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# Elevated Expression of Brain Indoleamine 2,3-Dioxygenase is Associated with Early Mouse Development

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## Abstract

Indoleamine 2,3-Dioxygenase (IDO) catalyzes the oxidative degradation of the essential amino acid L-tryptophan at the initial and rate-limiting step of the kynurenine pathway. Although IDO via L-tryptophan depletion may suppress the growth of various pathogens, its immunomodulatory features and the cascade kynurenine pathway catabolites may contribute, by reducing immune responses of T cells, to the development of immunodeficiency observed in diseases such as HIV, autoimmune disorders and cancer. This study has focused on the IDO activity change in mice and discovered a linear relationship between the mouse brain IDO activity increase and the logarithmic function of mouse age in the early development of the healthy mice up to 8 weeks. This relationship may reflect the increase in infectious pathogen inhibition capability as well as immunologic tolerance through the early development of mice. This finding enhances our understanding of the IDO function in the mouse brain and may facilitate the mouse model selection for IDO research and related drug development.

**Keywords:** Indoleamine 2,3-dioxygenase; L-Tryptophan; Brain; Kynurenine; Mouse; Age

## Introduction

The essential amino acid L-tryptophan (TRP) plays a critical role as a constituent of human proteins and serves as the original substrate for two biosynthetic pathways: the serotonin pathway, which has mood regulating capabilities and the kynurenine pathway, which is the major route for the catabolism of TRP in mammals [1]. At the first and rate-limiting step in the kynurenine pathway, indoleamine 2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) initiate the oxidation of the TRP [1-3]. While TDO is predominantly located in liver, IDO is widely expressed in extrahepatic tissues including brain and is stimulated by pro-inflammatory cytokines among which interferon-γ is one of the most potent inducers [4,5]. Since it is localized in both the peripheral organs and brain, and regulated by cytokines, IDO may be a link between the immune system and TRP metabolism in the brain [6].

The local cellular TRP depletion via IDO may be a part of the cytostatic and antiproliferative activity, and may inhibit the growth of various infectious pathogens as an immune defense mechanism. On the other hand, IDO and the kynurenine pathway metabolites may suppress T cell responses and mediate immune tolerance, which play crucial roles in various physiological and pathological processes of diseases including infectious diseases, organ transplantation, neuropathology, inflammatory and autoimmune disorders and cancer [1,7,8]. The increased levels of IDO, the kynurenine pathway metabolites and the resulting TRP deprivation upon immune stimulation in IDO-expressing cells can contribute to the development of immunodeficiency and hypervulnerability due to a decreased response of stimulated T cells [1,7,9]. The therapeutic strategies were taken into account for the immunodeficiency effect, and different treatment options and related kynurenergic drug development were attempted [7,10]. With the increasing importance of IDO in the kynurenine pathway of TRP depletion and its clinical relevance, the present study evaluated the IDO activity in the brain of healthy mice with different ages. Since abundant neurobiology data are available in mice [4], it is beneficial to elucidate the IDO activity change pattern in order to assist the selection of appropriate age groups of mice and to understand the disparity between different age groups.

## Materials and Methods

### Mice

B6D2F1 mice were used in this study and the animal protocol was approved by Childrens Hospital Los Angeles Institutional Animal Care and Use Committee (IACUC). The mice were housed in polypropylene cages under controlled environment at 20°C with 12 hours lights each day. The mice were allowed free access to food and tap water in groups of five.

### IDO activity measurement

Mice were sacrificed at ages ranging from 1 to 8 weeks. The entire brain was collected from each mouse and immediately homogenized in 400 µl of ice cold 0.14 M KCl, 20 mM phosphate buffer, pH 7.0 as described [4]. The homogenates were then centrifuged at 14,000g for 30 min at 4°C and IDO activity was measured similarly as stated [4,11]. In brief, the supernatant (100 µl) was added to 400 µl of the reaction mixture (400 µM L-tryptophan, 20 mM ascorbic acid neutralized with NaOH, 10 µM methylene blue, and 200 µg/ml catalase in 50 mM potassium phosphate buffer, pH 6.5). After 60 min incubation with agitation at 37°C, the reaction was stopped with the addition of 100 µl of 30% trichloroacetic acid followed by 30 min incubation at 50°C for the conversion of N-formylkynurenine to L-kynurenine.

