Pregnenolone Shifts Circulating Monocytes Toward a Regulatory Phenotype in People with HIV

476

Daire Daly[1,2], Hemi Park [1,2], Livia Brennan [1,2] Emily R. Rudmann[1,2], Jun Yan Loi [2,9], Janet Lo [7], Kiana Keller [1], Marco Loggia [5,6], Eva-Maria Ratai [5], Mark C. Poznansky[2], Christine E. Marx [8], Gregory K. Robbins[4], Patrick M. Reeves[*2], Ruxandra F. SÎrbulescu[*1,2], Shibani S. Mukerji[*1,2,3,4]

[1]Department of Neurology, Massachusetts General Hospital (MGH), Harvard Medical School (HMS), Boston, MA USA. [2]Vaccine and Immunotherapy Center, MGH. [3]HMS, Boston, MA, USA. [4]Division of Infectious Diseases, MGH, HMS. [5]Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, MGH, HMS. [6]Department of Anesthesia, Critical Care and Pain Medicine, MGH, HMS. [7]Metabolism Unit, Division of Endocrinology, MGH. [8]Duke University School of Medicine, Department of Psychiatry and Behavioral Sciences, Durham, NC, USA. School of Biological Sciences, [9]Nanyang Technological University Singapore. *Co-Senior Authors, Contributed Equally

Mass General Brigham

BACKGROUND

What is the impact of pregnenolone on immune activation in people with HIV (PWH) compared to those without HIV (PWoH)?

Pregnenolone, a parent neuroactive steroid (NAS), is an endogenous hormone synthesized in the mitochondria of adrenal glands, gonads, and brain cells. One of its downstream metabolites received FDA approval for postpartum depression[1] and showed efficacy in major depressive disorder among PWoH[2]. Preclinical studies suggest that pregnenolone has anti-inflammatory properties, and thought to decrease CNS inflammation, but the mechanisms in humans remain unclear[3]. **This research examined the effects of pregnenolone after TLR stimulation on monocytes derived from both PWH and PWoH using peripheral blood mononuclear cells (PBMCs).**

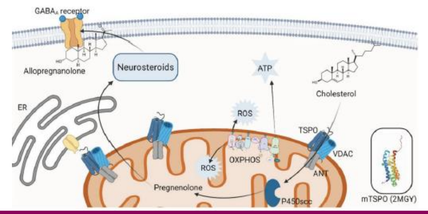


Fig 1. Schematic of the role of the mitochondria in the production of pregnenolone and its derivatives[4]

METHODS

Endogenous plasma NAS levels were measured via mass spectrometry in 20 PWoH (9 females (F), 11 males (M)) and 33 PWH (8F, 25M; viral load <200 copies/mL, all on ART)(full cohort). Correlations between pregnenolone and cytokines were evaluated using Pearson correlation coefficients and adjusted with Benjamini-Hochberg false discovery rate (BH-FDR). PBMCs from 4 PWoH (2F, 2M) and 5 PWH (3M, 2F) were stimulated overnight with the TLR4 agonist lipopolysaccharide (LPS) or the TLR1/2 agonist PAM3CSK4 (PAM). Following stimulation, cells were treated with brefeldin A to capture intracellular cytokines and pregnenolone at concentrations of 0.016 µM, 10 µM, or 50 µM for 6 hours. Phenotypic markers and intracellular cytokine expression were quantified using flow cytometry, and the effects were assessed using linear regression models.

RESULTS – Lower Endogenous Pregnenolone Correlated with Inflammation; No Significant Difference Between PWH and PWoH

