PBMC from Highly-Exposed Sero-Negative Individuals Inhibit Transmitted Founder HIV

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Abstract Highly-exposed Sero-Negative (HESN) individuals engage frequently in unprotected sexual activities, yet remain sero-negative. In order to avoid sero-conversion, these individuals likely restrict HIV infection soon after transmission. Transmission of HIV infection is established by an extremely small fraction of circulating virus. These Transmitted Founder viruses (TFV) likely arise from a single HIV clone. Recently, Parrish and Fenton-May have shown that TFV are type I interferon (IFN)-resistant compared to chronic viruses from the same individual. We hypothesize that HESN inhibit TFV more efficiently than normal donors after IFN-beta treatment.

We examined the ability of PBMC from 23 normal donors and 32 HESN donors to inhibit 10 TFV or an R5-tropic NL4-3 derivative, JM1186 after IFN-beta treatment. Two percent of media was removed for p24 concentration determination by alpha-LISA. HESN PBMC genotypes for selected genes were performed by custom Nimblegen Array and 454 Sequencing.

A significant proportion of HESN PBMC inhibited JM1186 infection >25% (p = 0.0017) but not normal PBMC (p = 0.0730). In a separate assay, 12 HESN PBMC and 8 normal PBMC were infected with CH040, SUMA, TFCH058, 6 month chronic virus (6mo) CH058, and JM1186 and treated or not with 75 IU/ml IFN-beta. In untreated samples, only infection with TF CHO58 resulted in a significantly lower p24 in HESN (p = 0.0473). Interestingly, this virus does not trigger an IFN response in normal monocyte-derived dendritic cells. In normal PBMC, the TFV CHO40 and SUMA were more resistant to IFN-beta than JM1186 (p = 0.0031 and 0.0446, respectively). For HESN samples, the median level of IFN-beta inhibition for all viruses was lower than normal, significantly so for CH040 and SUMA, (p = 0.0027 and 0.0181, respectively). All samples were ranked for their ability to inhibit all viruses. 6 of 9 HESN donors with ranks above the distribution of normal donors had variations in genes that were reported to interact with HIV Protease, Vpr, Vpu, Integrase, and Env.

PBMC from HESN respond to IFN in a manner that more efficiently inhibits some viruses, including two TFV. HESN appear able to also inhibit an IFN sensitive TFV without IFN treatment. Determining the mechanism of resistance, whether it is increased responsiveness to IFN,expression of variant genes which impede viral replication, or resistance to viral countermeasures, will uncover modes of HIV resistance relevant to in vivo transmission events.

[4]Proc Natl Acad Sci U S A. 2013 Apr 23;110(17):6626-33
[5]Retrovirology. 2013 Dec 3;10:146

Day 10 and Day 14 post-infection are within growth phase of TF and lab adapted viruses tested

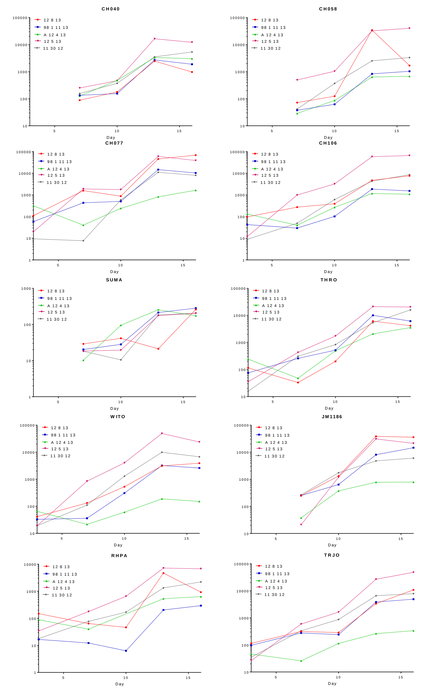


Figure 1 PBMC were thawed, cultured for 4 days with 10 IU/ml IL2, and then infected with 5 ng/ml p24 of each virus listed.2 ul of SN was removed at each timepoint and analyzed by p24 Alpha-LISA.

UCSD CFAR Cohort

HxDate	SexPart3mo	SexPart1mo
09/08/10	20	10
11/24/09	12	5
01/12/10	80	20
01/21/10	17	0
02/09/10	30	20
01/29/10	7	3
02/18/10	8	3
02/25/10	20	3
03/09/10	30	10
03/10/10	25	3
04/01/10	12	10
04/07/10	15	12
04/14/10	8	5
04/30/10	8	6
05/07/10	10	5
05/06/10	15	10
05/12/10	12	3
05/19/10	12	4
05/06/10	15	5
06/03/10	12	1
06/04/10	35	10
08/24/10	8	5
09/15/10	100	20
09/23/10	10	3

HESN from PBMC restrict Lab adapted HIV more efficiently than normal PBMC after IFN-beta treatment

