

# Activation of the Kynurenine Pathway at the Blood-Brain Barrier: A Mechanism For Persistence of HIV in the Brain?

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## Abstract

**Introduction:** Perivascular macrophages/microglia are considered to be the primary source/reservoir of productive HIV brain infection. The reasons for the persistence of HIV infection in such cells are unclear. Quinolinic acid (QUIN) is a neurotoxic product of the kynurenine pathway (KP) and is related to HIV infection. Activation of the KP leads to immune tolerance: depletion of tryptophan by IDO the first enzyme of the KP and by KP products including QUIN. We hypothesized that KP activation would be important in HIV persistence on the brain. However, it is unknown whether the cells associated with perivascular macrophages/microglia have functional enzymes of the KP present to be able to lead to an immune tolerant microenvironment. The KP expression is only known in macrophages/microglia and astrocytes. We therefore sought to map KP expression in human primary cultured Blood-Brain Barrier (BBB) Endothelial Cells (EC) & pericytes.

**Methods:** Cells were stimulated for 24 h with vehicle alone, human cytokines interferon-gamma, tumour necrosis factor-alpha or both. To assess downstream KP catabolism in HBMVEC, HBMVPC (BBB cells) and HDMEVC (non-BBB EC), cells were stimulated as above in the presence of the KP intermediates 3-hydroxyanthranilic acid or QUIN. KP metabolites in culture supernatants were quantified by HPLC & GC-MS. RT-PCR was performed on cDNA reverse-transcribed from total cell RNA using primer sets for 5 KP enzymes (IDO, KMO, KYNU, HAAO & QPRTase).

**Results:** By RT-PCR & HPLC/GC-MS, BBB EC have IDO and partial KP expression. They synthesize kynurenic acid (KA) constitutively & kynurenine (KYN) after immune activation. Pericytes also have IDO and partial KP expression. They produce small amounts of picolinic acid & after immune activation, KYN. SV40-BBB EC show no KP expression. Human umbilical vein EC express only low levels of KA after immune activation. Human dermal microvascular EC KP expression resembles that in BBB EC.

**Conclusions:** Immune activation of the cells associated with perivascular macrophages/microglia at the BBB leads to significant KP activation resulting in immune tolerance. This is likely an important contributor to persistence of HIV in the brain.

## Introduction

The kynurenine pathway (KP) is a major degradative pathway of L-Tryptophan (L-Trp) that ultimately leads to the production of NAD. The KP has been associated with many inflammatory brain diseases, especially AIDS Dementia Complex (ADC). It is involved in the killing of pathogenic organisms, development of immune tolerance and tumor evasion and the pathogenesis of cerebral malaria in a murine model.

Significant activation of the KP occurs with the interferons (IFNs), especially IFN-gamma (IFN-γ). There are three dominant products of the KP: quinolinic acid (QUIN), kynurenic acid (KA) and kynurenine (KYN). Not all cells, however, have all the component enzymes present to produce these and other KP products. The KP is fully expressed in monocytic lineage cells such as macrophages and microglia. Astrocytes lack an intermediate enzyme, kynurenine monooxygenase (EC 1.14.13.9), so they produce KA and KYN but not QUIN. The expression of the KP in other cells is unknown.

Given the importance of the KP in inflammatory brain diseases and that the blood-brain barrier (BBB) endothelial cell (EC) is the first brain-associated cell that a cerebral blood-borne pathogen encounters, we sought to determine which KP enzymes are present at the BBB by examining human primary cultured brain microvascular ECs and pericytes. For comparison we also examined EC from umbilical vein and dermis.

We show here that KP enzyme expression in BBB EC and pericytes differs to that which we have previously shown in astrocytes, and that the major products of the constitutively expressed KP in BBB EC is KA, and after immune activation, KYN. We discuss the implications of this for inflammatory brain disease pathogenesis.

## Hypothesis

The kynurenine pathway is expressed in cells of the blood-brain barrier.

## Aim

- To use RT-PCR to map constitutive and induced expression of KP enzymes in BBB cells;
- To use HPLC/GC-MS to quantify KP metabolites produced constitutively & after immune activation of BBB cells

## Conclusion

The KP is expressed at the BBB as follows:

- HBMVEC constitutively synthesizes KA, and after IFN-γ treatment, additionally synthesizes KYN;
- HBMVPC constitutively synthesizes small amounts of PIC; after IFN-γ+TNF-α treatment, additionally synthesizes KYN;
- The KP in SV40-immortalised human BBB EC was not functional.

