Modelling the Pathogenesis of HAND

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INTRODUCTION

An increasingly important consequence of HIV/AIDS is the neurological effects of chronic infection even when peripheral viral load is being controlled effectively. Improvement in anti retroviral therapies means that patients are now entering their 3rd decade living with HIV. However, significant numbers (up to 25%) with controlled viremia develop HIV-1 associated neurocognitive disorders (HAND) which greatly affects their daily quality of life. Detailed scientific understanding of how HIV affects the brain is poor because of difficulties in obtaining relevant clinical samples at appropriate times during infection. Model systems are required that will allow us to develop improved clinical treatments for HAND. The HPA is unique in the UK in the having facilities and scientific infrastructure to maintain the experimental infection of macaques with simian immunodeficiency virus (SIV). This model is considered by most scientists as the best for studying the processes of infection and disease. We have undertaken *in situ* analysis of brains from cynomolgus macaques (*M.fascicularis*) infected for 20-40 weeks with either neurotropic SIVmac17E-Fr, nef attenuated SIVmacC8 or its wild type equivalent SIVmacJ5. This is a non-accelerated disease progression model and at these time points peripheral viral replication was undetectable. The availability of an SIV/macaque model with an undetectable viral set point following the initial replicative burst enables analysis of neurological processes occurring in a setting equivalent to that seen following initiation of HAART and could detail whether supportive management of the CNS should be considered early during a patients treatment regime.

METHODS

Groups of four animals were inoculated intravenously with either SIVmacJ5 or the Δnef attenuated SIVmacC8. 20 weeks later these groups plus a third group of naive animals were inoculated with neurovirulent SIVmac17E-Fr. All animals were euthanized 23 weeks after SIVmac17E-Fr challenge at a time when no clinical symptoms were apparent. Formalin fixed paraffin embedded representative sections of cerebral cortex, cerebellum and brain stem were examined by in situ hybridisation (ISH) to detect viral RNA expression and by immunohistochemistry (IHC) to detect viral proteins, a range of host cell types and associated pathological changes. Brains taken at necropsy from SIV naive juvenile macaques were used as negative control samples to establish baseline staining levels.

RESULTS

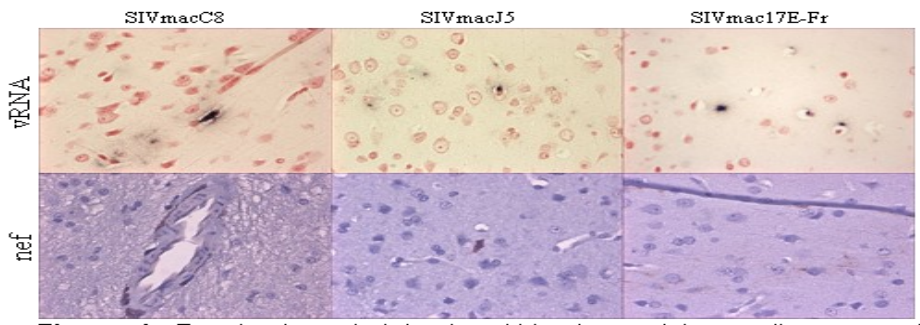

Figure 1: Despite low viral loads within the periphery, all groups of macaques showed evidence of SIV encephalitis as detected by both ISH and IHC.

- GFAP, CNPase1, FF1, CD68 and iba-1 expression was observed in SIV naive macaques. No CD163 expression or T cell influx was observed.
- SIVmacC8 infected animals showed increased expression of GFAP, CD68, iba-1, CD163 and influx of CD8 T cells.
- SIVmacJ5 infected animals showed increased expression of GFAP, CD68, iba-1,CD163 and CD8/CD4 T cell influx. Expression of CNPase1 and FF1 was decreased.
- SIVmac17E-Fr infected animals showed increased expression of GFAP, CD163 and CD8/CD4 T cell influx. Expression of GFAP, CNPase1, FF1 and

