Pyridoxal 5'-phosphate to mitigate immune dysregulation and coagulopathy in COVID-19

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Abstract

Although most cases of COVID-19 are paucisymptomatic, severe disease is characterized by immune dysregulation, with a decreased type I interferon response,¹ increased inflammatory indicators, surging IL-6, IL-10 and TNFα suggestive of cytokine storm,²–⁴ progressive lymphopenia, and abnormal blood clotting.⁵,⁶ Factors determining susceptibility to severe disease are poorly understood, although mortality correlates with increasing age and co-morbidities including diabetes and cardiovascular disease (CVD).⁷,⁸ Pyridoxal 5'-phosphate (PLP) tends to be insufficient in populations particularly vulnerable to COVID-19, including the elderly,⁹,¹⁰ the institutionalized,¹¹,¹² and people with diabetes,¹³,¹⁴ and CVD,¹⁵,¹⁶ and PLP becomes further depleted during infection and inflammation.¹⁷,¹⁸ In turn, low PLP results in immune imbalance, as PLP is an essential cofactor in pathways regulating cytokine production, in particular type I interferons and IL-6, and in lymphocyte trafficking and endothelial integrity.¹⁹ Furthermore, normalizing PLP levels attenuates abnormalities in platelet aggregation and clot formation.²⁰–²² Finally, PLP insufficiency induces excess secretion of renin and angiotensin,²³ and hypertension.²⁴ In inflammatory disease, pharmacological doses of PLP decrease circulating TNFα, IL-6²⁵ and D-dimer,²⁶ and animal studies demonstrate that supplemental PLP shortens the duration and severity of viral pneumonia.²⁷ Severe COVID-19 manifests as an imbalance in the immune response¹ and the clotting system.⁵ Pharmacological PLP supplementation may therefore mitigate COVID-19 symptoms by alleviating both the immune suppression underlying viral spread and the pathological hypersecretion of inflammatory cytokines, as well as directly bolstering endothelial integrity and preventing hypercoagulability.

Keywords: COVID-19, SARS-CoV-2, pyridoxal 5'-phosphate, pyridoxine, vitamin B6, immune response, IL-6, TNF, type I interferon, lymphopenia, blood clotting, coagulopathy, cytokine storm, sphingosine-1-phosphate, kynurenine, inflammasome, serine hydroxymethyltransferase 2 (SHMT2), hypertension, angiotensin

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COVID-19: a disease of systems imbalance

From the first case series reported in China\textsuperscript{6} to recent overviews of the clinical picture in COVID-19,\textsuperscript{2,4,5} the heterogeneity of the host response has been striking. While most infections are asymptomatic or mild, critically ill patients show dysregulation of the immune response, the blood clotting cascade, and the vascular system.\textsuperscript{2,5,6} Plasma IL-6 and TNFα tend to increase with increasing disease severity, and in patients requiring ICU admission.\textsuperscript{2,3} Despite the seeming hyperactivity of the immune system in terms of inflammatory cytokine production, the majority of hospitalized patients show progressive lymphopenia, which may correlate with poor prognosis,\textsuperscript{6,28} and more recently, low levels of type I interferon, a key mediator in the anti-viral response, has been described.\textsuperscript{1} Thus, viral clearance is impaired by the insufficient early interferon response and the progressive lymphopenia, while overexpression of inflammatory cytokines leads to tissue and organ damage, leaky vasculature, plummeting blood pressure and shock. This inappropriate immune response leads to a dilemma for clinicians: to treat with immunosuppressive drugs and risk aggravating viral pathology, or to let immune-mediated damage progress unchecked and exacerbate the patients’ pathology.\textsuperscript{4}

In parallel, the blood clotting cascade is also dysregulated, with early reports of elevated D-dimer\textsuperscript{6} confirmed by recent accounts of clotting abnormalities and endothelial damage.\textsuperscript{5} Furthermore, regulation of vascular tone may be compromised, exacerbating organ damage particularly in the lung and kidney.\textsuperscript{5}

