Pathogenesis of JC Virus Reactivation in Natalizumab-treated MS Patients

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BACKGROUND

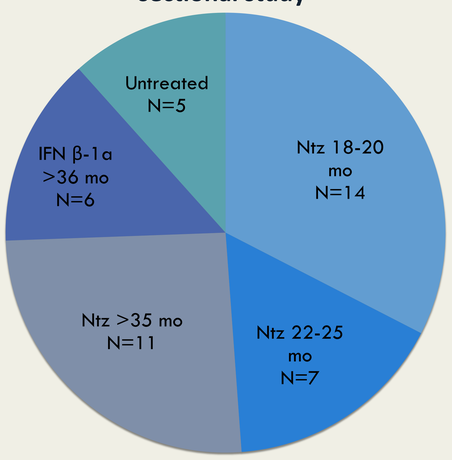
Progressive multifocal leukoencephalopathy (PML), caused by JC virus (JCV), remains a devastating brain opportunistic infection in HIV-infected individuals but has also become a complication of treatment with novel immunomodulatory agents. Natalizumab, a monoclonal antibody against integrin alpha-4, provides effective treatment for multiple sclerosis (MS) by preventing leukocytes from entering the brain. However, as of January 2014, 428 PML cases have been reported in natalizumab-treated MS patients. The pathogenesis of JCV reactivation remains elusive.

OBJECTIVES

1. [illegible] reactivation as duration of natalizumab treatment increases
2. Uncover the initial site of viral reactivation
3. Characterize JCV – specific cellular immune response in natalizumab-treated MS patients.

METHODS

N=43 JCV seropositive MS patients, cross-sectional study



CSF, blood and urine collection

-Blood: Ficoll, **PBMC** cytometry sorting:

CD34⁺ cells

B cells

Monocytes

T cells

NK cells

Polymorphonuclear cells

-DNA extraction and quantitative PCR for JCV

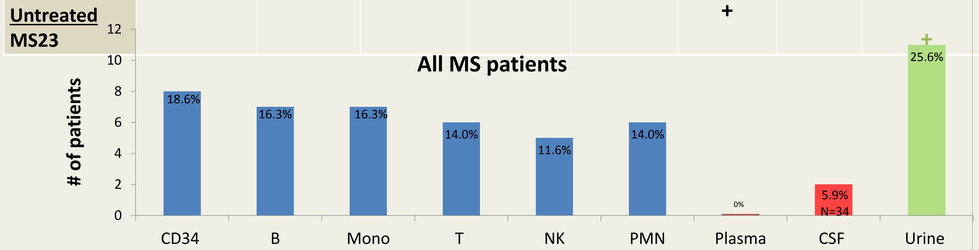
-JCV-specific T cells detection by **ELISpot** and **ICS**, both **ex vivo** and after **in vitro** stimulation with JCV VP1 peptide library

RESULTS

JCV DNA detection in CSF, PBMC subpopulations, plasma and urine

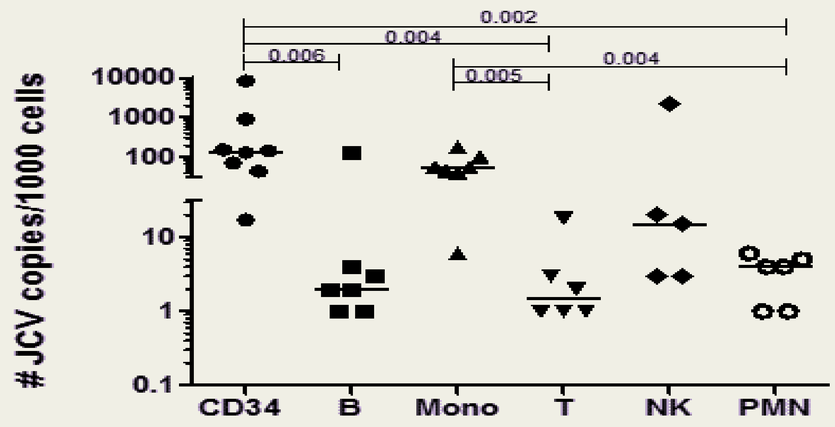
	Natalizumab 18-20 mo n=14	Natalizumab 22-25 mo n=7	Natalizumab >36mo n=11	IFN-β >36 mo n=6	Untreated n=5
CSF 2/34 (5.9%)	**1/11**	0	**1/11**	0/2	0/5
Plasma	0/14	0/7	0/11	0/6	0/5
PBMC 12/43 (27.9%)	4/14	2/7	3/11	3/6	0/5
Urine 11/43 (25.6%)	3/14	1/7	2/11	1/2	1/5