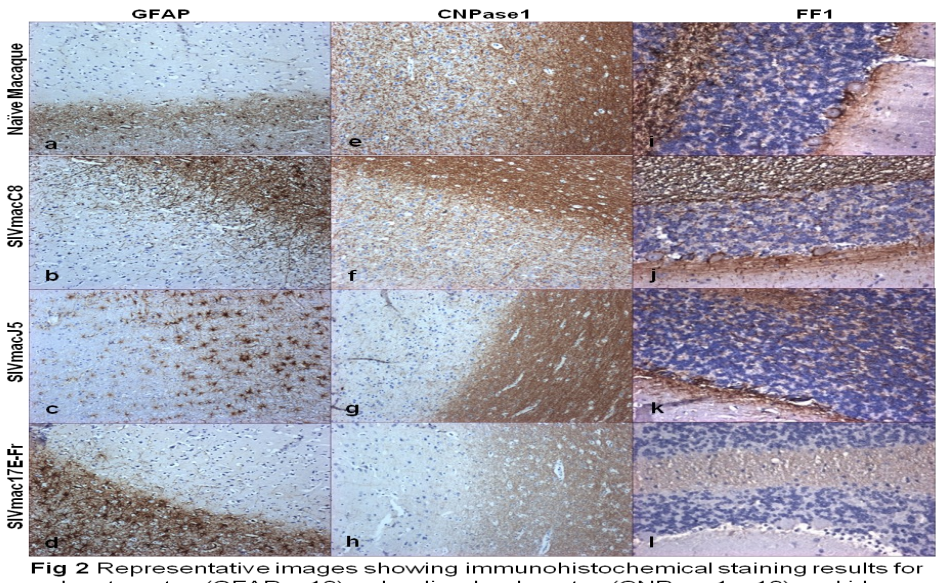

Fig 2 Representative images showing immunohistochemical staining results for a-d: astrocytes (GFAP x10), e-h: oligodendrocytes (CNPase1 x10) and i-l: neuronal phosphorylation (FF-1 x20) within a-h: frontal lobe and i-l: cerebellum

RESULTS

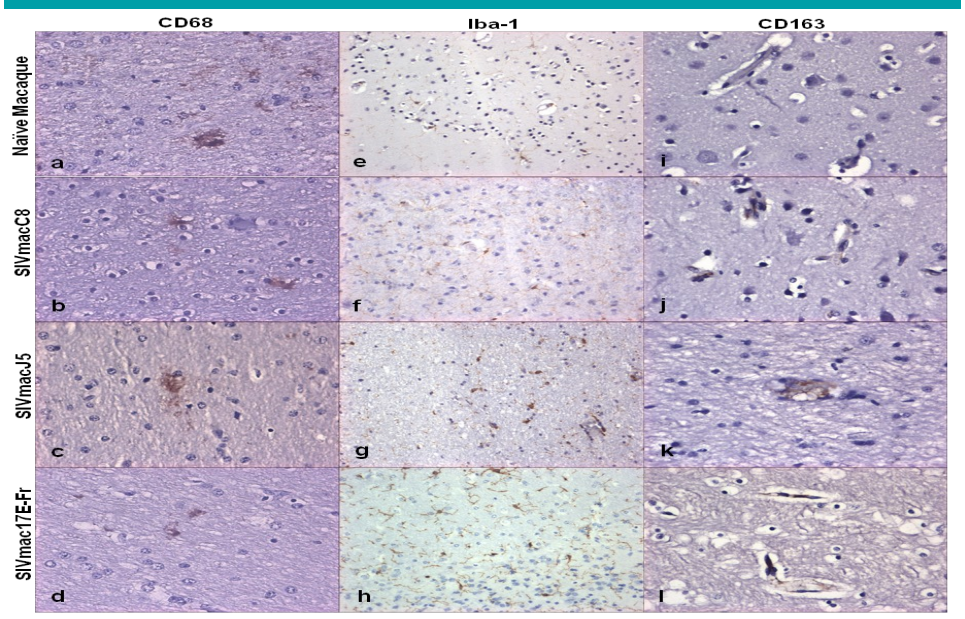

Fig 3: Representative images showing immunohistochemical staining results for a-d: macrophage (CD68, x40), e-h: microglia (iba-1, x20) and i-l: SIV infected microglia (CD163, x40) within frontal lobe