After centrifugation at 13,000g for 10 min at 4°C, the supernatant (125 µl) was transferred and mixed with 125 µl of 2% (w/v) p-dimethylaminobenzaldehyde in acetic acid in a 96-well microplate. The IDO activity was obtained by measuring the reaction mixture at 450 nm using a SPECTRAmax 250 microplate reader (Molecular Devices) with L-kynurenine as the standard.

### Protein concentration determination

The protein concentration in the supernatant of each brain extraction was determined by Biuret method with bovine serum albumin as a reference standard.

## Results and Discussion

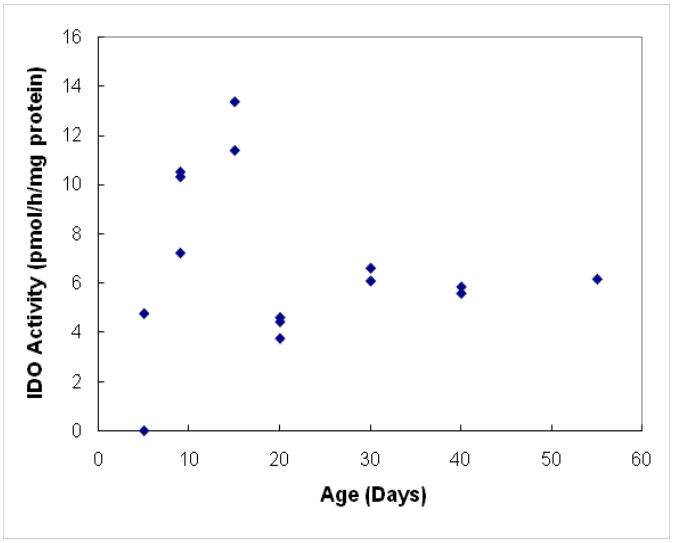


**Figure 1.** IDO activity in pmol/h/mg protein with the mouse age approximately between 1 and 8 weeks. A rapid increase was observed up to 15 days followed by a sharp drop between 15 and 20 days.

As shown in Figure. 1, while its rapid increase was seen from 5 days to 15 days, IDO activity (pmol/h/mg protein) dropped sharply between 15 and 20 days followed by a gradual increase between 20 and 55 days. On the other hand, before 15 days and after 20 days, the protein concentrations of the supernatants (mg/ml) increased gradually in proportion to mouse age while it exhibited a jump between 15 to 20 days, as indicated in Figure. 2. This rapid protein concentration increase was considered related to non-IDO specific proteins and caused the sharp decrease in IDO activity seen in Figure. 1. In conjunction with the results of IDO activities, it was suggested that the IDO activity unit of pmol/h/mg protein might not be suitable due to the presence of suspected non-IDO specific proteins. The concentrations of these non-IDO specific proteins were considered no relevance to IDO activity during the mouse brain development.