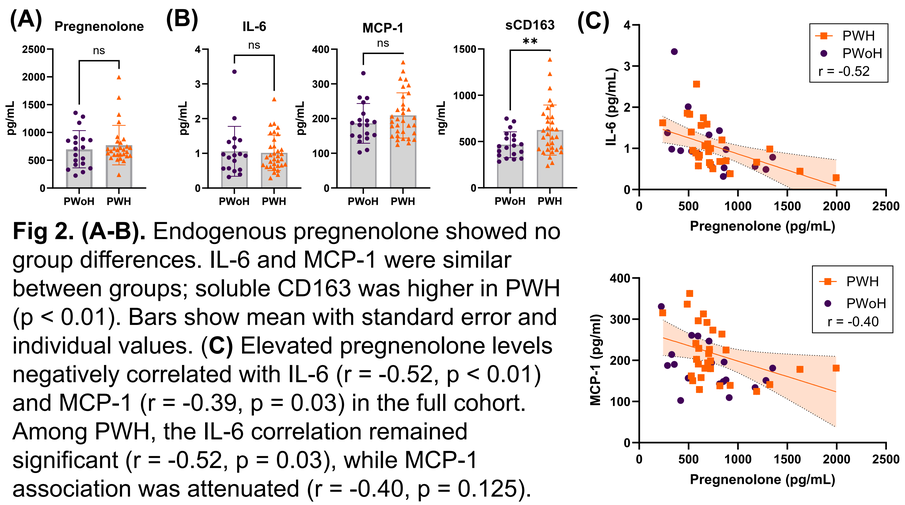


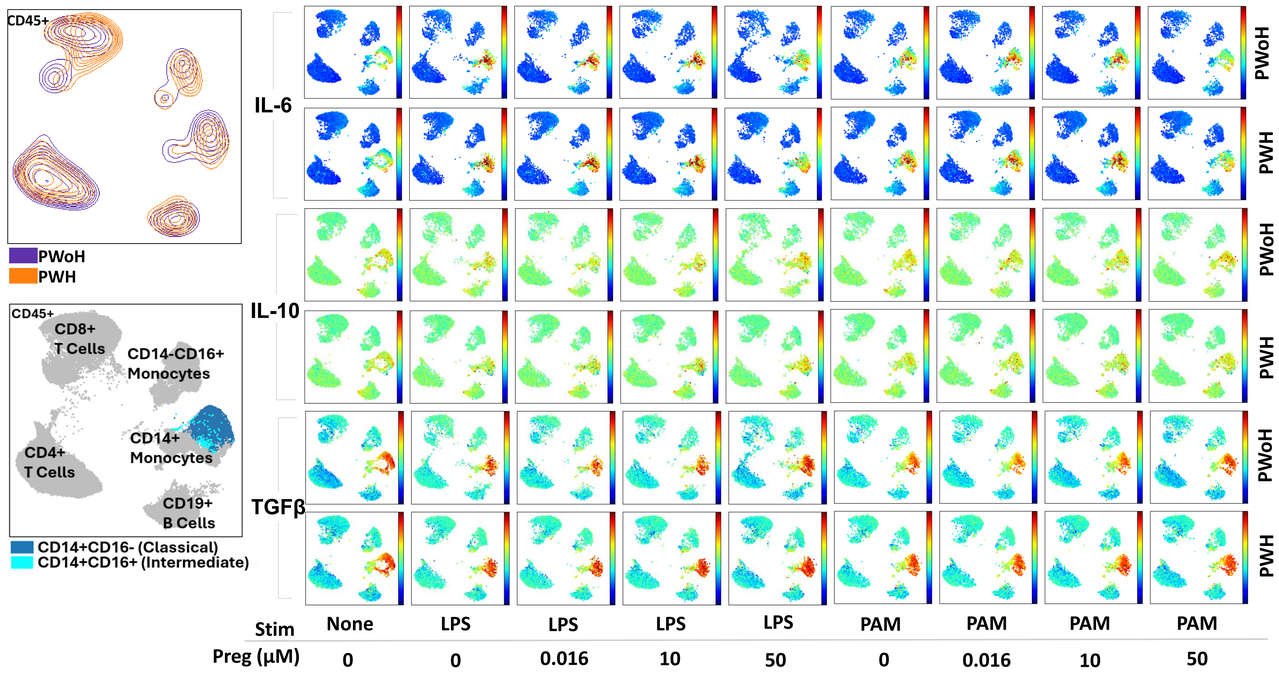
Fig 2. (A-B). Endogenous pregnenolone showed no group differences. IL-6 and MCP-1 were similar between groups; soluble CD163 was higher in PWH ($p < 0.01$). Bars show mean with standard error and individual values. **(C)** Elevated pregnenolone levels negatively correlated with IL-6 ($r = -0.52$, $p < 0.01$) and MCP-1 ($r = -0.39$, $p = 0.03$) in the full cohort. Among PWH, the IL-6 correlation remained significant ($r = -0.52$, $p = 0.03$), while MCP-1 association was attenuated ($r = -0.40$, $p = 0.125$).

MAIN FINDINGS

Pregnenolone reduces innate inflammatory immune activation in classical monocytes while increasing regulatory cytokines.

RESULTS – Monocyte Sensitivity to TLR Stimulation is Affected by Pregnenolone

Figure 3. Pregnenolone Modulates Pro- and Anti-Inflammatory Cytokine Profiles in Response to TLR Agonists. UMAP (left) showing CD45+ leukocyte clustering in PBMCs derived from PWH and PWoH. Phenotypic mapping of monocytes, T cells, and B cells in the full cohort. Density plots (right) show that pregnenolone (0.016–50 µM) dose-dependently reduces inflammatory cytokine IL-6 production in CD14+CD16- monocytes and modestly increases IL-10 and TGFβ across both cohorts.



