The Role of GM-CSF in the Neuropathogenesis of Simian Immunodeficiency Virus (SIV)

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Introduction

Why do neurocognitive disorders persist in people living with HIV while taking combination antiretroviral therapy (cART)?

- The brain is a known reservoir for HIV.
- HIV can act on myeloid cell populations like macrophages.
- Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is a hematopoietic growth factor and type I soluble cytokine that has been demonstrated to have a proinflammatory role over macrophages (and other myeloid cells) and can induce proliferation and migration across the blood brain barrier (Vogel 2015).
- GM-CSF has also been found to be present and is involved in the inflammation process of Simian Immunodeficiency Virus (SIV).
- Along with GM-CSF, the expression of GM-CSF receptors has been shown to be increased in SIV.

Hypothesis: In SIV infected regions of the midbrain, GM-CSF expression will be increased, which would contribute to the HIV neuropathogenesis by attracting inflammatory cells such as macrophages.

Methods

- Sections of midbrain were obtained from ten *Rhesus Macaque* animals in formalin fixed, paraffin embedded tissue slides.
- Immunohistochemistry (IHC) was performed on five SIV positive slides and five SIV negative slides of *Rhesus Macaque* brain tissue.

Primary Antibody Used	Specificity
GMCSF (1:100)	Granulocyte Macrophage Colony Stimulating Factor
CD163	Macrophage Marker
Phalloidin	Structural Protein
DAPI	Nuclei
CSF2RB	GM-CSF Receptors

- Images were taken on 4x, 10x, 40x and 100x
- DAPI was counted via a Macro in FIJI
- GMCSF and CSF3RB were manually counted in FIJI
- Percent of the number of positive GMCSF cells were counted per the number of positive nuclei. Calculations from these analyzed images were performed in excel.
- Statistic graphs were made in Graph Pad Prism
- Material for counting GM-CSF and CSF2RB was included if there was a DAPI nuclei present with the red or far-red cytokine/receptor (Fig. 1 and Fig. 2)
- Material for counting was included if there was both a blue nucleus (DAPI) and far-red macrophage (CD163) present in the same location (Fig. 5).

Results

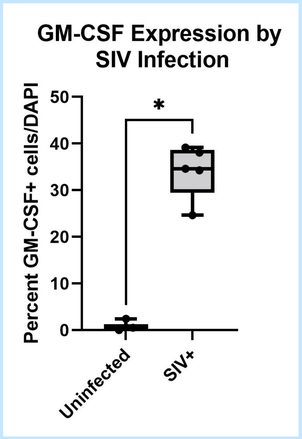


Fig. 1: Percentage of GM-CSF positive cells per the number of nuclei.

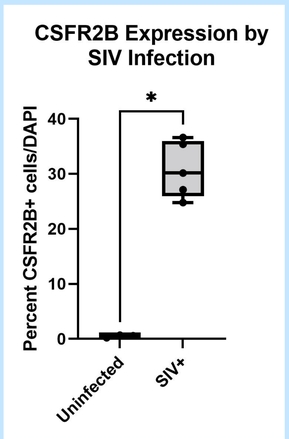


Fig. 2: Percentage of CSF2RB positive cells per the number of nuclei.

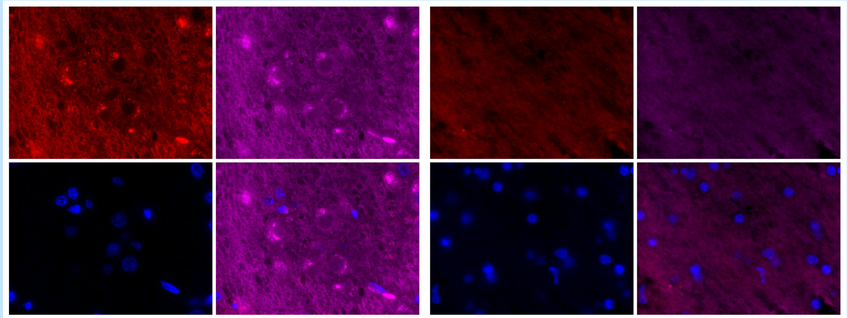


Fig. 3: Representative images of SIV positive animal 14-0681. GM-CSF is represented by red (top left), CSF2RB is represented by far-red (top right), and DAPI are represented in blue (bottom left. The last image is the overlay (bottom right).

Fig. 4: Representative images of SIV positive animal 15-0432. GM-CSF is represented by red (top left), CSF2RB is represented by far-red (top right), and DAPI are represented in blue (bottom left. The last image is the overlay (bottom right).

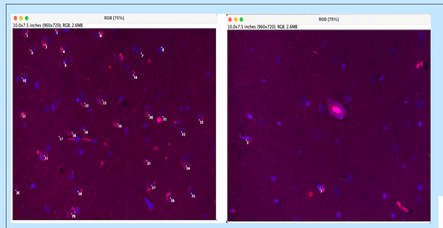
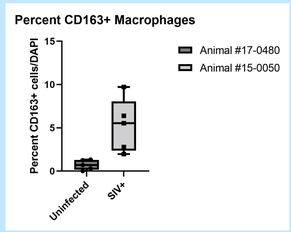


Fig. 5: Quantification of CD163 in animal 17-0780 (left) and animal 15-0050 (right).

Fig 6: Percent of cells positive for CD163+ per the number of nuclei present in an SIV positive (17-0480) and an SIV negative (15-0050 animal.



Conclusion

- Increased expression of GM-CSF and receptors in SIV positive animals.
- GM-CSF has a proinflammatory role over myeloid cells including macrophages.
- Brain is a reservoir for HIV.
- HIV can influence macrophages and can play a role in the viral persistence of HIV.
- Increased GM-CSF expression can lead to increased expression of macrophages (CD163)?
- Infected macrophages could spread HIV by using the ability to invade most body tissues.
- Increased amounts of macrophages can cause neuroinflammation and contribute to the pathophysiology of HIV in the brain.
- To help patients living with HIV and suffering from neurocognitive impairments, this research could present a potential treatment created by combining GM-CSF antibodies with antiretroviral therapies in the future.

Works Cited

Vogel, Daphne Y. S. Kooji, Gijis. Heijnen, Priscelilla D. A. M. Breur, Marjolein. Peferoen, Laura A. N. Van der Valk, Paul. De Vries, Helga E. Amor, Sandra. Dijkstra, Christine D. 2015. GM-CSF promotes migration of human monocytes across the blood brain barrier. European Journal of Immunology.