Epidemiology of PLP insufficiency parallels COVID-19 susceptibility

PLP, the active form of vitamin B6 (pyridoxine), is an essential co-factor in many inflammatory pathways and becomes depleted during inflammation.\textsuperscript{17,29} PLP deficiency leads to aberrant function of these inflammatory pathways, and their progressive dysregulation; PLP levels are inversely correlated with plasma IL-6 and TNFα in chronic inflammatory conditions.\textsuperscript{25,30} Population studies suggest that PLP levels decrease in older people (by 0.9 ng / ml / decade in unsupplemented
individuals), and up to 24% of Americans without supplemental vitamin intake may have insufficient levels. Institutionalized people, particularly hard hit by COVID-19 in many outbreaks, are more likely to suffer PLP deficiencies, with up to 70% of the institutionalized elderly in Canada and the US being PLP deficient. Interestingly, low PLP concentrations are more common in people with diabetes and confer an increased and independent risk for CVD.

Regardless of pre-existing PLP status, inflammation increases its utilization and leads to its depletion, suggesting that COVID-19 patients experiencing inflammation would become acutely depleted of PLP. Furthermore, depletion tends to occur rapidly at sites of inflammation, leaving those areas particularly vulnerable to the consequences of PLP deficiency. PLP is an essential cofactor in several pathways involved in immunity and inflammation, notably sphingosine metabolism and tryptophan catabolism, as well as in the regulation of endothelial integrity, platelet aggregation, the clotting cascade, and blood pressure regulation via renin-angiotensin secretion.

**Sphingosine-1-phosphate (S1P) metabolism**

S1P is a potent regulator of inflammation, acting specifically on lymphocyte trafficking, permeability of the endothelial barrier, and cytokine and chemokine production. PLP directly regulates the abundance of S1P, both as a gatekeeper for its production and as a bottleneck for its degradation by S1P-lyase. PLP is essential for S1P-lyase function. Inhibition of S1P-lyase results in trapping of lymphocytes in secondary lymphoid organs and sites of inflammation, with a consequent loss of lymphocytes in the circulation, resulting in lymphopenia and systemic immune suppression, while the activated lymphocytes trapped in inflamed tissue may secrete pro-inflammatory cytokines and exacerbate local tissue damage. Furthermore, a key function of PLP involves tuning the leakiness of endothelial barriers through regulation of tight junctions. When PLP is limiting, dysregulated S1P turnover may allow signaling via S1P receptors 2 and 3 (S1PR2 / S1PR3), resulting in leaky endothelial barriers, hypovolemic shock, and increased fluid retention in inflamed tissues such as the lung in COVID-19. Finally, S1P also
acts as an intracellular rheostat for inflammatory signaling. Changes in PLP abundance modulate intracellular cascades involved in TNF receptor, nuclear factor KB (NFKB), and some Toll-like Receptors (TLR) signaling, contributing to excessive cytokine production and inflammatory tissue damage.34 (Figure 1a). Pharmacological S1P modulators are used to treat autoimmune disease,37 and have shown promise in animal models of influenza- and SARS-induced cytokine storm.38 However, pharmacological modulation of the S1P system typically acts by inducing lymphocyte sequestration and immune suppression, which in the context of COVID-19 could trigger viral resurgence. In contrast, restoring the homeostatic function of the S1P system through PLP repletion may allow immune function to move toward normal balance, enabling the control of viral replication without uncontrolled cytokine expression. Similarly, loss of PLP during inflammation, especially in patients with a pre-existing deficiency, may exacerbate the loss of immune equilibrium characteristic of COVID-19 infection.

