Mechanistic perspective on the relationship between pyridoxal 5'-phosphate and inflammation

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A variety of inflammatory disease conditions have been found to be associated with low levels of plasma pyridoxal 5'-phosphate (PLP), the active form of vitamin B6, without any indication of a lower dietary intake of vitamin B6, excessive catabolism of the vitamin, or congenital defects in its metabolism. The present review was conducted to examine the existing literature in this regard. Current evidence suggests that the inverse association between plasma PLP and inflammation may be the result of mobilization of this coenzyme to the site of inflammation, for use by the PLP-dependent enzymes of the kynurenine pathway of tryptophan degradation, metabolism of the immunomodulatory sphingolipids, ceramide and sphingosine 1-phosphate, and for serine hydroxymethylase for immune cell proliferation.

INTRODUCTION

Pyridoxal 5'-phosphate (PLP), the active form of vitamin B6, functions as a cofactor for a wide variety of enzymes involved in the metabolism of proteins, lipids and carbohydrates, and in processes essential for the synthesis or metabolism of hemoglobin, neurotransmitters, nucleic acids, one-carbon units, immunomodulatory metabolites, etc.1-5 Vitamin B6 availability also plays a critical role in both the innate and adaptive immune responses.6 A variety of disease conditions have repeatedly been found to be associated with low levels of plasma PLP, including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), cardiovascular disease, deep vein thrombosis, diabetes, and cancer.7-14 An inverse relationship has been found between the inflammatory marker C-reactive protein (CRP) and plasma PLP status among participants of the Framingham Heart Study and the National Health and Nutrition Examination Survey (NHANES),15,16 as well as in case-control and cross-sectional studies on inflammatory conditions such as RA, IBD, or coronary disease.7,8,17-19 In addition, plasma concentration of PLP is negatively associated with the acute-phase protein alpha1-acid glycoprotein, tumor necrosis factor-α, and the proinflammatory cytokine interleukin-6 in RA and IBD.7,8,20

The inverse association between low plasma PLP status and inflammatory diseases has been interpreted to mean that low dietary intake of vitamin B6 confers an increased risk of disease. While this may be true in some instances,21,22 in most studies of inflammatory conditions, including RA, IBD, diabetes, and cancer, there is a lack of correlation between vitamin B6 intake and plasma PLP concentration and no indication of low dietary intake of vitamin B6.7,11,23-25 Despite the lower plasma PLP concentrations, RA patients exhibit normal measures of other indicators of long-term vitamin B6 status, including erythrocyte aspartate transaminase (EAST) activity coefficient and erythrocyte PLP concentration.7,23,26 Urinary excretion of 4-pyridoxic acid, which is a measure of vitamin B6 metabolism, was not correlated with plasma PLP in RA patients, suggesting that low plasma PLP in this condition is not due to increased metabolism of vitamin B6.23 In a rat model of RA, there was also no difference in urinary 4-pyridoxic acid excretion between control animals and those with experimental RA.27 The
temporal relationships between PLP and inflammation are best demonstrated in studies of RA and IBD, both of which are characterized by an overactive immune response accompanied by periods of intense inflammation, that are followed by periods of remission. Plasma PLP concentration of IBD patients with active disease is lower than that of patients in remission. In RA, plasma PLP concentration is inversely correlated with severity of the disease. Thus, it appears that the lower plasma PLP concentration is linked to a dietary inadequacy or to vitamin B6 deficiency in most cases, but rather is due to a metabolic phenomenon inherent to inflammation. Among the participants of the NHANES, the prevalence of low (<20 nmol/L) plasma PLP is about 2.5-fold higher in individuals with higher (>10 mg/L) CRP concentrations than in those with lower (<3 mg/L) CRP concentrations. These data suggest there is a higher need for plasma PLP by the immune system during active inflammation.

