HIV/AIDS

# Insights into Therapy: Tryptophan Oxidation and HIV Infection

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Published 19 May 2010; Volume 2 Issue 32 32ps23

**New data from Favre and colleagues strengthen the link between activation of the tryptophan oxidation (TOx) pathway—via the indoleamine 2,3-dioxygenase enzymes IDO1 and IDO2—and chronic inflammation in progressive HIV disease. It can now be appreciated that a pathogenic TOx activation cycle exists in HIV. TOx regulation is a therapeutic target for other diseases, such as cancer and autoimmune disorders. Here TOx control is examined with an eye to eventual therapeutic intervention in HIV disease.**

## HIV AND BEYOND

Microbial products, including lipopolysaccharide (LPS), the immunostimulatory endotoxin from the outer membrane of Gram-negative bacteria, are known to induce the tryptophan oxidation (TOx) pathway, which is the key degradation route for the essential amino acid L-tryptophan (L-Trp). The first (and rate-limiting) step in the pathway—the conversion of L-Trp to *N*-formyl-kynurenine—is catalyzed by the indoleamine 2,3-dioxygenases IDO1 and IDO2, two enzymes that are synthesized in both immune cells and other tissues and are the gatekeepers of the immune response via the TOx pathway. In this issue of *Science Translational Medicine*, Favre and colleagues add to a growing body of knowledge linking LPS-induced endotoxemia (the presence of bacterial endotoxins in the blood), activation of the TOx pathway, and the chronic inflammatory state of progressive HIV disease (*1*). Researchers currently are developing targeted therapies to alter TOx activation in a number of disease contexts, and a richer understanding of pathway regulatory mechanisms will help to guide these drug discovery and development efforts. Here various aspects of TOx regulation are examined from the perspective of potential therapeutic intervention.

## TOX PATHWAY PERMUTATIONS

As an essential amino acid, Trp cannot be synthesized in humans and must be obtained through the diet. Dietary Trp feeds into three major metabolic pathways: (i) protein synthesis; (ii) synthesis of the neurotransmitter serotonin; and (iii) TOx, to form either energy [adenosine triphosphate (ATP)], nicotinamide and related compounds [nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP)], or other side products (such as xanthurenic acid). In humans, oxidative catabolism of Trp can be initiated by one of three different enzymes: tryptophan-2,3-dioxygenase (TDO2) in the liver, as well as IDO1 and IDO2, which have incompletely overlapping nonhepatic tissue distributions. These three enzymes open the indole ring, causing the irreversible loss of Trp (*2*). The TOx pathway is interconnected with other metabolic networks (*3*) and is variously regulated in a number of tissues.

The liver is thought to be the only organ with substantial TDO2 activity, and this enzyme is not believed to drive the immunoregulatory effects of TOx. Instead, TDO2 is induced by dietary protein and corticosteroids, but not by cytokines such as interferon-γ (IFN-γ) (*4*). Recent work by Schmidt *et al.* demonstrated an in vitro immunoregulatory effect associated with TDO2 activity, which may implicate the activity of pathway metabolites more than a clinically significant in vivo effect, although a contribution to immunomodulation within the liver has not been excluded (*5*). The hepatic end products generated via activation of this pathway are ATP, $CO_2$, and water.

The metabolic pathway initiated by the extrahepatic enzymes IDO1 and IDO2 is shown in Fig. 1. IDO1 and IDO2 are inducible and less substrate-specific for Trp than TDO2. While there are many cytokine inducers of IDO-mediated TOx, the most effective pathway inducer appears to be IFN-γ (*4*). That said, there are important tissue-specific differences in cytokine induction, leading to different tissue responses to the same systemic signals. For example, in the brain it appears that interleukin-6, and not IFN-γ, drives IDO induction (*6*). Elucidation of the differences in IDO1- versus IDO2-induced cytokine responses will require further study. Many different microbial infections—bacterial, viral, and parasitic—have been implicated in IDO-induced TOx (*7*), and a number of microbial products can directly induce the pathway. Bacterial LPS and the HIV-Nef and HIV-Tat proteins are among the best-studied but are not likely to be unique in this capability.

