Early non-mac-tropic HIV-1 R5 envs efficiently trans-infect T-cells and infect ectocervices

Paul J. Peters[1], Olivia O'Connell[1], Thomas Musich[2], Maria Paz Gonzalez-Perez[1], Tiffany Moore-Simas[3], Cynthia Derdeyn[4], Paul R. Clapham[1]

[1]University of Massachusetts Medical School, Worcester, MA, [2]National Cancer Institute, Bethesda, MD, [3]University of Massachusetts Medical Center, Worcester, MA, [4]Emory University, Atlanta, GA

Paul Peters
373 Plantation ST
Biotech 2, Rm 315
Worcester MA, 01605
Paul.peters@umassmed.edu
Phone: (508) 856-5824



Background

Non-mac-tropic HIV-1 R5 viruses are preferentially transmitted and persist in immune tissue even in AIDS patients who carry highly mac-tropic variants in the brain.

Non-mac-tropic R5 Envs require high levels of CD4 for infection contrasting with highly mac-tropic Envs, which infect cells expressing low CD4.

Non-mac-tropic R5 Envs predominate in immune tissue and have been described as T-tropic.

Here, we investigated whether transmitted/founder (T/F), acute and late non-mac-tropic R5 Envs were more T-tropic compared to highly mac-tropic R5 Envs. We tested whether non-mac-tropic R5 Envs mediated more efficient infection of CD4+ T-cells and cervical explant cultures compared to highly mac-tropic R5 Envs.

Methodology

HIV-1+ envelope clones:

TABLE 1. HIV-1 env clones used to prepare GFP reporter Env+ pseudovirions.

Patient	Non-mac-tropic			Mac-tropic		
AIDS Patients	**Env**	**Origin**	**Full ID**	**Env**	**Origin**	**Full ID**
BaL				BaL.26	Lung	
NA20	LN8	LN		B59	Brain	
				B501	Brain	
NA420	LN40	LN		B33	Brain	
JR	JR-CSF	CSF		JR-FL	Brain	
P1114	98-27	Plasma		98-15	Plasma	
P7766	SP1	Spleen	SP13-33-41	FL2	Brain	FL19-56-66
P10017	SP2	Spleen	SP10-9-65	FL1	Brain	FL9-1-2
P6568	SP1	Spleen	SP6-11-9	FL1	Brain	FL11-1-249
CA110	SP2	Spleen	SP53-23-131	OC1	Brain	OC58-11-57
	SP3	Spleen	SP53-6-122	SP1	Spleen	SP52-13-34
	SP4	Spleen	SP52-16-50			
E21	LN58-3	LN		B37-6	Brain	
				B100-1	Brain	
KM21	BL1-45	Blood				
	S1-20	Semen				
T/F						
3T	3T	Plasma	p1054.TC4.1499			
6T	6T	Plasma	p63358.p3.4013			
15T	15T	Plasma	p700010040.C9.4520			
19T	19T	Plasma	pPRB958_06.TB1.4305			
Acute Stage						
R463F	R463F	Plasma	R463FPL16MAR07EnvE44			
R880F	R880F	Plasma	R880FPL12JAN07EnvA6			
Z185F	Z185F	Blood	Z185FPB24AUG02ENV3.1			
Z205F	Z205F	Blood	Z205FPB27MAR03ENV1.1			
R66M	R66M	Plasma	R66MPL7MAR06.3A9Env			
Z1792M	Z1792M	Plasma	Z1792MPL18DEC07.3G7Env2			
Z201M	Z201M	Plasma	Z201MPL7FEB03ENV2.1			
Z221M	Z221M	Plasma	Z221MPL7MAR03ENV2.1			
Z153M	Z153M	Plasma	Z153MPL13MAR02ENV5.1			

HIV-1+ envelope clones:
We selected a panel of envelopes that consisted of late disease macrophage-tropic and non-macrophage-tropic viruses, transmitted/founder (T/F), and acute stage viruses (Table 1).

Infectivity assays:
We used Env$^+$ pseudovirions that carried a GFP reporter gene to measure infection of CD4+ T-cells and ectocervical explant cultures.