	CD34	B	Mono	T	NK	PMN	CSF	urine
18-20 mo								
MS04	+	+	+	+	+	+		
MS17								+
MS19	+		+	+	+			
MS22							+	
MS25	+	+						+
MS33	+		+	+		+		
MS34								+
22-25 mo								
MS12				+				
MS29		+						
MS38								+
>36 mo								
MS02	+	+	+	+	+	+		
MS03	+	+	+	+	+	+		+
MS11							+	
MS18								+
MS27	+	+	+		+	+		
IFN-β>36 mo								
MS05	+	+						+
MS40								+
MS41								+
MS42			+					+
MS43								+
Untreated								
MS23								+



JCV DNA was detected in the CSF of 2/34 (5.9%) subjects tested with no symptoms or MRI lesions consistent with PML and in PBMC of 12/43 (27.9%) subjects but not in plasma. Detection within different PBMC subpopulations is shown above. Viruria was detected in 11/43 (25.6%) subjects.

JC viral load in PBMC subpopulations



JCV DNA load was higher in CD34⁺ cells compared to B (p=0.006), T (p=0.004) and polymorphonuclear (PMN) cells (p=0.002) and in monocytes compared to T cells (p=0.005) and PMN (p=0.004)

JCV-specific T cell detection by ELISPot and ICS

		Natalizumab 18-20 mo n=14	Natalizumab 22-25 mo n=7	Natalizumab >36 mo n=11	IFN-β >36 mo n=6	Untreated n=5	All subjects n=43
ELISpot	ex vivo	3/14(21.4%)	1/7(14.3%)	0/11(0%)	2/6(33%)	1/5(20%)	7/43(16.3%)
	in vitro	11/14(78.6%)	7/7(100%)	10/10(100%)	3/6(50%)	5/5(100%)	36/42 (85.7%)
ICS	ex vivo	8/14(57.1%)	5/7(71.4%)	9/11(81.9%)	3/6(50%)	2/5(40%)	27/43(62.8%)
	in vitro	14/14(100%)	7/7(100%)	11/11(100%)	6/6(100%)	5/5(100%)	43/43(100%)
CD4⁺	ex vivo	7/14(50%)	4/7(57.1%)	8/11(72.7%)	2/6(33%)	2/5(40%)	23/43(53.4%)
	in vitro	14/14(100%)	7/7(100%)	11/11(100%)	6/6(100%)	5/5(100%)	43/43(100%)
CD8⁺	ex vivo	5/14(35.7%)	2/7(28.6%)	8/11(72.7%)	3/6(50%)	2/5(40%)	20/43(46.5%)
	in vitro	12/14 (85.7%)	6/7 (85.7%)	11/11 (100%)	6/6(100%)	3/5(60%)	38/43(88.4%)

JCV-specific cellular response detection by ELISpot and ICS was significantly enhanced after in vitro stimulation with VP1 peptides, compared to ex vivo ($p<0.001$). ICS captured JCV-specific cellular response more frequently compared to ELISpot, both ex vivo ($p<0.001$) and in vitro ($p<0.01$). JCV-specific CD4⁺ - mediated response was more frequently detected in vitro, compared to CD8⁺ - mediated response ($p=0.05$). Presence of JCV-specific CD4⁺ T cells in the peripheral blood, as detected by ICS ex vivo, correlated with DNA detection in PBMC ($p=0.05$, not shown).

CONCLUSIONS

1. **Asymptomatic** JCV reactivation may occur in the CSF of natalizumab-treated MS patients after 18 months of therapy. Therefore, in the absence of new clinical or MRI findings, JCV detection in CSF is not equivalent to PML.
2. JCV is associated with multiple circulating mononuclear cell subpopulations and **viral load** is higher in **CD34⁺ cells and monocytes** compared to other cell types.
3. **Viremia** might trigger a JCV-specific **CD4⁺ - mediated response** that can be detected by ICS.
4. JCV DNA detection in **PBMC**, but not in plasma or urine, may be a surrogate marker for viral reactivation.
5. In vitro stimulation with JCV peptides allows enhanced detection of JCV-specific cellular response, which is highly prevalent in all MS patients, regardless of treatment, and is mediated by CD4⁺ and CD8⁺ T cells.

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