The second enzyme in the TOx pathway is arylformamidase (AFMID), which is responsible for converting the dioxygenase product (*N*-formyl-kynurenine) to L-kynurenine (L-KYN). L-KYN is subsequently converted to 3-hydroxy-L-kynurenine (3-HKA) by kynurenine 3-monooxygenase (KMO), and 3-HKA is metabolized to 3-hydroxy-anthranilic acid (3-HAA) by kynureninase (KYNU). Favre *et al.* noted that the 3-HKA and 3-HAA metabolites have effects on the T helper 17 cell ($T_H17$)–to–regulatory T cell ($T_{reg}$) ratio in vitro (*1*). It appears that 3-HAA is removed from the tissue microenvironment only by the action of another dioxygenase, 3-hydroxyanthranilate 3,4-dioxygenase (HAAO). This means that, in order to prevent the in vivo accumulation of 3-HAA and its potential effects on T cell ratios, the organism is dependent on the relative activity of this specific enzyme. Outside of the central nervous system (CNS), TOx pathway activation does not lead to the accumulation of quinolinic acid (QA), suggesting that the relative activities of the aminocarboxymuconate semialdehyde decarboxylase (ACMSD) and quinolinate phosphoribosyltransferase (QPRT) enzymes are sufficient to divert QA to other products. In a multistep process initiated by QPRT, the downstream metabolism of QA results in the formation of NAD, NADP, and nicotinamide. In general, it is presumed that the QPRT-driven pathway to nicotinamide and nicotinamide nucleotides occurs preferentially in the extrahepatic TOx pathway, and the ACMSD-driven subpathway to ATP appears to occur preferentially by hepatic TOx, although further detailed studies may alter this understanding (*4*). In the CNS, QA is produced locally in response to either local or systemic inflammatory signals and stimulates the *N*-methyl-D-aspartate (NMDA) receptor, a key component in learning and memory

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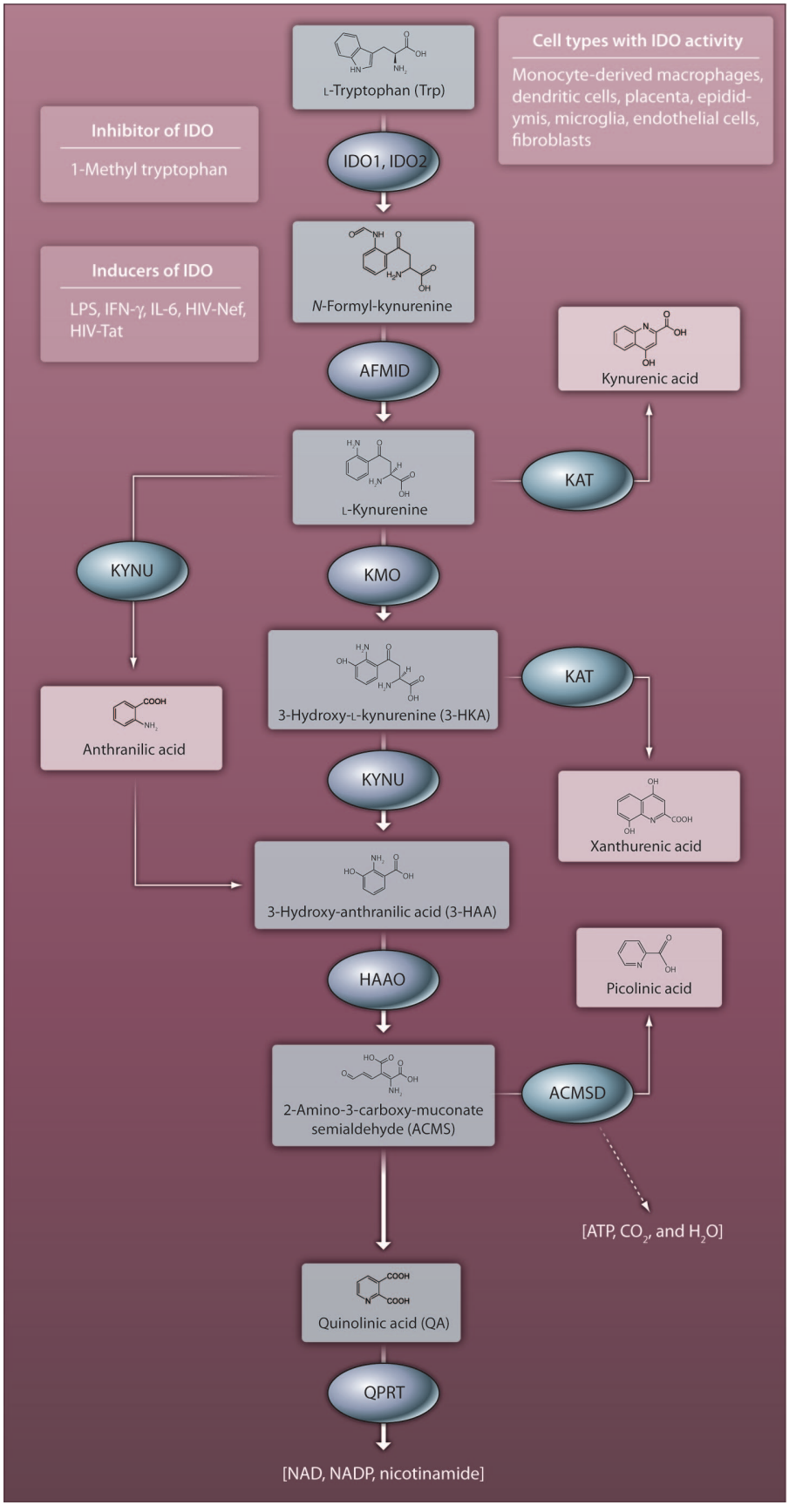