PROPOSED ROLE OF PLP IN INFLAMMATION

Current evidence points to the possibility that the inverse association between plasma PLP and inflammation is the result of mobilization of plasma PLP to sites of active inflammation for use by PLP-dependent enzymes that play a role in the inflammatory response. Approximately 80% of the body store of PLP is in muscle, bound to glycogen phosphorylase. The PLP in muscle is not readily released; thus, when there is an increase in demand for PLP during inflammation or when intake of vitamin B6 is low, PLP is supplied by liver and plasma stores. Hence, plasma PLP concentration would be highly susceptible to a sudden increase in PLP demand during an immune response. Mobilization of PLP for inflammatory processes was implied in a study of an animal model of RA, which found that, during active disease, a decrease in PLP was seen in plasma and liver, two readily accessible compartments, but not in other tissues. It is proposed here that the lower plasma PLP observed during inflammation is due to the mobilization of this coenzyme to the site of inflammation for degradation of tryptophan via the kynurenine pathway, metabolism of immunomodulatory sphingolipids, and the proliferation of immune cells.

PLP and indoleamine 2,3-dioxygenase-dependent degradation of tryptophan

Degradation of tryptophan through the indoleamine 2,3-dioxygenase (IDO) pathway is a hallmark of inflammation (Figure 1). IDO is ubiquitous in nonhepatic tissues and is present in an inducible form in myeloid tissues, such as macrophages, dendritic cells, and monocytes. In the liver, tryptophan degradation is initiated by tryptophan 2,3-dioxygenase (TDO), which catalyzes the same reaction as IDO, but is not involved in inflammation. IDO is induced by a number of proinflammatory molecules, including interferon gamma, lipopolysaccharide, and CD40 ligands.

The involvement of IDO-dependent degradation of tryptophan in immune response and immune tolerance has been demonstrated in multiple studies. A role for the TDO pathway in immune tolerance was demonstrated by showing that the activity of IDO is required to prevent T-cell-mediated rejection of allogeneic fetuses in pregnant mice. Induction of IDO-dependent tryptophan degradation in macrophages and dendritic cells allows them to block unwanted T-cell responses by suppressing their activation, thus resulting in immune tolerance. In a mouse model, IDO activity suppresses the development of RA and prevents tumor cells from being rejected by mice that have been immunized against the tumor. Inflammation response and mortality increase in colitis upon inhibition of IDO expression.

An important aspect of IDO-dependent degradation of tryptophan is that PLP functions as a cofactor for many of the enzymes of this pathway (Figure 1). These include enzymes that carry out the conversion of kynurenine to kynurenic acid or anthranilic acid, as well as the conversion of 3-hydroxy kynurenine to 3-hydroxyanthranilic acid or xanthurenic acid. It has been proposed that the IDO-dependent effect is linked to depletion of tryptophan, which will result in cellular starvation for this amino acid. However, there are a number of studies that show that the downstream products of tryptophan degradation, many of which are formed through the action of PLP-dependent enzymes, mimic the actions of the IDO-dependent immune modulation as described below. Activity of the flavin-dependent enzyme kynurenine monoxygenase is necessary for development of immune tolerance in vivo, suggesting a role for the downstream products of kynurenine. It has been shown that even in the absence of a functional IDO, addition of the tryptophan degradation product kynurenine can modulate immune response in vitro. 3-hydroxyanthranilic acid (HAA), a product of a PLP-dependent reaction (Figure 1), induces apoptosis of human T helper-1 (TH1) cells that are responsible for autoimmune response, but not that of T helper-2 (TH2) cells responsible for immunity to extracellular pathogens. Multiple tryptophan metabolites, HAA, 3-hydroxykynurenine, and N-3,4, dimethoxycinnamoyl) anthranilic acid (3,4-DAA), which is a synthetic derivative of anthranilic acid, inhibit production of the TH1 proinflammatory cytokines interleukin-2, interferon gamma, and tumor necrosis factor-α, thereby skewing the T-cell profile from TH1 to TH2. HAA also induces the death of T-cells activated by...
CD345 and inhibits T-cell proliferation by cytokines. It is shown that 3,4-DAA administration can reverse the paralysis of mice with experimental autoimmune encephalomyelitis, which is a model for multiple sclerosis. Rejection of an implanted heart in a rat model was shown to be delayed by administration of HAA in combination with donor’s bone marrow cells prior to the implant, potentially by eradicating the T-cell response to the graft. In mice, kynurenine and 3-hydroxykynurenine and xanthurenic acid, which is a product of a PLP-dependent reaction, contribute to development of immunotolerance in airway inflammation in allergic asthma. Additional evidence for involvement of PLP-dependent reactions of the kynurenine pathway in immune response comes from the accumulation of quinolate at micromolar concentrations or greater in lymphoid tissues and macrophages during immune response. Thus, it appears that tryptophan degradation via PLP-dependent reactions is involved in developing immune tolerance, suppression of inflammation, and reducing tissue damage due to immune response.

