor, it does not appear to play a mediating role in this regard – rather, serum amyloid A (SAA) is emerging as a potent driver of atherogenesis. The fact that statins inhibit hepatic production of both CRP and SAA may rationalize the protective utility of statin therapy in patients with moderate LDL but high CRP. Other agents which may have potential for down-regulating IL-6-driven SAA production include spirulina, salicylate, the AMPK activators metformin and berberine, and tocotrienols. Appropriate weight loss, control of infection, or thiazolidinedione therapy can often lower SAA levels by suppressing IL-6 overproduction. Good omega-3 status tends to decrease SAA both by diminishing IL-6 levels and by a direct effect on hepatocytes. Fenofibrate, a PPARalpha agonist, can decrease elevated acute phase proteins and lessen cardiovascular risk in metabolic syndrome; both it and omega-3s have the potential to suppress IL-6-driven SAA production via PPARalpha activation in the liver.

Interleukin-6, But Not C-Reactive Protein, Promotes Atherogenesis

Elevated C-reactive protein (CRP) is a clearly documented and potent risk factor for coronary disease and MI, whose impact is additive to that of LDL cholesterol (or apoB). Individuals with moderate LDL cholesterol but elevated CRP are at increased cardiovascular risk, and in the JUPITER trial targeting such subjects, rosuvastatin therapy decreased risk for cardiovascular events by 44% markedly while lowering CRP by 37%. Nonetheless, there is now good reason to suspect that CRP is not a mediating risk factor for cardiovascular disease; in mendelian randomization analyses, people who inherit alleles of the CRP gene associated with increased CRP levels have not been found to be at elevated risk. Likewise, rodent studies and cell culture studies have failed to generate convincing evidence that CRP is a mediator of atherogenesis.

CRP is an acute phase reactant, and most of the circulating CRP is thought to be of hepatic origin. As with other acute phase proteins, interleukin-6, along with interleukin-1, is a major driver of its hepatic expression. Interleukin-6 (IL-6) is produced by inflamed hypertrophied visceral adipose tissue, and elevated serum IL-6 is a characteristic feature of metabolic syndrome. Moreover, elevated serum IL-6 is predictive of cardiovascular events, just as CRP is. In two recent analyses, possession of the Asp358Ala allelic variant of the IL-6 receptor gene, known to be associated with decreased membrane expression of the receptor (as well as elevated serum levels of the soluble IL-6 receptor), was found to confer protection from coronary heart disease; this protection was roughly twice as great for those expressing two copies of the allele as compared to those expressing one. These findings strongly suggest that IL-6 signaling via intact membrane receptors (as opposed to the trans-signaling pathway
reflecting interaction of soluble IL-6/IL-6 receptor complexes with membrane gp130\(^{17}\) plays a significant mediating role in the genesis of cardiovascular disease – and that CRP association with cardiovascular risk may reflect its utility as a marker for IL-6 activity.

Since IL-6 receptor is expressed primarily by hepatocytes and leukocytes (endothelial cells fail to express it), it is reasonable to suspect that much of the impact of increased IL-6 activity may be indirect. Of particular pertinence are two recent studies showing that hepatocyte specific knockout of gp130 expression in mice (gp130 is a key component of the IL-6 receptor) protects mice from atherogenesis.\(^{18, 19}\) Suspicion naturally falls on the range of acute phase proteins whose hepatic synthesis is promoted by IL-6. Although the acute phase reactant fibrinogen, like CRP, correlates with cardiovascular risk, mendelian randomization analysis likewise casts doubt on fibrinogen as a mediating risk factor: people who inherit alleles of fibrinogen associated with modestly increased serum levels have not been found to be at increased cardiovascular risk.\(^{20}\)