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**Fig. 1. The TOx pathway.** The extrahepatic human TOx pathway is shown along with IDO inhibitors and inducers. IDO1, indoleamine 2,3-dioxygenase 1; IDO2, indoleamine 2,3-dioxygenase 2; AFMID, arylformamidase; KMO, kynurenine 3-monooxygenase; KAT, kynurenine aminotransferase; KYNU, kynureninase; HAAO, 3-hydroxyanthranilate 3,4-dioxygenase; ACMSD, aminocarboxymuconate semialdehyde decarboxylase; QPRT, quinolinate phosphoribosyltransferase.

(*8*). Overstimulation of NMDA receptors can cause neurotoxic effects that have been implicated in neurological diseases.

In HIV-infected patients, the amounts of QA measured in the brain and cerebrospinal fluid (CSF) are greatly increased compared to those in controls. In one study (*8*), a 100-fold variation in the amounts of QA in the CSF and brain tissue was detected among HIV patients, and these elevations did not correlate with serum QA concentrations (*8*). These data suggest that the degradation of Trp via the TOx pathway is stalled in the CNS. This tissue-specific accumulation of QA may result from a relatively low amount of QPRT activity compared to that of HAAO and other upstream enzymes (Fig. 1). Recent work by Connor and colleagues revealed that systemic stimuli such as LPS induce IDO and KMO activities in the mammalian brain but do not alter kynurenine aminotransferase (KAT) activity (*6*). A critical question is whether QPRT is also inducible and under what circumstances, because this effect would have the potential to draw down the local QA concentrations and interrupt the neurotoxic effects associated with high concentrations of this metabolite.

## OVERACTIVE TOX

The new work by Favre *et al.* builds on the relationship between LPS and TOx first reported in 1978 by Yoshida and Hayaishi (*9*) and the findings of Brenchley and others (in 2006) that HIV infection is marked by chronically elevated circulating amounts of LPS (*10*). Favre *et al.* place the observations of Terness and others regarding TOx metabolites into a new disease-specific context (*11*, *12*) and provide evidence that the intermediate TOx metabolites 3-HKA and 3-HAA are regulators of a specific T lymphocyte subset ratio; namely, the $T_H17$:$T_{reg}$ ratio (*1*). The loss of a normal $T_H17$:$T_{reg}$ ratio in the gut is associated with increased LPS endotoxemia and persistent induction

of TOx overstimulation in the setting of HIV (*13*). These data add to an impression that chronic TOx induction during HIV infection is a central pathogenic process that results in the disruption of normal T lymphocyte function and is driven by a combination of endogenous cytokines (particularly IFN-γ), viral products (the HIV-Tat and HIV-Nef proteins), and bacterial products (including LPS) (Fig. 2).