The production of KA may be a constitutive and inducible protective response of the BBB to immune activation, as KA is an antagonist of NMDA receptors at the glycine subunit. However much less KA is produced relative to QUIN. Also, the absence of the QUIN-degrading enzyme QPRTase in EC would augment the potential toxicity of QUIN in the brain, where it may exceed the low QPRTase activity which we have already demonstrated in human astrocytes (Guillemin, 2001). The similarities in KP expression in both brain and dermal microvascular EC suggest that that this expression may be a common feature of microvascular EC.

The biological significance of BBB KYN production is not known. The BBB is permeable to KYN via the large amino acid transporter and the possibility that peripherally produced KYN can be a substrate for further CNS KP metabolism has already been proposed by Stone (1993). The data presented here demonstrate that KYN produced at the BBB by cytokine stimulation might be another source of substrate for further processing by CNS-resident monocytic-lineage cells.

Pericyte-derived PIC blocks the neurotoxic (but not the neuroaxonal) effects of QUIN, possibly by Zn<sup>2+</sup> chelation; by countering QUIN-mediated neurotoxicity, PIC synthesis contributes to brain homeostasis until it is overwhelmed by de novo QUIN resulting from inflammatory insults.

IDO expression at the BBB results in L-Trp depletion, which restricts obligate intracellular cerebral parasites and viruses and leads to apoptosis of mainly CD4+ T cells, some subsets of monocytes, NK cells and dendritic cells.

The KP-mediated immune paresis particularly affecting T cells at the BBB is likely to facilitate selection of macrophage-tropic viral strains and contribute to persistence of viral infection in perivascular macrophages.

## Method

- Human BBB EC (HBMVEC) and pericytes (HBMVPC) obtained from Cell Systems Inc. Kirklind WA, USA.
- Human dermal microvascular ECs (HDMEVC) from Clonetics (Lonza Inc., Basel, Switzerland).
- SV40-transformed BBB EC (THBMVEC) obtained from KS Kim (Johns Hopkins University, Baltimore MD, USA).
- Human umbilical vein EC (HUVEC) obtained by collagenase digestion of umbilical vein of normal term cords.

Cell phenotype was confirmed by immunofluorescence using a panel of antibodies.

RT-PCR was used to examine expression of five key KP enzymes in quiescent and cytokine-stimulated cells (IFN-γ, TNF-α or both).

To assess downstream metabolism in HBMVEC & HBMVPC (brain) and HDMEVC (non-brain), cells were stimulated as above in the presence of KP intermediates 3-hydroxyanthranilic acid (3HAA) or QUIN.

KP metabolites in culture supernatants were quantified by HPLC/GC-MS.

## Results

### RT-PCR

Genes	Primers	Sequences
IDO	forward	5'-ACCACAAAGTCACAG CGCC-3'
	reverse	5'-CCCA GCGAGCA GCTCAAGG-3'
KMO	forward	5'-GATGAGGAAGATAAG CTGAGG-3'
	reverse	5'-CTTAAG GTTTC CCCCCTCTC-3'
KYNU	forward	5'-GACTA TTCACCTAGAAC GAGCA-3'
	reverse	5'-ACAGGAGACACAACTAAGT TC-3'
HAAO	forward	5'-GAGGGCCTTCTTGGTGTG-3'
	reverse	5'-GAT TGA TGTGTTGTGCTGG-3'
QPRTase	forward	5'-GGTCACA GGAC AGCAGAG-3'
	reverse	5'-AAGCCAGA GGAG CTGAC-3'
GADPH	forward	5'-ACACCAT GAGCA GCTCTG-3'
	reverse	5'-CTCAGTGTG GCGCA GAGTGC-3'

RT-PCR Primers for KP Enzymes  
 IDO: indoleamine 2,3-dioxygenase EC 1.14.11.17  
 KMO: Kynurenine monooxygenase EC 1.14.13.9  
 KYNU: L-Kynurenine Hydrolase EC 3.1.1.3  
 HAAO: 3-Hydroxyanthranilate 3,4-dioxygenase EC 1.13.11.6  
 QPRTase: Quinolate phosphoribosyltransferase EC 2.4.2.19  
 GAPDH: Glyceraldehyde phosphate dehydrogenase EC 1.2.1.12