**Serum Amyloid A – A Key Mediator of Inflammation-Driven Cardiovascular Risk**

However, there is growing reason to suspect that another acute phase protein, serum amyloid A (SAA), may in fact be a mediating risk factor for cardiovascular disease. Not surprisingly, as it is induced by a similar IL-6-dependent mechanism, serum SAA tends to correlate with levels of CRP and fibrinogen, and like them is predictive of cardiovascular risk.\(^{21-25}\) Polymorphisms in the SAA1 and SAA2 genes, the functional significance of which remain unclear, have been linked recently to carotid intima-media thickness and ankle-to-brachial index, both established surrogates for cardiovascular disease, in Chinese subjects.\(^{26, 27}\) Moreover, in physiologically relevant concentrations, SAA acts directly on endothelial cells (possibly via formyl peptide receptors) to exert a range of pro-inflammatory/pro-oxidative effects typically associated with endothelial dysfunction in vascular disorders.\(^{28, 29}\) These effects are antagonized by HDL particles, with which the SAA protein often associates; conversely, SAA blocks the anti-inflammatory effects of HDL on endothelium – suggesting that HDL and SAA are functional antagonists with respect to vascular health.\(^{25, 25, 30}\) SAA also acts on monocytes and smooth muscle cells to promote migration, tissue infiltration, and cytokine production.\(^{18, 31, 32}\) In atherosclerosis-prone apo-E-deficient mice fed a chow diet, injection with a murine SAA lentivirus that increases serum SAA levels was associated with a marked increase in aortic atherosclerotic lesion area and macrophage infiltration, along with increased vascular expression of MCP-1 and VCAM-1.\(^{33}\) And in the hepatocyte-specific gp130-deficient mice cited earlier, injections of recombinant SAA abolished the protection from vascular remodeling afforded by the loss of gp130.\(^{18}\) Finally, it is notable that statin therapy tends to decrease elevated SAA levels, just as it does elevated CRP levels.\(^{34-38}\)

In aggregate, these findings support that hypothesis that SAA elevation is a key direct mediator of the adverse impact of increased IL-6 activity (as seen in metabolic syndrome or other chronic inflammatory conditions) on cardiovascular health – and that the markedly favorable impact of rosuvastatin on cardiovascular risk in people with high CRP but moderate LDL may, in large measure, reflect a suppression of IL-6-triggered hepatic SAA production. Presumably, the mechanism whereby statins suppress hepatic SAA production is similar to that by which they suppress CRP production, as both SAA and CRP are acute phase proteins whose hepatic synthesis is boosted in a complementary manner by IL-6 and IL-1, and whose transcription is contingent on nuclear NF-kappaB and STAT3 complexes.\(^{10, 39, 40}\) Statins appear to suppress the transactivational activity of STAT3 by inhibiting the isoprenylation of Rac-
IL-6-mediated Rac-1 activation is required for the serine phosphorylation of STAT3 (Ser-727) that promotes its interaction with its coactivator CBP/p300. Notably, association of STAT3 with CBP/p300 plays a key role in IL-6-driven transcription of human SAA.

Ancillary Strategies for SAA Control – Spirulina, Salsalate, AMPK Activators, and Tocotrienols

If this hypothesis is correct, it behooves us to identify additional drugs or nutraceuticals capable of intervening in IL-6-mediated SAA induction. As noted, activation of NF-kappaB plays a key role in transcription of both CRP and SAA; this explains why IL-1 markedly potentiates the impact of IL-6 on induction of these proteins, even though by itself it has little effect. However, there is also evidence that IL-6 can moderately activate NF-kappaB in hepatocytes. Yoshida and colleagues have presented evidence consistent with the possibility that upstream activation of Rac-1 and NADPH oxidase in IL-6-treated hepatocytes (Hep3B cells) contributes to this activation of NF-kappaB. Notably, the NADPH oxidase inhibitor DPI, as well as N-acetylcysteine, inhibit induction of CRP in these cells. Free bilirubin functions physiologically to inhibit NADPH oxidase, and this may rationalize the numerous clinical reports that plasma bilirubin levels tend to correlate inversely with CRP. The biliverdin-derived spirulina chromophore phycocyanobilin (PhyCB) has recently been shown to mimic the NADPH oxidase-inhibitory activity of bilirubin, in vitro and in vivo; moreover, oral administration of PhyCB, either in free form or bound within the protein phycocyanin, exerts potent and versatile anti-inflammatory effects in rodent studies. Hence, it would be of interest to determine whether some adequate intake of spirulina (or of a PhyCB-enriched spirulina extract) might lessen oxidative stress and NF-kappaB activation in hepatocytes, thereby suppressing hepatic production of CRP and SAA. To date, there are no rodent or clinical studies reporting the impact of spirulina administration on CRP or SAA levels. PhyCB may also have the potential to act downstream from SAA, blunting its induction of oxidative stress in the vasculature; NADPH oxidase is a major source of superoxide in fibroblasts and neutrophils exposed to SAA.