The detrimental effects of TOx induction in progressive HIV disease contrast with the demonstrable benefit of TOx activation in the normal placenta and the presumed value of TOx in limiting microbial access to Trp in some nonviral infections (*14*). In HIV infection, the mediators of TOx pathogenicity appear to be the altered concentrations of the pathway products, in particular Trp, 3-HKA, 3-HAA, and QA.

The growing interest in targeted therapies to alter TOx activation in cancer management (*15*) needs to be extended to HIV infection, as well as reproduction, organ transplantation, neurodegenerative diseases, and autoimmune syndromes, in which TOx pathway activation has also been implicated in aspects of disease pathogenesis (*16*–*20*).

## TOX THERAPEUTIC TACTICS

There is accumulating evidence that small molecules that participate in the TOx pathway are capable of acting as immune regulators outside of their direct role in amino acid catabolism. Further study of the tissue-specific bioavailability of 3-HKA and 3-HAA is needed to confirm their in vivo significance in pathogenic immunoregulatory signaling; however, what emerges through the work to date is a model for chronic activation of IDO-associated TOx, leading to T lymphocyte dysregulation in HIV infection (Fig. 2). Given the number of diseases in which TOx activation appears to be linked to pathogenesis, it is likely that multiple clinical trials will emerge to test agents that might therapeutically manipulate this pathway. Oral Trp loading is one approach to avoid in going forward, except perhaps in a carefully controlled research setting, because it has been associated with both an increase in circulating TOx pathway intermediates (*21*) and the still poorly understood phenomenon of eosinophilia-myalgia syndrome (*22*).

As with any metabolic pathway, the pharmacological regulation of extrahepatic TOx can be approached in several ways. The approach that is currently being tried is inhibition of the first and rate-limiting step in the TOx pathway, namely IDO activity (both IDO1 and IDO2). This is being pursued with the competitive inhibitor 1-methyl-D-tryptophan (D-1MT), and there are currently at least four D-1MT trials (phase 1 and 2) in the clinical oncology domain (*23*). D-1MT was also used in a study of SIV–infected macaques, an animal model of HIV, with some benefit (*24*). A number of other IDO inhibitory molecules have been studied, but they have not yet progressed to clinical trials (*25*). There also is interest in the use of Trp catabolite mimetics, such as 3,4-DAA [*N*-(3,4-dimethyoxycinnamoyl) anthranilic acid], which is orally active and may represent a lead compound for inhibiting autoreactive $T_H1$ cells in autoimmune diseases (*20*). Furthermore, it may be desirable in some therapeutic settings to differentially regulate IDO1 and IDO2 (*26*). Because a functional *IDO2* gene product is not expressed in as many as 25 to 30% of certain human populations (*27*), the consequences of this natural experiment need to be better understood, so that they may guide our evolving understanding of how to successfully use pharmacological agents to inhibit this pathway.

Other conceptual therapeutic approaches that target TOx include (i) diverting the pathway from toxic intermediates or