Cervical explant infections:
Infections were done on 5 replicates for each tissue for at least 5 donors for each Env+ pseudovirion. GFP+ cells were quantified on day 7 post-infection. Most infections were carried out in the presence of PHA and IL-2. However, infection was still detected without stimulation, but at lower levels (Table 2). Alternative stimulation with anti-CD3, anti-CD28 mabs resulted in similar levels of infection as for PHA/IL-2 (data not shown).

Results

Mac-tropic R5 Envs confer highest infection of HeLa TZM-bl cells as well as macrophages
All Envs (which are all R5) conferred strong infection of HeLa TZM-bl cells that express high levels of CD4 and CCR5. However, mac-tropic envelopes conferred up to 20-fold higher infectivity titers compared to T/F/acute and late stage non-mac-tropic Envs (figure 1A).

All of the Envs previously determined to be mac-tropic conferred high levels of infectivity for macrophages and several orders of magnitude higher than for non-mac-tropic T/F or late disease Envs (figure 1B).

There was no significant difference between the amount of gp120 present in late stage mac-tropic and non-mac-tropic Env+ pseudovirions, although T/F/acute pseudovirions carried slightly less gp120 (figure 1C).

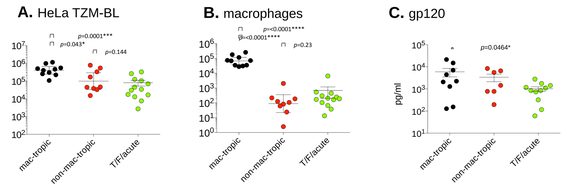

Figure 1. Env+ pseudovirion infection of (A) HeLa TZM-bl and (B) macrophages. (C) gp120 concentrations on pseudovirions.

Infection of CD4+ T-cells and MDDCs by Env+ pseudovirions
Both CD4+ T-cells and MDDCs were poorly susceptible to Env+ pseudoviruses (figure 2A), with highly mac-tropic Envs mediating slightly higher infection than non-mac-tropic T/F/acute and late disease R5 Envs, although this was not significant.

DEAE dextran and spinoculation rescue infection of CD4+ T-cell but not of MDDCs
DEAE dextran and spinoculation significantly enhanced CD4+ T-cell infection by all Env+ pseudovirions by an average of 10-100-fold (figure 2B). In contrast, there was little effect on the low levels of infection of MDDCs (figure 2A). These results show that infection of CD4+ T-cells (but not MDDCs) is greatly increased by enhancing the attachment of virus particles.

Infection of CD4+ T-cells by mac-tropic Envs trended higher than non-mac-tropic Envs but not significantly.

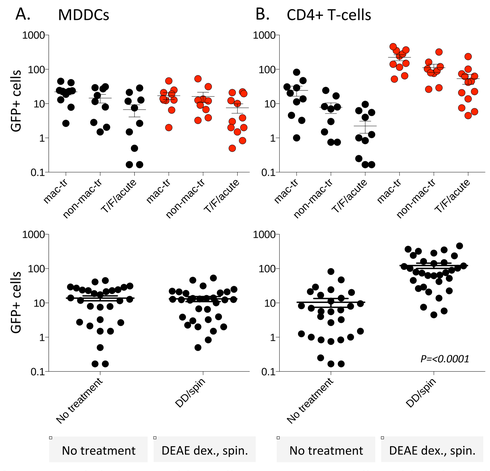

Figure 2. T/F/acute and late disease Envs mediated low infection of LPS treated MDDCs and CD4+ T-cells. Infection of CD4+ T-cells but not MDDCs is greatly enhanced by DEAE-dextran and spinoculation.

MDDCs mediate efficient T-cell infection by non-mac-tropic R5 Envs
Trans-infection via LPS treated MDDCs resulted in substantially higher levels of CD4+ T-cell infection compared to direct infection of T-cells or MDDCs alone (figure 3).

Trans-infection of CD4+ T-cells by late stage non-mac-tropic Envs mediated similar levels of T-cell infection as by highly mac-tropic Envs. However, the T/F/acute Envs mediated slightly lower levels of infection and this was significant.