**Tryptophan catabolism and the kynurenine system**

PLP is a cofactor in the tryptophan catabolism pathway (Figure 1b). When PLP is limiting, tryptophan catabolism to nicotinamide adenine dinucleotide (NAD+) is impaired and kynurenines accumulate.39,40 Kynurenines are a family of tryptophan catabolites which signal through the Aryl Hydrocarbon Receptor (AhR) and play numerous roles in inflammation.40,41 When PLP is limiting, NAD+ production though this pathway is compromised, limiting energy supply and stress resilience.39,40 Concomitantly, kynurenine metabolites, including kynurenine, kynurenic acid, 3-hydroxykynurenine, and quinolinic acid are overproduced, resulting in pleotropic effects on the immune system which depend on the relative abundance of the specific metabolites.40,41 Kynurenine tends to produce immune suppression by decreasing NK activity and T cell proliferation and increasing T cell apoptosis, while kynurenic acid may increase cytokine production, in particular IL-6, TNFα, IL-10 and IL-1, and amplify signaling through the IL-6 / JAK / STAT axis.40,41 Recently kynurenines have been reported to stimulate an IL-6 positive feedback cycle which could amplify harmful inflammation.42
a. PLP in sphingosine-1-phosphate metabolism

**PLP SUFFICIENCY**

![Diagram showing PLP in sphingosine-1-phosphate metabolism]

**Tonic S1P signaling:**
- appropriate trafficking
- tight endothelial barrier

**PLP DEPLETION**

- S1P accumulation
- TNFR, TLR, NFKB
- Dysregulated S1P signaling:
  - trapped lymphocytes / lymphopenia
  - leaky endothelial barrier
  - amplified inflammatory signaling

b. PLP in tryptophan catabolism

**PLP SUFFICIENCY**

- Tryptophan degradation
- Kyn / KynA
- Balanced host response

**PLP DEPLETION**

- Tryptophan degradation
- Kyn / KynA
- Dysregulated host response
- Amplified IL-6 cascade

Figure 1. PLP regulates inflammation via S1P and tryptophan pathways.

a. PLP is a cofactor for S1P-lyase (S1PL) and regulates S1P abundance and tissue distribution. S1P gradients control lymphocyte trafficking, including retention at sites of inflammation and within lymphoid tissues, which can lead to lymphopenia in the circulation. S1P receptors (S1P-R1,2,3) differentially modulate tight junctions in the endothelium, and reduced S1PL function results in loss of barrier integrity. S1P may
also amplify TNFα, TLR, and NFκB intracellular signaling cascades, further exacerbating leaky vasculature / shock and boosting inflammation, in turn further depleting PLP.

b. PLP is a cofactor for kynureninase in the production of NAD⁺ from tryptophan. When PLP is limiting, NAD⁺ production decreases, limiting energy supply. Concomitantly, kynurenine metabolites (including kynurenine, Kyn, and Kynurenic Acid, KynA) accumulate. Kyn and KynA signal through the Aryl Hydrocarbon Receptor (AhR), resulting in immune suppression and paradoxically increased cytokine production (IL-6, TNFα and IL-10) and amplified signaling through the IL-6 / JAK / STAT axis, potentially resulting in an IL-6 positive feedback loop. In addition, other neurotoxic kynurenine metabolites are produced, including neuroinflammatory 3-hydroxykynurenine and excitotoxic quinolinic acid (not shown).⁴⁰,⁴³

This illustration leaves out the complex cross-talk and cross-amplification between the S1P and AhR downstream effectors. In addition, PLP deficiency also upregulates IL-1β and ROS production via the NLRP3 inflammasome and suppresses type I interferons via serine hydroxymethyltransferase 2 (not shown).

Other inflammatory pathways

PLP suppresses IL-1β production and the production of reactive oxygen species (ROS) by inhibiting the NLRP3 inflammasome, and thus PLP deficiency may predispose to excessive IL-1β secretion and ROS-mediated tissue damage.⁴⁴

PLP regulates serine hydroxymethyltransferase 2 (SHMT2), an enzyme best known for its role in folate metabolism.¹⁹,⁴⁵ In addition, SHMT2 also regulates type I interferon production.⁴⁵ Type I interferons are critical in the early host response to viral infections and may underlie successful host control of viral replication.⁴⁶ However, unregulated expression of type I interferons may result in significant pathology.⁴⁵,⁴⁶ PLP modulates the availability and conformation of SHMT2, and thus is a critical regulator in the type I interferon pathway.⁴⁵ Here again, availability of PLP determines the balance between host control of viral replication, and cytokine-mediated pathology.
Blood clotting and vascular effects of PLP