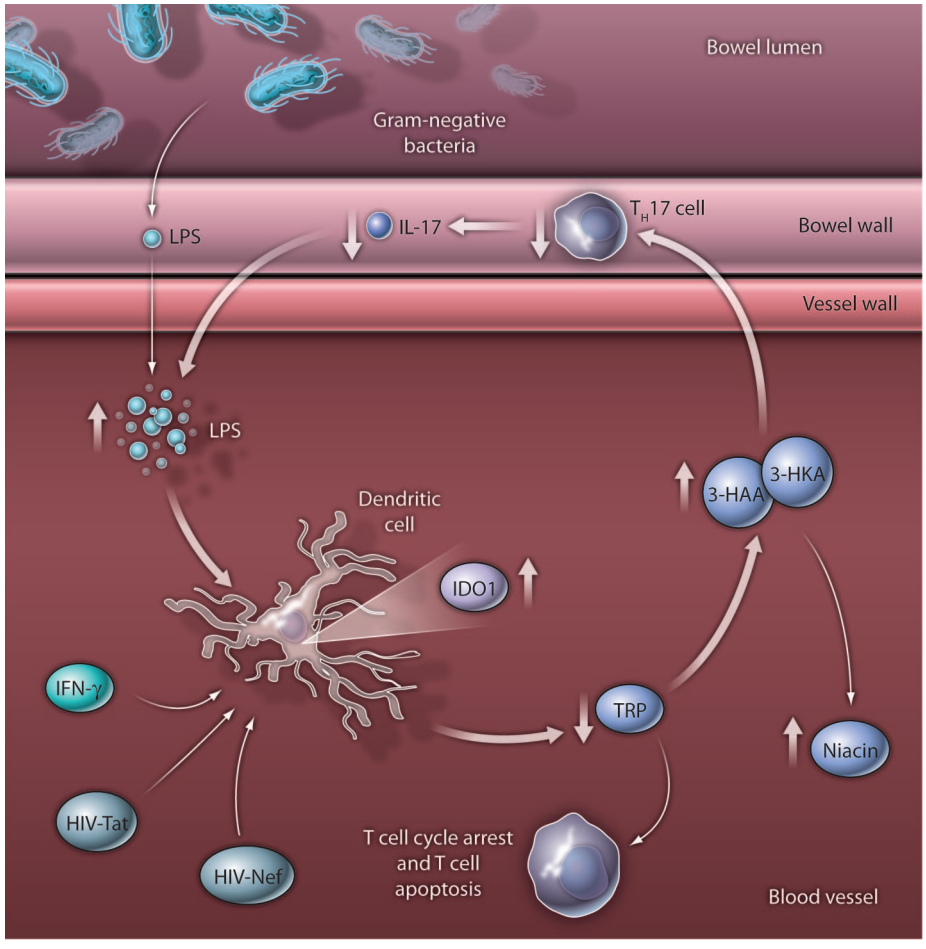


**Fig. 2. TOx activation cycle in HIV.** Data from Favre *et al.* and other sources (*4*, *10*, *11*, *13*, *27*) are summarized in this model of chronic IDO activity in HIV infection, leading to LPS endotoxinemia (abnormally high LPS concentrations in blood) and T lymphocyte dysregulation. The activation cycle in progressive HIV infection is initiated in dendritic cells (DC) and other tissues via upregulated IDO activity induced by IFN-γ and HIV antigens. This in turn lowers available Trp and increases 3-HAA and 3-HKA, which is expected to result in lower $T_H17$ cell concentrations in the bowel wall. The decrease in $T_H17$ cells and local IL-17 concentrations in the bowel wall diminishes the body's ability to prevent the translocation of LPS from the Gram-negative bacteria, which enters the circulation and contributes to further IDO-associated TOx. Other affects of decreased Trp include T cell–cycle arrest and apoptosis, and an increase in the concentration of niacin.

end products by inducing benign metabolic side-product formation, (ii) inducing downstream pathway activity to drive end-product formation and avoid the accumulation of toxic intermediates, and (iii) feedback inhibition of the pathway via increased end-product concentrations. On the basis of data that suggested that HIV-infected patients display a metabolic drive toward increased available nicotinamide (*28*), we initiated a very small clinical trial of oral nicotinamide in part to provide potential feedback inhibition of the TOx pathway (*29*). We found that oral nicotinamide administration resulted in increased circulating Trp in HIV-infected patients, but followup is needed to determine the clinical implications of this result (*4*, *29*). Nicotinamide and other endogenous TOx-related compounds may ultimately prove to be useful adjuncts to therapy or lead compounds for the development of new pharmacological agents to treat HIV and other diseases associated with TOx-induced pathogenesis.