Recent studies demonstrate the potential mechanisms by which kynurenine metabolites regulates immune response. Kynurenine and two of its metabolites, kynurenic acid and xanthurenic acid, have been shown to be endogenous ligands for the arylhydrocarbon receptor. These processes that may reduce immune surveillance of malignant cells and promote tumor progression. Kynurenic acid is also a ligand of the G protein-coupled receptor, GPR35, which is primarily expressed in immune cells and gastrointestinal tissues. Kynurenic acid has been shown to suppress the lipopolysaccharide-induced tumor necrosis factor-α secretion in mice and cultured immune cells that express GPR35.

In humans, IDO-dependent degradation of tryptophan has been implicated as an important modulator of activity in a variety of diseases, including immune deficiency syndromes, amyotrophic lateral sclerosis, RA, coronary artery disease, and various types of cancers, many of which have also been associated with low plasma PLP status. Most human cancers express IDO and TDO constitutively and it has been reported that women with early-stage breast cancer have higher tryptophan degradation than controls, suggesting a means by which some neoplasms might evade the immune system. Occur-
rence of increased tryptophan degradation in these diseases is manifested by higher plasma kynurenine: tryptophan concentration ratios. Participation of PLP-dependent enzymes during enhanced tryptophan degradation of these diseases is suggested from the imbalance of metabolites from PLP-dependent reactions in cases compared to healthy controls. A high kynurenine: tryptophan ratio has been reported to predict major coronary events, and there is an inverse relationship between plasma PLP concentration and 3-hydroxykynurenine, one of the degradation products of tryptophan, in coronary heart disease patients with one or more inflammation markers in the upper tertile.

**PLP-dependent metabolism of sphingolipids**

Reduced availability of PLP is associated with reduction in several components of the immune response, including significant decreases in lymphocyte numbers, especially T-helper cells and IL-2 production in humans. Lymphocytes isolated from vitamin B₆-deficient subjects also show reduction of lymphoproliferative responses to mitogens that activate both T and B cells when grown in a culture medium containing adequate concentration of PLP. This has been attributed to the lower numbers of T-helper cells and IL-2 production in humans. Lymphocytes isolated from vitamin B₆-deficient subjects. Maturation and egress of lymphocytes, especially T-cells, from thymus and lymph nodes relies on the gradient of sphingosine-1-phosphate (S1P). PLP-dependent enzymes play a major role in the synthesis and breakdown of S1P, which is a potent metabolite that regulates inflammation and immune response processes such as cell growth, survival, differentiation, lymphocyte trafficking, vascular integrity, and cytokine and chemokine production. PLP is required for the activity of serine palmitoyltransferase that catalyzes the condensation of serine and palmitoyl CoA into 3-keto-dihydrosphingosine, which is then converted to S1P in a series of reactions. PLP is also a cofactor for sphingosine-1-phosphate lyase, which irreversibly cleaves S1P to regulate its concentration. A gradient of S1P is required for lymphocyte egress from thymus and peripheral lymphoid organs, which is maintained by S1P lyase. Administration of vitamin B₆ antagonist 4' deoxypyridoxine interferes with the S1P gradient, results in accumulation of mature lymphocytes in the thymus, and depletes B- and T-lymphocytes from lymph causing lymphopenia. These conditions can be reversed by providing excess vitamin B₆ in the diet. During inflammation, S1P concentration increases in the inflamed peripheral tissues, which functions as a chemoattractant for the inflammatory cells.

