Progesterone Alters Kynurenine Pathway Activation in IFN-γ-Activated Macrophages – Relevance for Neuroinflammatory Diseases

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ABSTRACT: We have previously demonstrated that the kynurenine pathway (KP), the major biochemical pathway for tryptophan metabolism, is dysregulated in many inflammatory disorders that are often associated with sexual dimorphisms. We aimed to identify a potential functional interaction between the KP and gonadal hormones. We have treated primary human macrophages with progesterone in the presence and absence of inflammatory cytokine interferon-gamma (interferon-γ) that is known to be a potent inducer of regulating the KP enzyme. We found that progesterone attenuates interferon-γ-induced KP activity, decreases the levels of the excitotoxin quinolinic acid, and increases the neuroprotective kynurenic acid levels. We also showed that progesterone was able to reduce the inflammatory marker neopterin. These results may shed light on the gender disparity in response to inflammation.

KEYWORDS: kynurenine pathway, microglia, macrophage, tryptophan, inflammation

CITATION: Bie et al. Progesterone Alters Kynurenine Pathway Activation in IFN-γ-Activated Macrophages – Relevance for Neuroinflammatory Diseases. *International Journal of Tryptophan Research* 2016:9 89–93 doi: 10.4137/IJTR.S40332.

TYPE: Rapid Communication

RECEIVED: June 10, 2016. **RESUBMITTED:** October 02, 2016. **ACCEPTED FOR PUBLICATION:** October 04, 2016.

ACADEMIC EDITOR: Gabor Mocz, Editor in Chief, *Biochemistry Insights*

PEER REVIEW: Five peer reviewers contributed to the peer review report. Reviewers' reports totaled 1118 words, excluding any confidential comments to the academic editor.

FUNDING: Authors disclose no external funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Introduction

KP, neuroinflammation, and psychiatric disease. The KP is the major catabolic pathway for tryptophan (TRP) and is known to be altered in a number of neurodegenerative diseases, including amyotrophic lateral sclerosis,[1] Parkinson's disease,[2] and Alzheimer's disease.[3] Neurodegenerative diseases are associated with chronic inflammation.[4] The KP has been shown to be upregulated by different inflammatory mediators such as proinflammatory cytokines, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).[5,6] Our group has previously shown that the KP is activated by IFN-γ in human macrophages.[7] The KP is also known to be strongly altered in a number of neuropsychiatric disorders, including schizophrenia,[8,9] depression,[10] and autism.[11] These disorders are also associated with chronic, and possibly causative, neuroinflammatory features.[12,13] In human beings, the levels of cytokine interleukin-6 have been found to be significantly higher in men than in women of the same age.[14] Recent molecular data of primary CD4+ T cells support a sexually dimorphic immune response in human beings.[15] In this study, gender was the most significant attributable source of genetic variation for the immune response. While the interplay between gonadal hormones and the immune system is likely to be a key factor in gender disparity for neurodegenerative and psychiatric disorders,[16] the mechanisms for this complex interplay are still unclear.

Gender and pathology. Gender disparity in onset and progression is observable in most neurodegenerative and psychiatric disorders that are all associated with neuroinflammation.[17] While mechanisms are still unclear, evidence is mounting that the immune system is influenced by sex hormones. Bini et al showed increased immune activity and survival in female and castrated male mice in a tuberculosis challenge experiment. Survival and immune activity were immediately improved after gonadectomy in males.[18] Another study showed an increase in IFN-γ and TNF-α expression with progesterone treatment in female mice and a decrease in testosterone in infected males. Infection and subsequent inflammation have been shown to induce expression of the progesterone receptor in mice, further compounding evidence for a complex interplay between sex hormones and the immune response in animal models.[19] Research on associations between progesterone levels and cognition in schizophrenia is sparse and with mixed results. However, progesterone has been shown to have some neuroprotective benefits.[20] Progesterone actions contribute to positive effects on cognition, sensory motor performance, and other aspects of neuropsychiatric illnesses,[21] suggesting possible ways in which progesterone may beneficially affect symptomatology in female patients. There are no published studies evaluating the benefit of progesterone as a treatment for schizophrenia.