## REFERENCES AND NOTES


1. D. Favre, J. Mold, P. W. Hunt, B. Kanwar, P. Loke, L. Seu, J. D. Barbour, M. M. Lowe, A. Jayawardene, F. Aweeka, Y. Huang, D. C. Douek, J. M. Brenchley, J. N. Martin, F. M. Hecht, S. G. Deeks, J. M. McCune, Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of Th17 to regulatory T cells in HIV disease. *Sci. Transl. Med.* **2**, 32ra36 (2010).
2. H. J. Ball, H. J. Yuasa, C. J. Austin, S. Weiser, N. H. Hunt, Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *Int. J. Biochem. Cell Biol.* **41**, 467–471 (2009).
3. A. J. Muller, P. A. Scherle, Targeting the mechanisms of tumoral immune tolerance with small-molecule inhibitors. *Nat. Rev. Cancer* **6**, 613–625 (2006).
4. K. Schroecksnadel, R. Zangerle, R. Bellmann-Weiler, K. Garimorth, G. Weiss, D. Fuchs, Indoleamine-2, 3-dioxygenase and other interferon-gamma-mediated pathways in patients with human immunodeficiency virus infection. *Curr. Drug Metab.* **8**, 225–236 (2007).
5. S. K. Schmidt, A. Müller, K. Heseler, C. Woite, K. Spekker, C. R. MacKenzie, W. Däubener, Antimicrobial and immunoregulatory properties of human tryptophan 2,3-dioxygenase. *Eur. J. Immunol.* **39**, 2755–2764 (2009).
6. T. J. Connor, N. Starr, J. B. O'Sullivan, A. Harkin, Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: A role for IFN-gamma? *Neurosci. Lett.* **441**, 29–34 (2008).
7. C. R. MacKenzie, K. Heseler, A. Müller, W. Däubener, Role of indoleamine 2,3-dioxygenase in antimicrobial defence and immuno-regulation: Tryptophan depletion versus production of toxic kynurenines. *Curr. Drug Metab.* **8**, 237–244 (2007).
8. M. P. Heyes, K. Saito, A. Lackner, C. A. Wiley, C. L. Achim, S. P. Markey, Sources of the neurotoxin quinolinic acid in the brain of HIV-1-infected patients and retrovirus-infected macaques. *FASEB J.* **12**, 881–896 (1998).
9. R. Yoshida, O. Hayaishi, Induction of pulmonary indoleamine 2,3-dioxygenase by intraperitoneal injection of bacterial lipopolysaccharide. *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3998–4000 (1978).
10. J. M. Brenchley, D. A. Price, T. W. Schacker, T. E. Asher, G. Silvestri, S. Rao, Z. Kazzaz, E. Bornstein, O. Lambotte, D. Altmann, B. R. Blazar, B. Rodriguez, L. Teixeira-Johnson, A. Landay, J. N. Martin, F. M. Hecht, L. J. Picker, M. M. Lederman, S. G. Deeks, D. C. Douek, Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* **12**, 1365–1371 (2006).
11. P. Terness, T. M. Bauer, L. Röse, C. Dufter, A. Watzlik, H. Simon, G. Opelz, Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: Mediation of suppression by tryptophan metabolites. *J. Exp. Med.* **196**, 447–457 (2002).
12. T. Hayashi, J. H. Mo, X. Gong, C. Rossetto, A. Jang, L. Beck, G. I. Elliott, I. Kufareva, R. Abagyan, D. H. Broide, J. Lee, E. Raz, 3-Hydroxyanthranilic acid inhibits PDK1 activation and suppresses experimental asthma by inducing T cell apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 18619–18624 (2007).
13. D. Favre, S. Lederer, B. Kanwar, Z. M. Ma, S. Proll, Z. Kasakow, J. Mold, L. Swainson, J. D. Barbour, C. R. Baskin, R. Palermo, I. Pandrea, C. J. Miller, M. G. Katze, J. M. McCune, J. Luban, Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection. *PLoS Pathog.* **5**, e1000295 (2009).
14. E. R. Pfefferkorn, M. Eckel, S. Rebhun, Interferon-gamma suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophan. *Mol. Biochem. Parasitol.* **20**, 215–224 (1986).