Hepatic activation of NF-kappaB might also be suppressed with salicylic acid, which blocks the canonical pathway of NF-kappaB activation through direct inhibition of IKK-beta. Although the impact of salicylate therapy on SAA appears not to have been reported, serum levels of CRP dropped significantly by 34% in obese young adults receiving 4 g of salsalate daily. (Salsalate is a well-tolerated delivery vehicle for salicylate that is currently being studied an adjuvant for diabetes management.) Moreover, in mice with hepatocyte-specific knockout of SOC3 – in which the acute phase response is up-regulated – salicylate administration not only lowered acute phase protein expression, but also inhibited the activating phosphorylation of STAT3, for reasons that remain unclear. Since salicylate also has the potential to favorably influence the function of vascular endothelium and foam cells in at-risk subjects, it may have particular merit as an agent for controlling SAA. Its drawback is that reversible ototoxicity (tinnitus, partial hearing loss) can have a dose-limiting impact on its clinical use – albeit most patients tolerate 3 g daily well.

Agents capable of activating AMP-activated protein kinase (AMPK) – such as the anti-diabetic agents metformin and berberine – also have the potential to suppress SAA production. In a human hepatocarcinoma cell line (HepG2), the ability of IL-6 exposure to boost expression of SAA and other acute phase reactants was antagonized by either metformin or the AMPK agonist AICAR; this effect was reversed by siRNA targeting AMPK. AMPK exerted this effect, at least in part, by inhibiting the IL-6-
mediated activating tyrosine phosphorylation of STAT3 – albeit it is not yet clear which protein AMPK targets directly. AMPK also might also suppress SAA expression by inducing an inhibitory phosphorylation of coactivator CBP/p300, which interacts with STAT3 in the transcriptional complex that promotes SAA transcription.\textsuperscript{39, 73} Clinically, there is a recent report that metformin therapy lowers serum SAA in women with polycystic ovary syndrome.\textsuperscript{74, 75} While the clinical impact of berberine on SAA has not been reported, reduction of CRP in berberine-treated patients with acute coronary syndrome has been noted recently.\textsuperscript{76} Hence, the impact of metformin and of berberine on SAA levels in at-risk patients merits further evaluation.

Tocotrienols, phytochemical relatives of the tocopherols, share the ability of statins to suppress HMG-CoA reductase activity and hence isoprenylation reactions.\textsuperscript{77-79} (Their utility in this regard reflects feedback inhibition of HMG-CoA reductase expression; their clinical impact on LDL levels tends to be more modest than that of statins.) They might therefore be expected to mimic, to a limited degree, the down-regulatory impact of statins on expression of SAA and CRP. However, tocotrienols also have a poorly understood anti-inflammatory activity that might influence the acute phase response.\textsuperscript{80} In several cancer cell lines gamma-tocotrienol has been found to suppress STAT3 signaling – an effect which may reflect induction of the tyrosine phosphatase SHP-1.\textsuperscript{81, 82} In a recent Malaysian clinical study, subjects receiving 150 mg daily of a tocotrienol-rich supplement daily experienced a significant drop in CRP.\textsuperscript{83} The clinical impact of tocotrienol administration on serum SAA levels warrants investigation.

SAA overproduction can also be controlled by decreasing the elevated IL-6 levels which drive this production – via appropriate weight loss, control of infections, or thiazolidinedione therapy.\textsuperscript{84} The possibility that PhyCB and salicylate might also lessen IL-6 production by inflamed visceral adipose tissue should also be considered. Indeed, spirulina supplementation has been reported to lower serum IL-6 levels in elderly Koreans, and salicylate administration decreased IL-6 expression in the adipose tissue of fat-fed mice.\textsuperscript{85, 86}

\textbf{The Omega-3 Factor and Fibrates}

A number of studies have correlated relatively good omega-3 status, as assessed by dietary intake or tissue levels, with decreased markers of systemic inflammation, usually including CRP and IL-6, but sometimes also SAA.\textsuperscript{87-92} For example, in one of these studies, SAA averaged 28% lower in subjects who ingested at least 300 g of fish weekly, as opposed to those who did not. In controlled clinical studies, SAA dropped significantly by about 23% in dyslipidemic men ingesting 15 of flaxseed oil (providing about 8 g alpha-linolenic acid) daily; a similar finding was reported in a study which administered 5 g of alpha-linolenic acid in flaxseed flour to morbidly obese volunteers.\textsuperscript{93, 94} Supplementation with 3 g of DHA daily for 3 months achieved significant reductions in CRP (15\%) and IL-6 (23\%), but a trend towards a modest reduction in SAA was not significant.\textsuperscript{95} Although some of the reduction in SAA and CRP associated with good omega-3 status is likely attributable to the concurrently observed reductions in IL-6, there is a possibility that omega-3 fatty acids in the liver might blunt the increase in SAA and CRP expression driven by IL-6. Indeed, one recent study found that pre-incubation of HepG2 cells with EPA or DHA led to a dose-dependent decrease in the expression of CRP mRNA and protein evoked by IL-6 exposure; this reflected a suppression of IL-6 triggered tyrosine phosphorylation of STAT3.\textsuperscript{96} Other factors being equal, one would expect that EPA/DHA would exert a similar effect on SAA expression. On the other hand, there is a report that DHA exposure increases SAA expression in SK-HEP-1 hepatoma cells.
cells, owing to an increase in the C/EBPbeta transcription factor, which is required for optimal expression of both SAA and CRP.97 Evidently, the direct impact of omega-3 fatty acids on hepatocyte response to IL-6 requires further evaluation. In any case, it is quite reasonable to conclude that ample intakes of omega-3, whether from flaxseed, fish, or algae, can exert a systemic anti-inflammatory effect that includes a reduction in SAA.