We hypothesize that the KP provides a mechanistic framework in which the immune system and gonadal hormones interact and affect the health of central nervous system. We expect gonadal hormones to alter the KP

metabolism of immune cells, their microenvironment, and their cellular interactions.

Materials and Methods

Cultures of human monocyte-derived macrophages. This research complied with the principles of the Declaration of Helsinki. Subjects gave their written, informed consent to participate in the research, which was approved by the Macquarie University Human Research Ethics Committee. Briefly, human peripheral blood mononuclear cells were isolated from 100 mL of blood from healthy volunteers using a standard Ficoll-Paque (Pharmacia) density separation method.[7,22] Monocyte-derived macrophages were obtained using a classic adherence method. After eight days, the classic medium was replaced by AIM-V, a serum-free medium (Life Technologies) to limit any aspecific activation. After 11 days, 98% of the adherent cells expressed macrophage markers, including CD68+++, CD11c++, and CD16+.[23]

Treatment. Progesterone stock was made from progesterone (Sigma-Aldrich Co.) and reconstituted in 100% ethanol before being diluted in a cell culture medium with a final concentration of <0.02% (v/v) of ethanol using a stock concentration of 2 mg/mL at 100 nM, 10 μM, and 100 μM, and then incubated for 48 hours at 37 °C in a humidified atmosphere containing 5% CO_2.

Quantification of the KP metabolites. As previously optimized, production of the KP metabolites was quantified at 48 hours.[11] We used ultra-high performance liquid chromatography to quantify TRP, kynurenine (KYN), and kynurenic acid (KYNA) and gas chromatography–mass spectrometry to quantify quinolinic acid (QUIN) and picolinic acid (PIC).[24]

Statistics. IBM SPSS statistics software version 21 was used for the analysis (IBM Corporation). Owing to the heteroscedastic nature of the metabolite data, raw values were log transformed to achieve normal distribution for analysis. One-way analysis of variance was used to assess the differences in the treatment groups, with post hoc Bonferroni tests for differences between treatment and incubation time groups.

Results

We found progesterone attenuates IFN-γ-induced KP activation and alters IFN-γ-induced metabolism in human macrophages. As previously reported,[7] KYN/TRP ratio increases in the culture supernatant of IFN-γ-treated human macrophages. We found that an attenuation of this KYN/TRP ratio increased following treatment with 100 μM progesterone ($P = 0.001$; Fig. 2D). In these IFN-γ-stimulated cells, treatment with progesterone significantly increased KYNA levels at 100 nM ($P < 0.001$), 10 μM ($P = 0.043$), and 100 μM ($P = 0.003$; Fig. 1C) and increased TRP levels at 100 μM ($P < 0.001$). KYNA/QUIN is significantly increased at 100 nM ($P < 0.001$) and 10 μM ($P = 0.003$) but not at 100 μM ($P = 0.483$; Fig. 2E). PIC/QUIN ratio is increased at 100 μM ($P = 0.001$; Fig. 2F).

In unstimulated cells, progesterone treatment for 48 hours decreased PIC levels at 10 μM ($P = 0.014$) and 100 μM ($P = 0.002$; Fig. 1E), decreased QUIN levels at 100 μM ($P < 0.001$; Fig. 1F), increased KYNA/QUIN ratio at 100 nM ($P = 0.026$; Fig. 2B), and increased PIC/QUIN ratio at 100 μM ($P < 0.001$; Fig. 2C). Treatment of unstimulated cells with 10 μM and 100 μM of progesterone for 48 hours decreased neopterin levels ($P = 0.001$, $P = 0.007$; Fig. 3A), and combined treatment with IFN-γ and 100 μM progesterone decreased neopterin levels ($P < 0.001$; Fig. 3B).

Discussion

We showed that progesterone is able to influence the production of both neopterin and KP metabolites. Neopterin is a well-known marker of immune system activation.[25] The dose-dependent reduction of neopterin levels following progesterone treatment indicates an anti-inflammatory effect. This effect persists in combined treatment with IFN-γ and progesterone but seems to be present only when treated with 100 μM of progesterone. This is a much higher concentration of progesterone than would occur under normal physiological conditions. However, high progesterone doses are prescribed to women in "at-risk" pregnancies, which could likely cause localized concentrations to approach 100 μM.