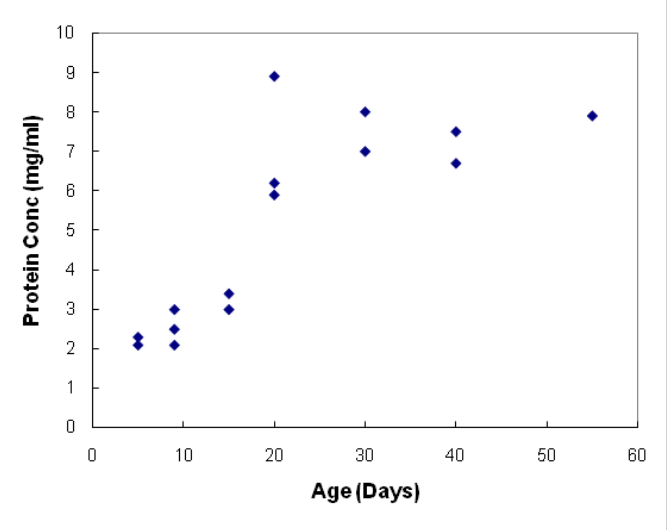


**Figure 2.** Protein concentration in mg/ml as a function of mouse age. While there was no significant change in protein concentration before 15 days and after 20 days, a sharp increase was seen between 15 and 20 days, which is considered a non-IDO specific protein contribution.

Alternately IDO activity in the unit of pmol/h/ml of the supernatant was evaluated as exhibited in Figure. 3a and 3b. While Figure. 3a presented the results of IDO activities on a direct scale of mouse age, Figure. 3b showed the results as the logarithmic function of mouse age. It was demonstrated that a linear relationship exists in IDO activity vs. the logarithmic function of mouse age up to 8 weeks. Since each mouse brain was homogenized with 400 µl of the buffer, the unit of IDO activity in pmol/h/ml of supernatant approximately corresponds to the IDO activity in the entire mouse brain from a single mouse without protein concentration correction. Although this non-corrected IDO activity is subject to a certain sample preparation variation with an increased volume of the homogenates due to an increased size of the mouse brain with age increase, it is still considered a more reliable unit measure for assessing IDO activity in this study. Future studies may achieve more accurate volumes of the homogenates to correct for the variability associated with the size of the mouse brain.

A study by Moroni, et al. [12] has indicated a rapid increase of kynurenic acid, one of the kynurenine pathway metabolites, in the rat brains for the first 60 days, followed by a slower increase

afterwards between 2 and 18 months. Although the exact relationship was unknown between IDO and kynurenic acid, a downstream metabolite of kynurenine pathway, it was indicated that the IDO activities obtained by Moroni et al. showed a similar trend as this study up to approximately 2 months. It was consistent with the catalytic role of IDO to the kynurenine pathway metabolites including kynurenic acid.