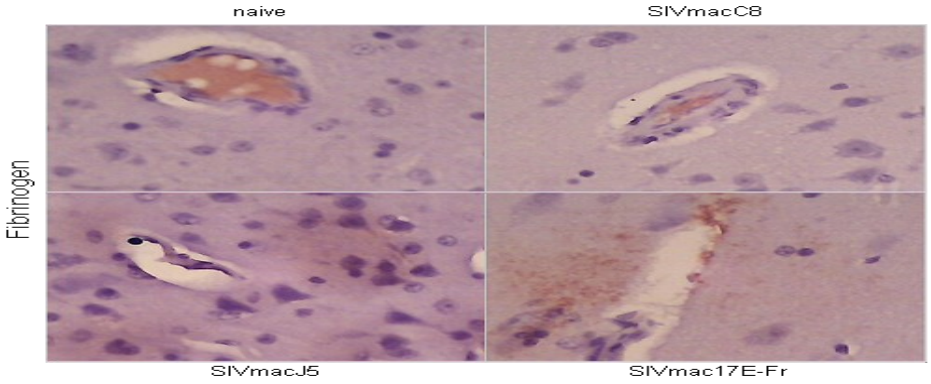

Figure 4: Pathological changes did not correlate with breakdown of the blood brain barrier as it appeared intact in SIVmacC8 infected animals with no leakage of fibrinogen into the brain.

	Blood 14dpc				Blood 14dpc 17E-Fr			Termination 20 weeks post SIVmac17E-Fr							
									DNA PCR						
Virus Wk 0	vRNA log_{10}	DNA PCR C8	DNA PCR J5	Virus Wk20	vRNA log_{10}	DNA PCR C8/J5	DNA PCR 17E-Fr	vRNA log_{10}	Blood C8/J5	Blood 17E-Fr	Spleen C8/J5	Spleen 17E-Fr	MLN C8/J5	MLN 17E-Fr	PLN C8/J5	PLN 17E-Fr
SIVmacC8	3.30	+	n/a	SIVmac 17E-Fr	3.00	+	-	3.09	+	-	-	-	+	-	+	-
	4.00	+	n/a		2.15	+	-	1.35	-	-	+	-	+	-	+	-
	4.25	+	n/a		2.06	+	-	1.98	-	-	+	-	+	-	+	-
	4.60	+	n/a		-	+	-	1.30	-	-	+	-	+	-	+	-
SIVmacJ5	5.20	n/a	+	SIVmac 17E-Fr	2.31	+	-	2.45	+	-	+	-	+	-	+	-
	4.40	n/a	+		2.11	+	-	-	+	-	+	-	+	-	+	-
	5.49	n/a	+		4.46	+	-	4.27	+	-	+	-	+	-	+	-
	5.97	n/a	+		-	+	-	-	+	-	+	-	+	-	+	-
----	n/a	n/a		SIVmac 17E-Fr	4.22	-	+	-	n/a	-	n/a	+	n/a	+	n/a	+
	n/a	n/a			4.92	-	+	-	n/a	-	n/a	-	n/a	+	n/a	+
	n/a	n/a			4.57	-	+	-	n/a	-	n/a	+	n/a	+	n/a	+
	n/a	n/a			5.21	-	+	-	n/a	+	n/a	-	n/a	+	n/a	+

Table 1: Viral RNA within peripheral blood was determined 14 days post viral challenge and at termination. DNA PCR discriminated between viral species within peripheral blood and tissues. - vRNA levels below detectable level of $log_{10}1.30$.

CONLUSIONS

- Pathological changes were observed following infection by SIV isolates not recognised as neurotropic, at a time when peripheral vRNA was undetectable and BBB damage not always apparent.
- Detailed examinations from early time points following infection would provide further information regarding the amount and timings of CNS viral invasion required for detrimental pathologies to develop.
- This non-accelerated model system representing different points of the clinical spectrum of HIV induced neuropathology in the absence of classical peripheral markers of disease progression could inform current debates regarding early intervention for CNS viral suppression and potential requirements for complementary therapies to support neurocognitive function.

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Neuropathology of wild-type and *nef*-attenuated T cell tropic simian immunodeficiency virus (SIVmac32H) and macrophage tropic neurovirulent SIVmac17E-Fr in cynomolgus macaques

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