We also found that progesterone can significantly influence the production of several KP metabolites. We previously described that the levels of KYN/TRP increase in response to IFN-γ stimulation in macrophages.[7] We observed a significant attenuation of this increase by treatment with 100 μM of progesterone ($P < 0.001$). This is likely to be associated with an anti-inflammatory effect of progesterone that has been described previously.[26,27]

Progesterone increases the production of the immunomodulatory KYN and the neuroprotective KYNA and decreases biosynthesis of the excitotoxin and proinflammatory QUIN by IFN-γ-induced macrophages (Fig. 2). KYN is known for its immunomodulatory ability, especially during pregnancy.[28,29] Both KYNA and KYN directly activate the aryl hydrocarbon receptor, a receptor known to have both pro- and anti-inflammatory effects.[30] KP modulation by progesterone could represent a mechanism for immune regulation. Interestingly, KYNA is also an alpha-7 nicotinic acetylcholine receptor (α7nAChr) antagonist. This receptor seems to play a crucial role in cholinergic regulation of macrophage activity.[31] Antagonism of the α7nAChr is associated with suppression of the anti-inflammatory effect of vagal nerve signaling, generally producing a net pro-inflammatory effect. Additionally, a depletion of TRP by increased KP activity may affect serotonin and subsequent melatonin production. Melatonin is also an α7nAChr agonist.[32] A reduction in an α7nAChr agonist would be expected to reduce anti-inflammatory effect on macrophages.[33] In this study, inflammatory marker neopterin is reduced with increased KP activity and the resulting KYNA production. The nature of the study (*in vitro*) and the