In 1963, a report in *Nature* designated vitamin B6 as a new anticoagulant.\textsuperscript{47} Treatment with physiological or supraphysiological doses of PLP prolongs bleeding time, reduces platelet aggregation, and decreases thrombin generation.\textsuperscript{20–22} In patients with homocysteinuria and clotting abnormalities, high-dose PLP treatment restored antithrombin III activity to normal, increased factor VII, and decreased beta-thromboglobulin,\textsuperscript{48} while lower doses of PLP were sufficient to reduce D-dimer levels,\textsuperscript{26} although these effects were not seen at the lowest levels of supplementation.\textsuperscript{49}

PLP deficiency may also disrupt blood pressure regulation and compromise cardiac energy metabolism. An animal model of hypertension may be produced simply by decreasing PLP in the diet.\textsuperscript{24} In PLP-deficient rats, production of both renin and angiotensin is significantly increased.\textsuperscript{23} In COVID-19, angiotensin-converting enzyme 2 (ACE2) serves as a receptor for the SARS-CoV-2 virus, and hypertension is a risk factor for severe COVID-19 pathology. Furthermore, even marginal PLP deficiency significantly attenuated citric acid cycle metabolite levels in the heart, suggesting that low PLP levels impair cardiac energy metabolism.\textsuperscript{50} Finally, critically ill patients with inflammation presented about six times more risk of cardiovascular complications when they were PLP-deficient, compared with matched non-PLP-deficient patients.\textsuperscript{29}

PLP as a weapon against COVID-19

Good nutrition and adequate vitamin intake have been recommended for COVID-19 prevention,\textsuperscript{51} with vitamin D specifically implicated in immune protection.\textsuperscript{52} However, PLP deserves particular investigation because it controls the pathways which are specifically dysregulated in COVID-19; because it becomes rapidly and critically depleted during inflammation; and because in supraphysiological doses, it has previously been shown to decrease pro-inflammatory cytokine levels and indicators of coagulopathy.
**Figure 2. The systemic interactions of PLP deficiency with COVID-19 pathology**

**a** Adequate PLP levels allow homeostatic function of 1. the innate immune response, where type I interferons (IFN) restrain viral proliferation and spread (areas of viral infection shown highlighted in yellow), and opposes excessive proinflammatory cytokine secretion; 2. the adaptive immune response, where PLP supports lymphocyte proliferation, appropriate trafficking, and regulated cytokine production; 3. the vascular system, where PLP is essential for endothelial barrier integrity (shown in close-up view), which prevents edema and maintains blood pressure (BP); and acts as an anti-coagulant by regulating platelet aggregation and components of the clotting cascade.

**b** Insufficient PLP leads to dysregulated physiological systems as biochemical flow through multiple pathways is disrupted, intermediates back up, and end-products are not formed. 1. Viral spread (yellow) escapes Type I IFN control due to compromised regulation of SHMT2. 2. Lymphocytes are trapped in lymph nodes and inflamed tissues as SP1-lyase becomes hypofunctional, leading to lymphopenia in the circulation, and...
exacerbating tissue damage through excessive cytokine secretion and ROS production via multiple dysregulated pathways, including S1P, kynurenines, inflammasomes, and lack of downregulatory opposition from Type I IFN. Lymphopenia (from compromised S1P-lyase) and hypofunctional lymphocytes (from immunosuppressive kynurenine accumulation) further contribute to viral spread. 3. The vascular system, already susceptible to direct viral pathology due to expression of ACE2, is further compromised by PLP deficiency as endothelial barrier function fails under the dual assault of S1P dysregulation and excessive proinflammatory cytokines, leading to edema and eventually plummeting BP, shock and organ failure; clots form as the anticoagulant effects of PLP on platelet aggregation and clotting factor activation are lost, in combination with endothelial damage from cytokines and viral cytopathology; and PLP deficiency stimulates hypersecretion of renin and angiotensin, leading to constriction of susceptible vascular beds despite systemic low blood pressure. Callouts illustrate how systemic dysregulation come together to exacerbate organ damage when PLP is deficient. In the brain, seizures refractory to antiepileptic medications may occur in critically ill patients with low PLP, as PLP is required for the biosynthesis of γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter; stokes may occur due to clot formation and emboli; and delirium is exacerbated in ICU patients by the accumulation of neurotoxic kynurenine metabolites, including neuroinflammatory 3-hydroxykynurenine and excitotoxic quinolinic acid. In the lung, viral cytopathology is exacerbated by cytokine- and ROS-mediated tissue damage, and gas exchange is compromised by edema and vascular constriction. In the kidneys, excessive proinflammatory cytokines with or without local viral cytopathology may cause tissue damage, low systemic BP leads to hypoperfusion, and vascular constriction may further compromise perfusion.