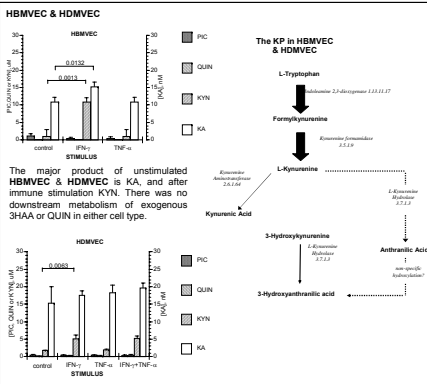
	IDO	KYNU	KMO	HAAO	QPRTase
HBMVEC	X	+	X	?	?
HBMVPC	X	+	X	+	+
THBMVEC	X	+	X	X	+
HUVEC	X	+	X	X	X
HDMEVC	X	X	X	X	+

Summary of constitutive KP enzyme expression by RT-PCR in BBB cells and non-BBB cells  
 + expressed; X not expressed

	IDO	KYNU	KMO	HAAO	QPRTase
HBMVEC	+	(+)	weak	?	?
HBMVPC	+	(+)	X	(+)	(+)
THBMVEC	+	(+)	X	X	(+)
HUVEC	+	(+)	(?)(IFN+TNF)	X	X
HDMEVC	+	(+)(TNF)	X	+(IFN+TNF)	(+)

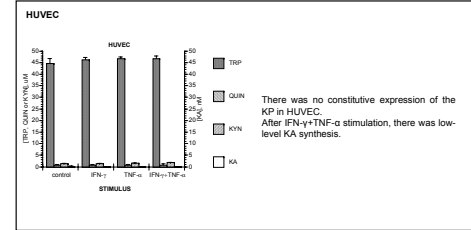
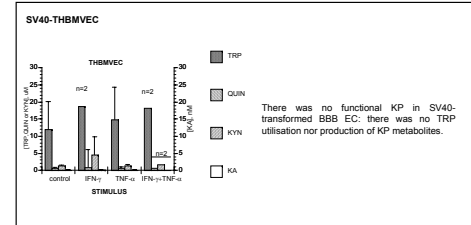
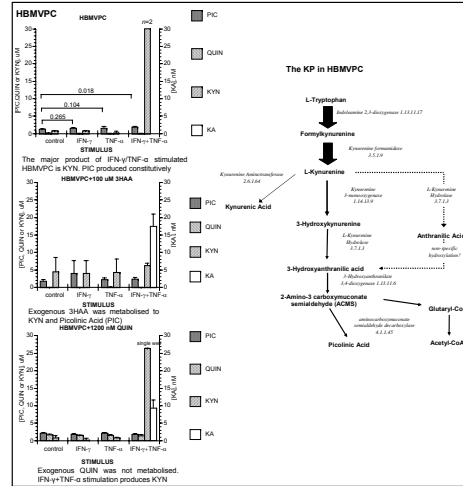
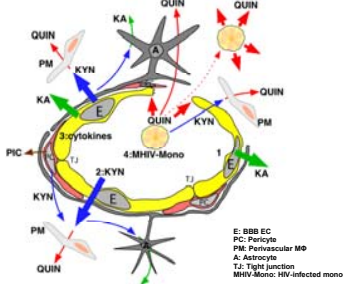
Summary of cytokine-inducible KP enzyme expression by RT-PCR in BBB cells and non-BBB cells  
 + expressed; X not expressed

### HPLC/GC-MS



### Hypothetical Model of KP metabolism at the BBB:

The data collected in this project using primary human cultured BBB cells can now be distilled into a hypothetical model of how the KP might function at the BBB in four scenarios:



- The quiescent BBB constitutively produces KA.
- Circulating, peripherally produced KYN permeates freely through the BBB, where it can be further processed by perivascular macrophages to QUIN and by BBB astrocytes to KA.
- Cytokine (IFN-γ) stimulation induces BBB EC to produce KYN, which can be further processed by perivascular macrophages to QUIN and by BBB astrocytes to KA. IFN-γ+TNF-α cause pericytes to produce PIC and KYN, which can be further metabolised by perivascular macrophages to QUIN. The resulting local L-Trp depletion by activation of EC IDO both restricts growth of intracellular pathogens and dampens the T cell-mediated immune response.
- M-tropic HIV-infected monocytes, which fully express the KP, produce KYN and QUIN. KYN freely diffuses through the BBB to be processed by perivascular macrophages to QUIN.

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