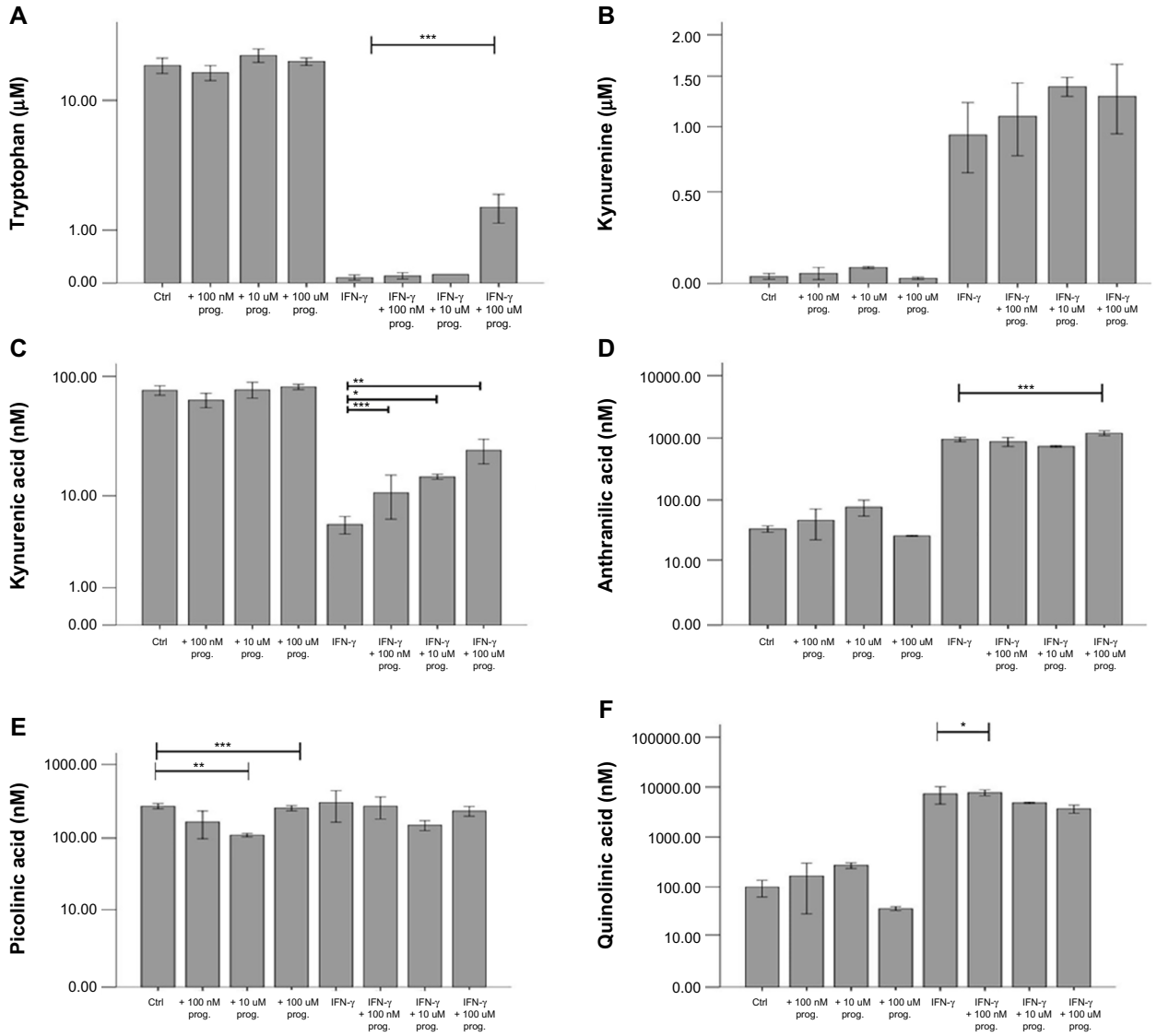

Figure 1. Effects of 100 nM, 10 µM, and 100 µM progesterone on primary human macrophages in the absence and presence of IFN-γ: (**A**) TRP production, (**B**) KYN production, (**C**) production of KYNA, (**D**) production of anthranilic acid, (**E**) production of PIC, and (**F**) production of QUIN. Error bars denote standard deviation ($n = 3$).

subsequent lack of nicotinic or vagal stimulation may possibly account for this effect. With no anti-inflammatory signaling to be suppressed and additional immunomodulation coming from increased KYN, the overall pattern is one of decreased immune activation. This pattern may well shift in *in vivo* conditions.

The potentiation of KYNA production by progesterone does not appear to be dose dependent. The increase in KYNA and decrease in QUIN in IFN-γ-stimulated and progesterone-treated samples resulted in an overall increase in KYNA/QUIN ratio. KYNA is an *N*-methyl-D-aspartate-receptor (NMDAR) antagonist[34] and is usually considered as a neuroprotective molecule,[35] whereas QUIN is an NMDAR agonist and a well-known neurotoxin, gliotoxin, and pro-inflammatory molecule.[36] The overall effects of progesterone can be considered as beneficial, resulting in a decrease in deleterious QUIN together with an increase in neuroprotective KYNA and a decrease in the inflammation marker neopterin. It is important to mention that, among all the human cell types, activated macrophages are able to produce the highest levels of QUIN, but produce far less KYNA.

These results may have a specific relevance to schizophrenia. It has become increasingly evident that inflammation is involved in the development of schizophrenia.[37] This anti-inflammatory role of progesterone may explain at least, in part, why schizophrenia develops differently in females. A shift in KYNA/QUIN ratio has been previously reported in schizophrenia, with a reduction in QUIN[38] and an increase in cerebrospinal fluid KYNA.[39] The relationship of progesterone levels to clinical schizophrenic presentation and treatment response in either gender is still unclear due to limited and conflicting data. It is possible that progesterone, via its action