Long-chain omega-3s induce activation of the PPARalpha transcription factor,98 and there is good reason to suspect that PPARalpha agonism mediates, at least in part, the impact of omega-3 nutrition on hepatocyte responsiveness to IL-6. The cardiovascular drug fenofibrate, a PPARalpha agonist, has reduced levels of CRP and SAA in some though not all studies; a recent meta-analysis of short-term randomized studies with fenofibrate concludes that its impact on CRP is real, with an average reduction of 29% across studies.99,100 Not surprisingly, greater response was seen in subjects with higher baseline CRP levels – whereas the impact was minimal in those with low-normal levels. The ACCORD study group, which studied the impact of fenofibrate on risk for heart attack, stroke, and cardiovascular death in simvastatin-treated type 2 diabetics, did not see benefit in the group overall, but did observe risk reduction in a sub-group characterized by metabolic syndrome – relatively high triglycerides and low HDL cholesterol.101 It is tempting to speculate that this responsive subgroup was enriched in subjects with high SAA – as would be typical of a population with metabolic syndrome.102,103 The molecular biology underlying PPARalpha’s impact on acute phase reactants requires further clarification. In mice, fenofibrate was found to induce a liver-specific down-regulation of gp130, an obligate component of the IL-6 receptor.104 If such an effect occurs in humans, it evidently could explain why fenofibrate and dietary omega-3s tend to decrease CRP and SAA levels – and why long-chain omega-3s inhibit STAT3 phosphorylation and CRP expression in HepG2 cells. In mice, PPARalpha activation also decreases hepatic expression of C/EBPbeta104 – a finding diametrically opposite to that reported in DHA-exposed SK-Hep-1 cells.

Sesamin, the major lignan in sesame seeds, appears to function as a PPARalpha agonist in rats – albeit it is much less effective in this regard in mice and hamsters, owing to differences in its metabolism.105-107 Nutraceuticals providing 500 mg sesamin per capsule are now commercially available; it would be of interest to determine whether this phytochemical could influence SAA levels in humans.

**Summing Up**

In summary – the increase in IL-6 activity associated with metabolic syndrome and other states of chronic inflammation is a notable mediator of cardiovascular risk, albeit largely indirectly via effects on the liver. The acute phase reactants CRP and fibrinogen, whose hepatic synthesis is promoted by IL-6 and which correlate with cardiovascular risk, nevertheless do not appear to act as mediators of this risk. In contrast, cell culture studies, rodent studies, and the findings of epidemiological studies all suggest that SAA can act directly on the vasculature to promote atherogenesis and cardiovascular events. Statin therapy can decrease elevated SAA, and it likely does so in a way analogous to its suppressive impact on hepatic CRP production; this may rationalize the findings of the JUPITER trial. Moreover, there is suggestive evidence that treatment with spirulina, salsalate, metformin or berberine, and tocotrienols likewise has the potential to decrease hepatic SAA production by intervening in the NF-kappaB/STAT3-dependent signaling pathway by which IL-6 promotes SAA transcription. Now that SAA is emerging as an important mediating risk factor, further clinical studies are needed to clarify the most effective
drug/nutraceutical regimens for controlling elevated SAA. A wholly nutraceutical regimen comprised of red yeast rice (a natural source of statins\textsuperscript{108,109}), spirulina, berberine, and tocotrienols might be envisioned. Fenofibrate therapy or good omega-3 status – whether achieved with flaxseed, fish, fish oil, or algal-derived DHA – also appear to be helpful in this regard, likely owing to hepatic PPARalpha activation.

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