PLP regulates homeostatic lymphocyte trafficking, numbers and functions; cytokine secretion and signaling; endothelial barrier integrity, platelet aggregation, and blood clotting parameters (Figure 2). As PLP becomes depleted during infection, normal regulation of these systems is lost – lymphocytes are trapped at inflammatory sites, causing local tissue damage and edema, exacerbated by uncontrolled cytokine release; lymphocytes in the circulation disappear and function poorly; endothelial barrier function deteriorates under the dual assault of S1P dysregulation and IL-6 / TNFα overproduction, causing blood pressure to plummet; increasing renin and angiotensin worsens constriction in lung and kidney vascular beds, leading to organ dysfunction. PLP repletion, or
supraphysiological supplementation, may therefore represent a safe, inexpensive and readily available method of restoring some balance to the dysregulated systems of COVID-19 patients.

Clinical trials in rheumatoid arthritis patients showed that doses of 100 mg of PLP daily, but not 50 mg, were sufficient to decrease plasma TNFα and IL-6 levels. In studies on patients with elevated homocysteine, commonly used dosages of PLP (3 to 25 mg daily) had only moderate effects on bleeding time and platelet aggregation, but doses of 50 mg reduced D-dimer and 300 mg normalized antithrombin III, factor VII and beta-thromboglobulin in patients with elevated homocysteine and coagulopathy. In ICU patients with low PLP levels who developed seizures refractory to antiepileptic medications, intravenous PLP (100 mg every 12 hours) controlled the seizures, and the patients remained seizure-free on 100 mg oral PLP daily.

PLP has an excellent safety profile, although prolonged supraphysiological supplementation may lead to progressive peripheral neuropathy, and a dose of 43 mg/kg per day (> 1 gram per day) in a 4 year old patient with hemophilia was associated with recurrent spontaneous hemarthroses. It should also be noted that very high doses of pyridoxine inhibit the active PLP form in vitro. Therefore, it may be prudent to attempt treatment and trials, at least initially, with the PLP form of the vitamin.

Taken together, the biology and epidemiology of PLP suggest that PLP status may be an additional factor along with age, comorbidities, genetics, and dose and route of infection, affecting susceptibility to SARS-CoV-2 and COVID-19 severity. Only randomized controlled trials at multiple dosages of PLP can establish definitively whether PLP repletion or high-dose supplementation decreases COVID-19 severity, length of hospitalization, requirements for oxygen, ventilation, ICU admission, and mortality. Even a small beneficial effect would reduce the burden on health care systems, as well as to individual patients. However, it will also be important to determine whether pre-existing PLP status affects disease severity and outcome. It would be interesting to correlate PLP levels in banked
patient sera with their length of hospitalization, particularly in vulnerable subgroups such as the very elderly, institutionalized, or people with hypertension. Such data might inform a preventative strategy for vulnerable populations. In vitro experiments could determine whether supplemental PLP ameliorates the deficient type I interferon response to SARS-CoV-2, which would suggest a role for PLP in improving early immunological control and clearance of the virus and therefore in shortening the often protracted course of COVID-19. Improved immunological control could reduce the time during which virus is shed, as well as the magnitude of viral shedding, in both hospitalized and community-dwelling patients, with important implications for public health.

References


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