BRAIN PET IMAGING OF MICROGLIA IN MACAQUES WITH SIV ENCEPHALITIS

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Introduction

The exact cause of neurocognitive decline despite peripheral control of HIV infection remains incompletely understood. One of the hypothesized causes is a constant neuroinflammatory burden resulting in a vicious cycle of microglial activation and release of proinflammatory chemokines/cytokines, eventually resulting in neuronal loss/dysfunction[1]. The exact role of neuroinflammation in the earliest stages of the infection when cerebrospinal fluid (CSF) Viral loads (VL) can be very high[2], is however unclear.

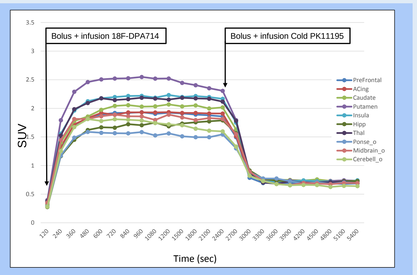
Neuroinflammation can be quantified using PET imaging with ligands targeting the translocator protein (TSPO), an outer mitochondrial membrane receptor known to be upregulated under inflammatory conditions[3].

Project Overview

In this study we wanted to image brain TSPO expression using [18F]-DPA714 PET[4], in macaques infected with a neurovirulent SIV strain (SIVsm804E). Our goal was to quantify neuroinflammatory changes before and after inoculation and correlate to HIV biomarkers in the periphery and in the CNS.

Methods

Dynamic PET imaging with [18F]-DPA714 followed by displacement with cold PK11195 (TSPO antagonist) was performed in 5 SIV-infected macaques at baseline as well as different time points following inoculation, until euthanasia.



Regional and whole brain volumes of interest were drawn on the respective monkey MR images. SUVmean at equilibrium which reflects total uptake (specific + non-specific uptake) and Binding Potential (BP) values ((Total binding/non specific binding) -1) were calculated. We achieved appropriate displacement in four out of five animals. To assess whether clinically relevant biomarkers (CSF and plasma VL, CD4 cell counts as well as CSF levels of MCP-1, IL-1ra, Il-2, IL-8, IL-10, IL-15 and IL-18) are predictive of SUVmean(total) and BP, each measurement was first included individually in a statistical model as an explanatory variable, with the total SUVmean or BP as the response variable. The measurements were then included together in one model to evaluate their combined relationship with SUVmean(total) or BPnd. Because of the repeated-measures nature of the data, a mixed model was fit to the data.

Multiplex post-mortem immunofluorescent (IF) staining for microglia/macrophages (iba1), neurons (NeuN) and apoptosis (cleaved caspase-3/PARP) was performed in all animals and compared to 3 normal monkey brains.

Results

When the last time point (prior to euthanasia) was compared to pre-inoculation baseline, [18F]-DPA714 total binding was decreased in 4 animals (Fig. 1A) and increased in one animal (Fig. 1C). We found an inverse relationship between binding and CSF VL: binding decreased when VL increased and vice versa (Fig. 1B and D).

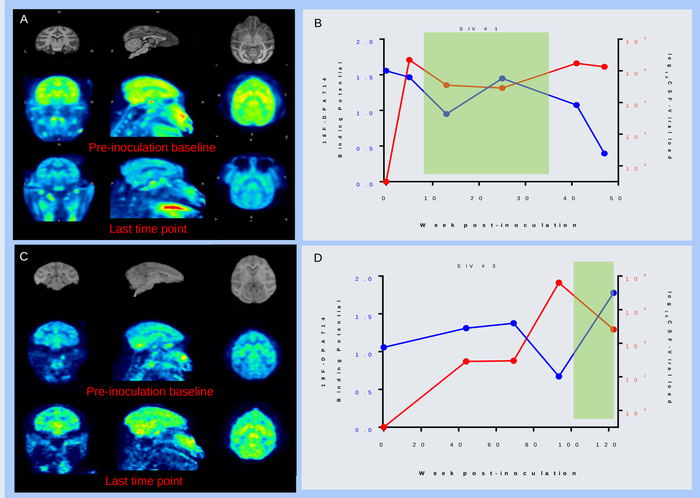


Fig 1: A and C show baseline and post inoculation, pre-euthanasia [18F]-DPA714 PET scans from two animals. While SIV#1 showed decreased uptake, SIV #3 showed increased uptake at the last time point. Over the course of their disease, both animals showed an inverse relationship between CSF VL and BP values. Green boxes reflect treatment (ART) courses.

Mixed effect model fitting of the variables showed significant negative correlation between SUVmean(total) (n=5) and CSF viral load (p= 0.024) and a trend positive correlation with CSF MCP-1 levels (p=0.067). BP (n=4) showed borderline significant correlation with CSF VL (p=0.064).