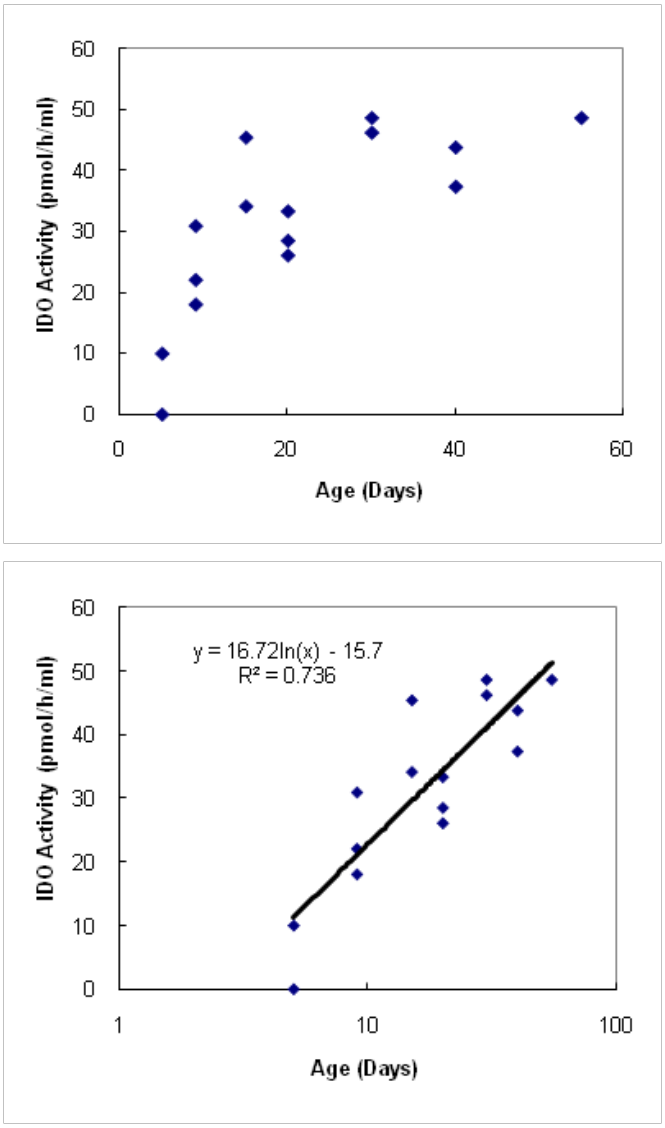


Figure. 3. (a) IDO activity change in pmol/h/ml with an increase of mouse age. A sharp increase was exhibited during the initial 15 days, followed by a slower upward trend. (b) Linear relationship between IDO activity in pmol/h/ml and the logarithmic function of mouse age was established up to 8 weeks in this study.

Traditionally the unit in pmol/h/mg protein was utilized in order to correct for the assay errors from sample preparations [6,12]. This study, however, suggests the use of the unit in pmol/h/ml of the supernatant instead of pmol/h/mg protein due to a suspected rapid increase of non-IDO related proteins along the development of the mouse brain around 15-20 days. This phenomenon may be significant in study designs when a mouse with a steady level of an enzyme such as IDO is needed at an early age development and a specific period like 15-20 days may be avoided with regard to the total protein present. On the other hand, when IDO activity in pmol/h/ml of the supernatant is employed, a linear relationship between the activity and the logarithmic function of the mouse age is established and can be used as a guide for the IDO activity estimation at the early mouse development up to 8 weeks. This relationship may also reflect the enhancement of the infectious pathogen inhibition and immunologic tolerance through the early development of mice.

Further studies consisting of more data points may be needed to provide statistically significant predictions and evaluations of this IDO trend as well as those for mice aging beyond a period of 8 weeks. Immunological techniques such as western blot and Enzyme-Linked Immunosorbent Assay (ELISA) may be used to confirm IDO content change with the mouse age, which indirectly demonstrate the presence of non-IDO specific proteins.

It has been found that dysregulation of the tryptophan-kynurenine metabolism pathways may contribute to the pathogenesis of age-associated neuroendocrine disorders (AAND)(hypertension, obesity, dyslipidemia, diabetes type 2, menopause, late onset depression, vascular cognitive impairment, impairment of immune defense, and some forms of cancer, e.g., breast and prostate) [13,14]. The shift of tryptophan metabolism from serotonin synthesis to formation of kynurenines may be triggered by activation of IDO. Decreased serotonin production might lead to mental depression while the development of AAND is associated with increased formation of kynurenines via their apoptotic, neurotoxic, and pro-oxidative effects, and upregulation of inducible nitric oxide synthase, phospholipase A2, arachidonic acid, prostaglandin, 5-lipoxygenase, and leukotriene cascade[13]. It has been demonstrated that the leukotriene pathway may contribute to the pathogenesis of neonatal bacterial meningitis by regulating E. coli K1 invasion of human BMEC and penetration into the brain [15,16]. Therefore, the IDO-related tryptophan-kynurenine metabolism pathways might be the convergent point for both pathogenesis and therapeutics of various AAND conditions.

## Conclusions

This study demonstrated a linear relationship between the brain IDO activity increase and the logarithmic function of mouse age in the early development of mice up to 8 weeks. It suggested that the IDO activity unit of pmol/h/ml, representing IDO activity in the entire brain from a single mouse, be utilized instead of the traditional unit of pmol/h/mg protein due to the possible presence of non-IDO specific proteins which might have exhibited a rapid and non-linear increase during early mouse development. Collectively these data indicate that IDO activation is associated with the brain development of mice. Changes in IDO expression induced by microbial and non-microbial factors may contribute to both pathogenesis and therapeutics of age-associated CNS inflammations.

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