

# **Clinical Potential of Phycocyanobilin for Induction of T Regulatory Cells in the Management of Inflammatory Disorders**

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## **Abstract**

Exposure of human mononuclear cells to phycocyanin *in vitro* is reported to promote generation of Treg cells. Induction of heme oxygenase-1 (HO-1) in lymphocytes has a similar effect, and it is not likely to be accidental that a key product of HO-1 activity, biliverdin, is homologous to the structure of phycocyanin's chromophore phycocyanobilin (PhyCB). Moreover, Treg induction is observed in mice injected with bilirubin, biliverdin's chief metabolite. These considerations suggest that bilirubin, generated within lymphocytes by HO-1 activation, may play a physiological role in the promotion of Treg immunomodulation. This effect of bilirubin is likely to be independent of NADPH oxidase inhibition, since the NADPH oxidase activity of macrophages is *necessary* for Treg induction, possibly because it contributes to HO-1 induction in lymphocytes. In light of numerous reports that oral phycocyanin is beneficial in various rodent models of autoimmune disorders, it is reasonable to suspect that PhyCB-enriched spirulina extracts may have clinical potential for boosting Treg activity in human autoimmune or allergic syndromes, mimicking the physiological role of HO-1 induction in this regard.

## **Bilirubin and Phycocyanin Promote Treg Generation**

Recently, Penton-Rol and colleagues have demonstrated that exposure of human peripheral blood mononuclear cells to phycocyanin *in vitro* leads to rapid up-regulation of the expression of key markers for Treg cells: Foxp3, CD25, interleukin-10, and TGF-beta.<sup>1</sup> This may help to rationalize their further finding that parenteral administration of phycocyanin has both preventive and therapeutic benefit in experimental autoimmune encephalomyelitis, the standard murine model of multiple sclerosis. Notably, pre-administration of phycocyanin prior to injection of the encephalitogen completely prevents the syndrome.

A literature search reveals that induction of heme oxygenase-1 (HO-1) activity in lymphocytes markedly increases Treg activity, boosting expression and synthesis of the same markers induced by phycocyanin: Foxp3, CD25, IL-10, and TGF-beta.<sup>2-8</sup> It is unlikely to be accidental that a product of HO-1 activity, biliverdin, is structurally homologous to the chromophore of phycocyanin, phycocyanobilin (PhyCB).<sup>9</sup> Indeed, this homology reflects the fact that biliverdin is the biosynthetic precursor for PhyCB in spirulina. A reasonable interpretation of the findings of Penton-Rol et al. is that phycocyanin is taken into lymphocytes via pinocytosis, where it is

degraded to liberate PhyCB or PhyCB oligopeptides, which in turn mimic the physiological impact of biliverdin on Treg induction.

Conceivably, the proximate mediators of these effects are bilirubin and its homolog phycocyanorubin. This is suggested by a recent report that parenteral bilirubin likewise promotes induction of Treg activity in mice subjected to mismatched islet transplantation.<sup>10</sup> Moreover, parenteral bilirubin has previously been shown to be therapeutic in experimental autoimmune encephalomyelitis.<sup>11-13</sup> Within cells, biliverdin and PhyCB are rapidly converted to bilirubin and phycocyanorubin (respectively) via biliverdin reductase activity.<sup>14</sup>

### **Treg-Inductive Effect of Bilirubin is Likely Independent of NADPH Oxidase Inhibition**

Although bilirubin and phycocyanorubin both have the potential to inhibit certain isoforms of NADPH oxidase,<sup>9, 15-18</sup> it is not clear that this is the mechanism responsible for Treg induction. So far, there is no literature indicating a role for lymphocyte NADPH oxidase in modulation of Treg activity. Moreover, there is evidence that some level of NADPH oxidase activity in macrophages may be *necessary* for Treg induction (possibly because it aids HO-1 induction in the lymphocytes?);<sup>19</sup> indeed, mice in whom phagocytic NADPH oxidase activity is genetically absent appear to be more prone to autoimmune disorders, likely owing to diminished suppressor cell performance.<sup>20-22</sup> These considerations suggest that biliverdin/bilirubin may be acting through a second target when they boost Treg induction. The nature of such a second target is unclear, but Bach and colleagues have proposed that modulation of the conformation of biliverdin reductase – an intriguing enzyme which may double as a transcription factor and a dual-specificity kinase<sup>23</sup> - is a likely candidate.<sup>24</sup> In any case, it should be straightforward to analyze the signaling pathways evoked by biliverdin during Treg induction. A failure of low-dose DPI or apocynin – other inhibitors of NADPH oxidase – to replicate the activity of biliverdin in this regard, would confirm the presence of a second target.

Induction of IL-10 transcription by biliverdin/bilirubin may be key to the Treg inductive effect of these agents, inasmuch as IL-10 itself can promote Treg induction.<sup>25, 26</sup> Indeed, HO-1 induction fails to induce increased Foxp3 expression in IL-10-deficient mice.<sup>4</sup> Moreover, there is evidence that HO-1 or biliverdin may induce IL-10 in other cells such as macrophages;<sup>24, 27-35</sup> if so, this effect may have anti-inflammatory implications broader than Treg induction, since IL-10 exerts a wide range of anti-inflammatory effects on tissues.<sup>36-38</sup> Conversely, IL-10 promotes HO-1 induction in various tissues;<sup>39-43</sup> thus, the two may constitute an anti-inflammatory “tag team”.

### **Clinical Implications**

Since there are no rich natural sources of biliverdin or bilirubin – which moreover are expensive to synthesize – whereas spirulina, a safe natural food, can contain 20% or more phycocyanin by dry weight, it should be an urgent priority to study the impact of spirulina or of PhyCB-enriched spirulina extracts on autoimmune disorders. Indeed, the recent study by Penton-Rol and colleagues was prompted by the anecdotal observation that interferon-treated multiple sclerosis

patients tend to have a better clinical course when they concurrently ingest spirulina.<sup>1</sup> In numerous rodent studies, orally administered whole spirulina or phycocyanin has exerted profound anti-inflammatory effects; hence, orally administered PhyCB may be capable of replicating the antioxidant and anti-inflammatory benefits achieved physiologically by biliverdin/bilirubin.<sup>9, 44</sup> A safe and practical strategy for boosting Treg induction would likely be clinically beneficial in a wide range of autoimmune, allergic, and inflammatory disorders, and also would have potential for preventing graft rejection.<sup>25, 45-48</sup> Arguably, PhyCB-enriched extracts rather than whole spirulina might be more uniformly effective in inflammatory disorders, inasmuch as spirulina polysaccharides have the potential to promote inflammation via activation of monocyte TLR2 receptors.<sup>49</sup>

Conversely, the Treg inductive potential of PhyCB might prove counterproductive in serious or chronic infections, perhaps necessitating a temporary discontinuation of administration. However, this deduction is complicated by the fact that PhyCB may have potential for preventing septic shock or inflammatory lung congestion.<sup>15, 33, 50, 51</sup> Thus, whereas PhyCB might diminish antigen-specific immune attack on pathogens, it might simultaneously ameliorate the pro-inflammatory mechanisms whereby infections often induce tissue injury and death. When PhyCB is used to treat clinical cancer – in light of its potential to slow the growth of cancers in which constitutive tumor NADPH oxidase activity promotes tumor aggressiveness<sup>52</sup> – concurrent suppression of Treg activity with measures such as metronomic cyclophosphamide might be indicated,<sup>53</sup> and whole spirulina might be the preferable delivery vehicle.

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