15. A. J. Muller, J. B. DuHadaway, P. S. Donover, E. Sutanto-Ward, G. C. Prendergast, Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat. Med.* **11**, 312–319 (2005).
16. C. Uyttenhove, L. Pilotte, I. Théate, V. Stroobant, D. Colau, N. Parmentier, T. Boon, B. J. Van den Eynde, Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat. Med.* **9**, 1269–1274 (2003).
17. A. L. Mellor, P. Chandler, G. K. Lee, T. Johnson, D. B. Keskin, J. Lee, D. H. Munn, Indoleamine 2,3-dioxygenase, immunosuppression and pregnancy. *J. Reprod. Immunol.* **57**, 143–150 (2002).
18. G. Brandacher, R. Margreiter, D. Fuchs, Clinical relevance of indoleamine 2,3-dioxygenase for alloimmunity and transplantation. *Curr. Opin. Organ Transplant.* **13**, 10–15 (2008).
19. G. J. Guillemin, B. J. Brew, C. E. Noonan, O. Takikawa, K. M. Cullen, Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus. *Neuropathol. Appl. Neurobiol.* **31**, 395–404 (2005).
20. M. Platten, P. P. Ho, S. Youssef, P. Fontoura, H. Garren, E. M. Hur, R. Gupta, L. Y. Lee, B. A. Kidd, W. H. Robinson, R. A. Sobel, M. L. Selley, L. Steinman, Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. *Science* **310**, 850–855 (2005).
21. C. M. Forrest, G. M. Mackay, N. Stoy, M. Egerton, J. Christofides, T. W. Stone, L. G. Darlington, Tryptophan loading induces oxidative stress. *Free Radic. Res.* **38**, 1167–1171 (2004).
22. S. R. Daniels, J. I. Hudson, R. I. Horwitz, Epidemiology of potential association between L-tryptophan ingestion and eosinophilia-myalgia syndrome. *J. Clin. Epidemiol.* **48**, 1413–1427, discussion 1429–1440 (1995).
23. Information produced by a search of D-1MT on ClinicalTrials.gov, a Web-based registry of federally and privately supported clinical trials conducted in the United States and around the world: http://clinicaltrials.gov/ (accessed 4 April 2010).
24. A. Boasso, M. Vaccari, D. Fuchs, A. W. Hardy, W. P. Tsai, E. Tryniszewska, G. M. Shearer, G. Franchini, Combined effect of antiretroviral therapy and blockade of IDO in SIV-infected rhesus macaques. *J. Immunol.* **182**, 4313–4320 (2009).
25. U. F. Röhrig, L. Awad, A. Grosdidier, P. Larrieu, V. Stroobant, D. Colau, V. Cerundolo, A. J. Simpson, P. Vogel, B. J. Van den Eynde, V. Zoete, O. Michielin, Rational design of indoleamine 2,3-dioxygenase inhibitors. *J. Med. Chem.* **53**, 1172–1189 (2010).
26. A. K. Witkiewicz, C. L. Costantino, R. Metz, A. J. Muller, G. C. Prendergast, C. J. Yeo, J. R. Brody, Genotyping and expression analysis of IDO2 in human pancreatic cancer: A novel, active target. *J. Am. Coll. Surg.* **208**, 781–787, discussion 787–789 (2009).
27. The National Center for Biotechnology Information Single Nucleotide Polymorphism database: http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=169355 (accessed 4 April 2010).
28. M. F. Murray, Tryptophan depletion and HIV infection: A metabolic link to pathogenesis. *Lancet Infect. Dis.* **3**, 644–652 (2003).
29. M. F. Murray, M. Langan, R. R. MacGregor, Increased plasma tryptophan in HIV-infected patients treated with pharmacologic doses of nicotinamide. *Nutrition* **17**, 654–656 (2001).
30. M.F.M. is an inventor on U.S. patent 7,012,086, "Treatment of retrovirus induced derangements with niacin compounds."


10.1126/scitranslmed.3001082

**Citation:** M. F. Murray, Insights into therapy: Tryptophan oxidation and HIV infection. *Sci. Transl. Med.* **2**, 32ps23 (2010).