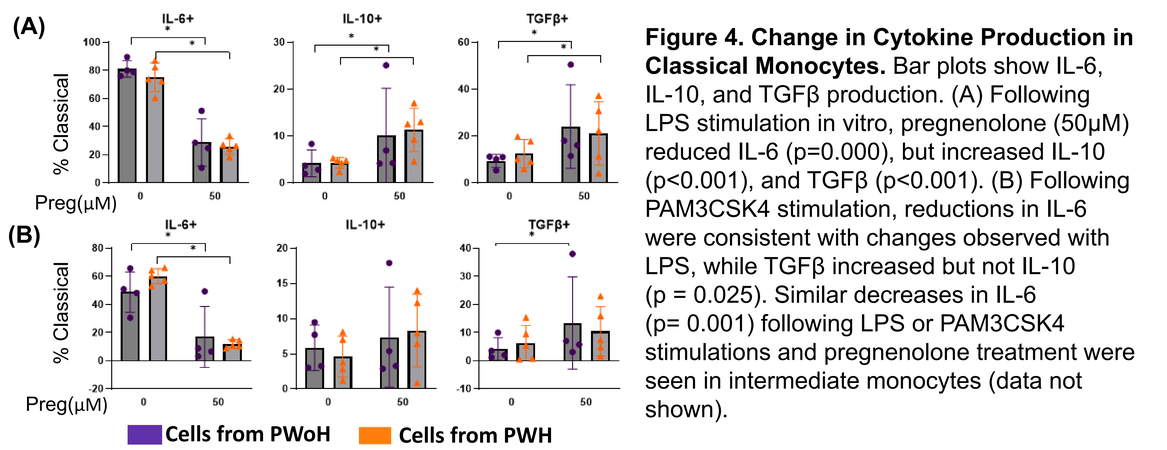


Figure 4. Change in Cytokine Production in Classical Monocytes. Bar plots show IL-6, IL-10, and TGFβ production. (A) Following LPS stimulation in vitro, pregnenolone (50µM) reduced IL-6 ($p=0.000$), but increased IL-10 ($p<0.001$), and TGFβ ($p<0.001$). (B) Following PAM3CSK4 stimulation, reductions in IL-6 were consistent with changes observed with LPS, while TGFβ increased but not IL-10 ($p = 0.025$). Similar decreases in IL-6 ($p= 0.001$) following LPS or PAM3CSK4 stimulations and pregnenolone treatment were seen in intermediate monocytes (data not shown).

Contact

Daire Daly
dddaly@mgh.harvard.edu
Shibani Mukerji
smukerji@mgb.org

CONCLUSIONS

1. **Correlation with IL-6:** Endogenous pregnenolone is inversely associated with IL-6 in both PWH and PWoH, suggesting a possible protective role in immune activation.
2. **Targeted Cytokine Modulation:** In vitro results show that pregnenolone attenuates pro-inflammatory signaling (IL-6) while enhancing regulatory pathways (IL-10, TGFβ).
3. **Cell-Specific Activity:** This immunomodulatory effect is primarily observed in monocytes suggesting a specific role in regulating innate immune activation.

Clinical Implications

Previous metabolomics profiling showed PWH with depression showed low levels of NAS compared to those without depression. These preclinical findings support the current clinical trial (NCT05570812) examining the effects of pregnenolone in PWH diagnosed with Major Depressive Disorder (MDD).

REFERENCES

1. Zawilska JB, et al. Neuroactive Steroids as Novel Promising Drugs in Therapy of Postpartum Depression-Focus on Zuranolone. Int J Mol Sci. 2025 Jul 7;26(13):6545.
2. Mukerji SS, et al. Low Neuroactive Steroids Identifies a Biological Subtype of Depression in Adults with Human Immunodeficiency Virus on Suppressive Antiretroviral Therapy. J Infect Dis. 2021 May 20;223(9):1601-1611.
3. Murugan S, et al. The neurosteroid pregnenolone promotes degradation of key proteins in the innate immune signaling to suppress inflammation. J Biol Chem. 2019 Mar 22;294(12):4596-4607.
4. Rupprecht R, Wetzel CH, Dorostkar M, Herms J, Albert NL, Schwarzbach J, Schumacher M, Neumann ID. Translocator protein (18kDa) TSPO: a new diagnostic or therapeutic target for stress-related disorders? Mol Psychiatry. 2022 Jul;27(7):2918-2926.