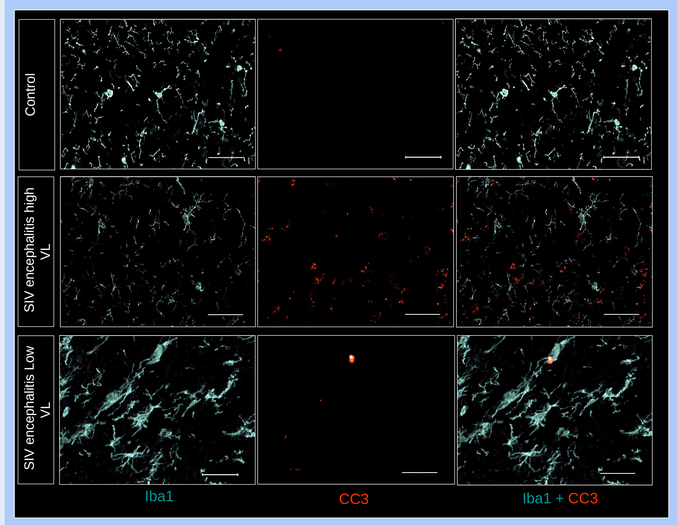


Fig 2: Iba1 (green) and CC3/PARP (red) staining in a control and two infected animals . SIV#1 (CSF VL=1.8E+06) shows decreased Iba1 and increased CC3/PARP staining suggesting microglial loss/dysfunction and increased apoptosis compared to control. SIV#3 (CSF VL=6.7 E+04) shows increased microglial staining with amoeboid shaped cell bodies and truncated processes consistent with microglial activation. There is lower CC3/PAR staining compared to SIV#1 but higher than control (scale bar=100 µm).

Pathology results in 4 of the animals compared to 3 uninfected monkey brains showed unchanged or mildly decreased Iba1 staining in the background compared to controls with increased diffuse CC3/PARP staining (apoptosis) (Fig.2, 2nd row). One animal (SIV#3) that showed upregulated TSPO on PET had increased Iba1 staining with amoeboid shaped microglial cell bodies and truncated processes suggestive of microglial activation (Fig.2, 3rd row).

Results

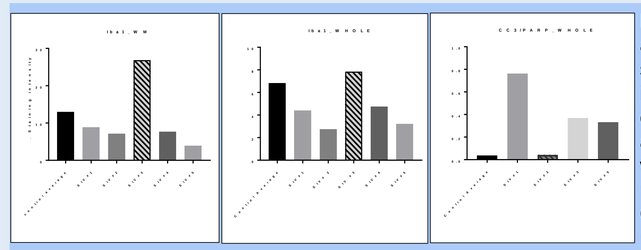


Fig 3: quantitative assessment of staining in all five infected animals compared to average control values (WM: white matter, Whole: Gray and white matter).

There was evidence of multiple microglial nodules with increased Iba1 and CC3/PARP staining in all animals (Fig. 4). The only animal showing increased binding on PET at the last time point (Fig.1C and D) showed a combination of diffuse and focal microglial activation on IF staining.

Noticeably, besides the microglial nodules, although there was suggestion of some apoptotic microglia/macrophages, CC3/PARP staining mainly co-localized with NeuN staining suggesting that the majority of apoptotic cells were neurons rather than glial cells (Fig.4).

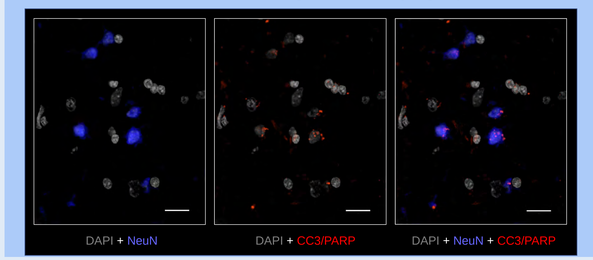


Fig 4: DAPI (gray), NeuN (purple) and CC3/PARP (red) staining obtained in SIV# 3. In animals with very high CSF VL, apoptotic markers mainly co-localized with neuronal markers (scale bar=20 µm).

Conclusions

We found a negative correlation between total [18F]-DPA714 binding on PET and CSF VL in macaques with severe SIV encephalitis. Imaging findings were further confirmed by IF staining.

Our results suggest that at very high CSF VL, microglial loss/dysfunction occurs rather than microglial activation. Marked neuronal apoptosis is also seen in this setting. On the other hand, microglial activation is seen when CSF VL decreases. Our findings provide a new insight into the role/status of microglia/macrophages in early stages of the disease when very high CSF viral loads are observed (Feibig stages II-IV)[2].

References

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