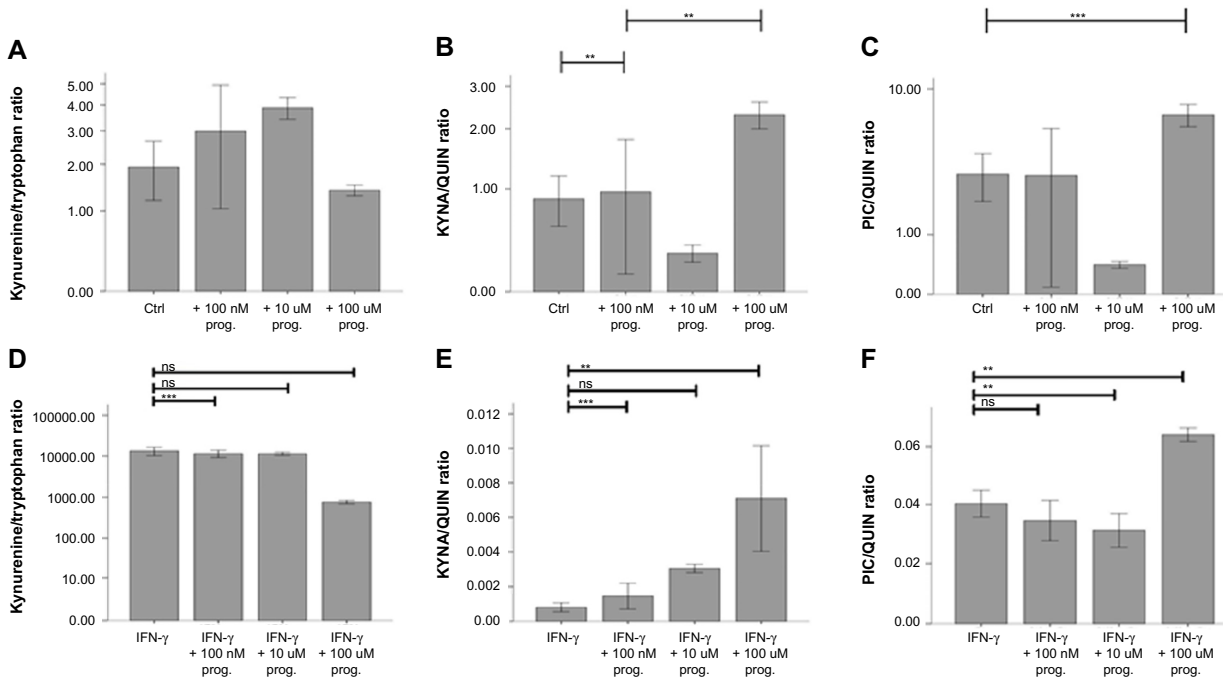

Figure 2. Effects of 100 nM, 10 μM, and 100 μM progesterone on primary human macrophages in the absence and presence of IFN-γ: (**A**, **D**) KP activity as described by KYN/TRP ratio, (**B**, **E**) KYNA/QUIN ratio, and (**C**, **F**) PIC/QUIN ratio. Error bars denote standard deviation ($n = 3$).

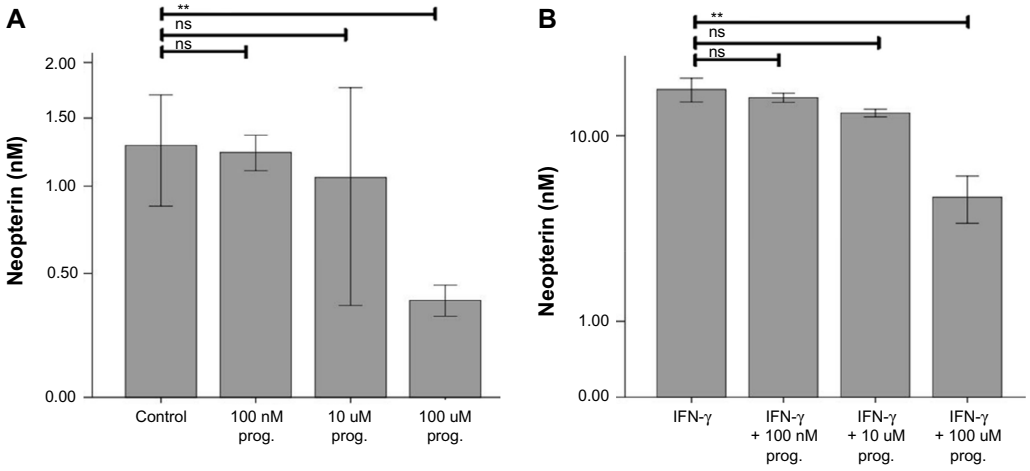

Figure 3. Effects of 100 nM, 10 μM, and 100 μM progesterone on neopterin production in primary human macrophages: (**A**) in the absence of IFN-γ and (**B**) in the presence of IFN-γ. Error bars denote standard deviation ($n = 3$).

on the KP, could play a key role in the development, symptomology, and severity of schizophrenia.