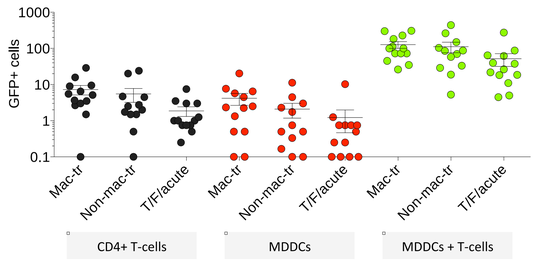

Figure 3. MDDCs trans-infection enhances T-cell infection by mac-tropic Envs, non-mac-tropic R5 Envs, and T/F/acute Envs.

Infection of ectocervical explant cultures
Infected GFP+ cells were visible by fluorescent microscopy (figure 4A). Pseudovirions carrying the lab-adapted, highly mac-tropic Bal Env consistently mediated high levels of infection, while infectivity levels of T/F/acute, and late disease R5 Envs were more variable (figure 4B). There was no statistical difference in explant infection between mac-tropic and non-mac-tropic R5 Envs from late disease. However, T/F/acute Envs mediated significantly lower levels of infection compared to late disease mac-tropic Envs. These observations do not support a cervical tissue tropism conferred by T/F Envs.

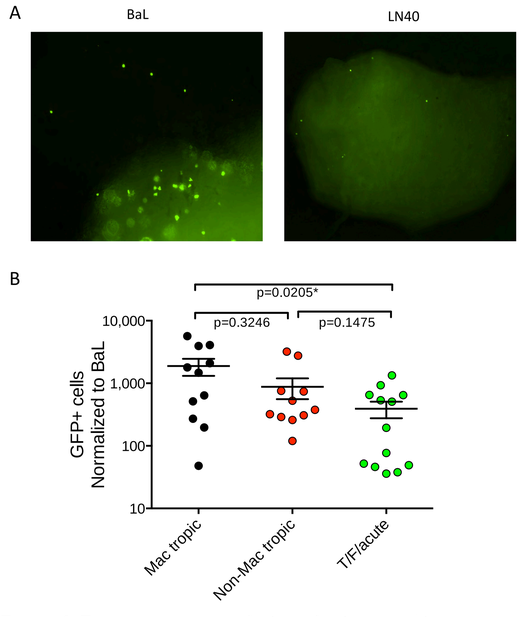

Figure 4. Env+ pseudovirion infection of ectocervical tissue explants. (A) Infected GFP+ cells are easily visible and quantified. (B) Late stage mac-tropic and non-mac-tropic R5 Envs mediate similar levels of Infection, while T/F/acute Envs conferred lower infection.

Identification of CD4+ T-cells as the main targets for infection in cervices
Using an immunomagnetic selection approach, we showed that T-cells were the main target for infection in cervical explant cultures (Table 2). In the absence of stimulation, T-cells were still the main target cell for BaL infection, although numbers of infected cells were low.

There was little evidence to support macrophage infection even for highly mac-tropic R5 Envs.

TABLE 2. Identification of Env+ pseudovirus, GFP+ infected cells in cervical explant cultures

Explant	Env+ pseudo viruses	PHA, IL-2	T-cells positive selection (CD3)		Monocyte, macs Negative selection		DCs Negative selection	
			T-cell +	T-cell -	mono/ mac+	mono/ mac -	DC+	DC-
Cx134	BaL 26	+	747	1	0	136	1	42
		+	139	2				
Cx135	BaL26	+	82	2	0	66	1	251
Cx147	BaL26	+	104	5	2	125		
		-	7	0	0	14		
	NA20 B59	+	79	7	5	116		
	7766 FL2	+	15	0	0	41		

Conclusions

1. T/F, acute or immune tissue-derived non-mac-tropic R5 Envs are NOT more T-tropic than highly mac-tropic Envs from brain tissue.

2. Trans-infection following capture of virions by DCs supports maximal infection by non-mac-tropic R5 Envs that is equivalent to infection by highly mac-tropic Envs.

3. T/F/acute R5 Envs are NOT preferentially tropic for cervical tissue.

4. Late stage non-mac-tropic Envs mediate similar (but not higher) levels of cervical explant infection as highly mac-tropic Envs.

5. CD4+ T-cells are the main targets in cervical explants, even by virions carrying highly mac-tropic Envs.

Acknowledgements

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