One of the intermediate products during the synthesis of S1P from 3-keto-dihydrosphingosine is ceramide, which plays an important role in inflammatory processes. Ceramide functions as a second messenger mediating the effects of tumor necrosis factor-α and interferon-γ on programmed cell death and regulating senescence. An increase in cellular ceramide concentration is observed in cystic fibrosis, experimental autoimmune encephalomyelitis, and diet-induced insulin resistance, all of which are marked by chronic inflammation. The importance of ceramide in these diseases is demonstrated by the fact that manipulation of ceramide concentration via inhibition of serine palmitoyl transferase or mutation of sphingomyelinase, reverses the pathology of the disease. Ceramide-1-phosphate, which is derived from ceramide, activates mast cells that mediate inflammation.

Thus, it is possible that a higher demand exists for PLP during inflammation due to the role of PLP in the synthesis of S1P and ceramide, and maintenance of S1P gradient.

**Other PLP-dependent reactions necessary for immune cell proliferation**

There is increased turnover of immune cells in inflammation. Proliferation of immune cells requires N5, N10-methylene tetrahydrofolate (a key folate intermediate and the source of carbons 2 and 8 of purine), as well as the methyl group for the synthesis of thymidylate and methionine synthesized in a PLP-dependent reaction via serine hydroxyl methyltransferase (SHMT). Studies on vitamin B₆-deficient mice have shown that DNA synthesis, as determined by the incorporation of labeled precursors of nucleic acid into DNA, depends on the availability of vitamin B₆. Adequate vitamin B₆ nutrition is required for the activity of SHMT and for protein turnover.

The addition of vitamin B₆ antagonist 4' deoxypyridoxine inhibits induction of SHMT in activated lymphocytes. SHMT activity is 5- to 20-fold higher under conditions where there is high cell division, as in leukemia, and in activated lymphocytes. Hence, during inflammation, the increased proliferation of immune cells may result in an increased requirement for PLP.

**CONCLUSION**

A variety of inflammatory disease conditions, including RA, IBD, and diabetes, have been found to be associated with low concentrations of plasma PLP, the active form of vitamin B₆, without any indication of a lower dietary intake of vitamin B₆, excessive catabolism of the vitamin, or congenital defects in its metabolism. Current evidence suggests the inverse association between plasma PLP and inflammation may be the result of mobilization of this coenzyme into inflammatory sites for PLP-dependent reactions of tryptophan degradation via the kynurenine
pathway for developing immune tolerance, generation of the immunomodulatory sphingolipids SIP and ceramide, maintenance of the gradient of SIP, and for the proliferation of immune cells. The majority of the body store of PLP is in muscle\(^1\) and is not readily released when there is an increase in demand for PLP during inflammation\(^2\) or when intake of vitamin B\(_6\) is low\(^3\); instead, PLP is supplied by liver and plasma.\(^2\) This makes plasma PLP highly susceptible to the higher demand for this coenzyme during inflammation. The possibility that PLP is mobilized for these important aspects of the immune response, however, needs to be verified by targeted studies using both animal and cell culture models.

Acknowledgments

Funding. Support was received from United States Department of Agriculture cooperative agreement 51520-008-04S. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the United States Department of Agriculture.

Declaration of interest. The authors have no relevant interests to declare.

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