Our findings on treatment with progesterone support our hypothesis that gender disparity in diseases may be due, at least in part, to hormonal effects on the KP and inflammation.

Author Contributions

Conceived and designed the experiments: CKL, JdB. Analyzed the data: CKL, JdB. Wrote the first draft of the manuscript: JdB. Contributed to the writing of the manuscript: JdB, GJG. Agree with manuscript results and conclusions: JdB, CKL, GJG. Jointly developed the structure and arguments for the paper: JdB, GJG. Made critical revisions and approved final version: JdB, GJG. All authors reviewed and approved of the final manuscript.

Abbreviations

IFN-γ: interferon gamma
KP: kynurenine pathway
KYN: kynurenine
KYNA: kynurenic acid
NMDAR: *N*-methyl-D-aspartate-receptor
PIC: picolinic acid

QUIN: quinolinic acid
TRP: tryptophan
CNS: central nervous system
PBMC: peripheral blood mononuclear cell.

REFERENCES

1. Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy states. *Int J Tryptophan Res*. 2009;2:1–19.
2. Lim CK, Fernández-Gomez FJ, Braidy N, et al. Involvement of the kynurenine pathway in the pathogenesis of Parkinson's disease. *Prog Neurobiol*. April 2016. doi:10.1016/j.pneurobio.2015.12.009. (epub publication) see https://www.ncbi.nlm.nih.gov/pubmed/27072742.
3. Guillemin GJ, Brew BJ. Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease. *Redox Rep*. 2002;7:199–206.
4. Quintanilla RA, Orellana DI, González-Billault C, Maccioni RB. Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. *Exp Cell Res*. 2004;295:245–57.
5. Guillemin GJ, Smith DG, Smythe GA, Armati PJ, Brew BJ. Expression of the kynurenine pathway enzymes in human microglia and macrophages. *Adv Exp Med Biol*. 2003;527:105–12.
6. Saito K, Markey SP, Heyes MP. Chronic effects of gamma-interferon on quinolinic acid and indoleamine-2,3-dioxygenase in brain of C57BL6 mice. *Brain Res*. 1991;546:151–4.
7. Jones SP, Franco NF, Varney B, et al. Expression of the kynurenine pathway in human peripheral blood mononuclear cells: implications for inflammatory and neurodegenerative disease. *PLoS One*. 2015;10:e0131389.
8. Erhardt S, Blennow K, Nordin C, Skogh E, Lindström LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neurosci Lett*. 2001;313:96–8.
9. Jospeh MH, Baker HF, Crow TJ, Riley GJ, Risby D. Brain tryptophan metabolism in schizophrenia: a post mortem study of metabolites of the serotonin and kynurenine pathways in schizophrenic and control subjects. *Psychopharmacology (Berl)*. 1979;62:279–85.
10. Myint AM. Kynurenines: from the perspective of major psychiatric disorders. *FEBS J*. 2012;279:1375–85.
11. Lim CK, Essa MM, de Paula Martins R, et al. Altered kynurenine pathway metabolism in autism: implication for immune-induced glutamatergic activity. *Autism Res*. 2015;9(6):621–31.
12. Müller N, Myint A-M, Schwarz MJ. Inflammation in schizophrenia. *Adv Protein Chem Struct Biol*. 2012;88:49–68.
13. Müller N, Myint AM, Schwarz MJ. Inflammatory biomarkers and depression. *Neurotox Res*. 2011;19:308–18.
14. Guest J, Grant R, Mori TA, Croft KD. Changes in oxidative damage, inflammation and [NAD(H)] with age in cerebrospinal fluid. *PLoS One*. 2014;9:1–8.
15. Qu K, Zaba LC, Giresi PG, et al. Individuality and variation of personal regulomes in primary human T cells. *Cell Syst*. 2015;1:51–61.
16. de Bie J, Lim CK, Guillemin GJ. Kynurenines, gender and neuroinflammation; showcase schizophrenia. *Neurotox Res*. 2016;30(3):285–94.
17. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. *Front Neuroendocrinol*. 2014;35:347–69.
18. Bini EI, Mata Espinosa D, Marquina Castillo B, et al. The influence of sex steroid hormones in the immunopathology of experimental pulmonary tuberculosis. *PLoS One*. 2014;9:e93831.
19. Vargas-Villavicencio JA, Larralde C, De León-Nava MA, Morales-Montor J. Regulation of the immune response to cestode infection by progesterone is due to its metabolism to estradiol. *Microbes Infect*. 2005;7:485–93.
20. da Silva TL, Ravindran AV. Contribution of sex hormones to gender differences in schizophrenia: a review. *Asian J Psychiatr*. 2015;18:2–14.
21. Zheng P. Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. *Prog Neurobiol*. 2009;89:134–52.
22. Kerr SJ, Armati PJ, Pemberton LA, Smythe G, Tattam B, Brew BJ. Kynurenine pathway inhibition reduces neurotoxicity of HIV-1-infected macrophages. *Neurology*. 1997;49:1671–81.
23. Guillemin GJ, Kerr SJ, Pemberton LA, et al. IFN-beta1b induces kynurenine pathway metabolism in human macrophages: potential implications for multiple sclerosis treatment. *J Interferon Cytokine Res*. 2001;21:1097–101.
24. Guillemin GJ, Cullen KM, Lim CK, et al. Characterization of the kynurenine pathway in human neurons. *J Neurosci*. 2007;27:12884–92.
25. Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. *Curr Drug Metab*. 2002;3:175–87.
26. Aisemberg J, Vercelli CA, Bariani MV, Billi SC, Wolfson ML, Franchi AM. Progesterone is essential for protecting against LPS-induced pregnancy loss. LIF as a potential mediator of the anti-inflammatory effect of progesterone. *PLoS One*. 2013;8:e56161.
27. Fukuyama A, Tanaka K, Kakizaki I, et al. Anti-inflammatory effect of proteoglycan and progesterone on human uterine cervical fibroblasts. *Life Sci*. 2012;90:484–8.
28. Johnson TS, Munn DH. Host indoleamine 2,3-dioxygenase: contribution to systemic acquired tumor tolerance. *Immunol Invest*. 2012;41:765–97.
29. Mándi Y, Vécsei L. The kynurenine system and immunoregulation. *J Neural Transm*. 2012;119:197–209.
30. Nguyen NT, Hanieh H, Nakahama T, Kishimoto T. The roles of aryl hydrocarbon receptor in immune responses. *Int Immunol*. 2013;25:335–43.
31. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature*. 2003;421(6921):384-388. doi:10.1038/nature01339.
32. Jeong and Park. (2015)
33. Muxel SM, Pires-Lapa MA, Monteiro AWA, et al. NF-κB drives the synthesis of melatonin in RAW 264.7 macrophages by inducing the transcription of the arylalkylamine-N-acetyltransferase (AA-NAT) gene. *PLoS One*. 2012;7:e52010.
34. Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. *J Neurosci*. 2001;21:7463–73.
35. Breda C, Sathyasaikumar KV, Sograte S, Notarangelo FM, Estranero JG. Ameliorates neurodegeneration by modulation of kynurenine pathway metabolites. *Proc Natl Acad Sci U S A*. 2016;113:5435–40.
36. Guillemin GJ. Quinolinic acid, the inescapable neurotoxin. *FEBS J*. 2012;279:1356–65.
37. Fillman SG, Weickert TW, Lenroot RK, et al. Elevated peripheral cytokines characterize a subgroup of people with schizophrenia displaying poor verbal fluency and reduced Broca's area volume. *Mol Psychiatry*. 2016;21(8):1090–1098. doi:10.1038/mp.2015.90.
38. Gos T, Myint A-M, Schiltz K, et al. Reduced microglial immunoreactivity for endogenous NMDA receptor agonist quinolinic acid in the hippocampus of schizophrenia patients. *Brain Behav Immun*. 2014;41:59–64.
39. Kegel ME, Bhat M, Skogh E, et al. Imbalanced kynurenine pathway in schizophrenia. *Int. J. Tryptophan Res*. 2014;7:15–22.