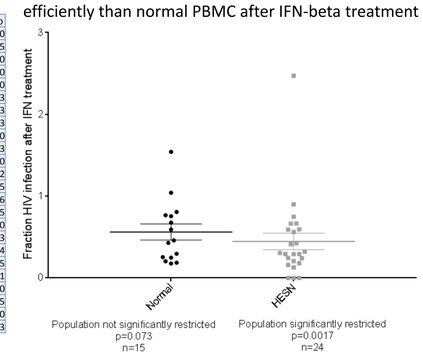


Figure 2 Cryopreserved PBMC were obtained from the UCSD Center for AIDS Research. Highly Exposed Sero-Negative individuals are negative by individual donation HIV nucleic acid testing (NAT) on the day that their risk data are collected. The HIV NAT used is the Procleix Ultrio HIV-1/HCV/HBV assay with an HIV RNA detection threshold of about 25 copies/ml. Individuals had a median of 13.5 partners for unprotected sex over three months. PBMC were thawed, cultured for 4 days with 10 IU/ml IL2, treated with 75 IU/ml IFN-beta for 18 h, and then infected with 5 ng/ml p24 JM1186, an NL4-3 variant with an R5 V3 crown in gp120. At day 14 post-infection, SN concentration of p24 was determined by Alpha-LISA. Shown is the fraction of p24 after treatment compared to the untreated samples for each donor. Wilcoxon Signed Rank Test was used to determine if each population demonstrated a greater than 25% inhibition in p24 concentration after IFN-beta treatment.

MACS HESN and Normal donors support similar levels of replication, but have significantly different abilities to restrict TF virus after IFN-treatment.

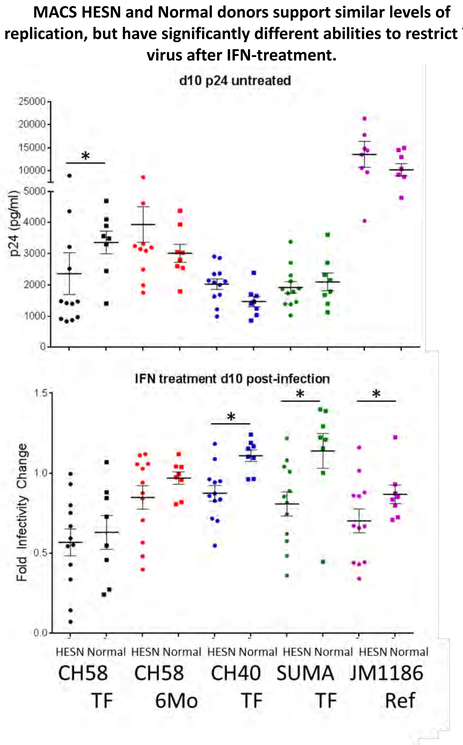


Figure 3 Thawed cryo-preserved PBMC from Normal and MACS HESN (>300 sex partners with no HIV+ test) samples were cultured in IL2 10% FBS RPMI for 4 days, **A** infected with 5 ng/ml of virus or **B** treated with 75 IU/ml IFN-B for 18 h and infected with 5 ng/ml of virus. P24 concentration was measured by alpha-LISA (Perkin-Elmer) at day 10 post-infection. For each donor, the ratio of the IFN-treated p24 concentration to the untreated concentration was determined. (* p value < 0.05, ** p value < 0.005)

TFV are resistant to IFN-beta compared to 6 mo chronic virus from same donor

CH58 virus does not induce Interferon response in MDDCs

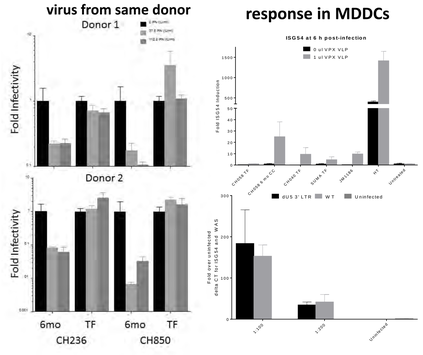


Figure 4 CD14+ monocytes were isolated from PBMCs and cultured for 5 days in the presence of 50 ng/ml GM-CSF and 200 ng/ml IL4. Cells were lifted and reseeded at 30K cells per well of a 96-well plate. One day later, cells were spinfected with 7.5 ng/ml p24 for 90 minutes at room temperature at 1400xg and incubated for an additional 4.5 h at 37 °C. Lipofectamine 2000 was used to transfect 50 ng Herring Testes (**HT**) DNA per well. SIV3+ was pseudotyped with VSV G and was added at the same time as HIV. Cells were lysed and RNA was harvested and converted to cDNA by iScript RT. Sybrgreen reagents were used to measure ISG54 and WAS RNA copies by QPCR. Reported are the ISG54 delta Ct from WAS divided by that value for the uninfected samples.

9 of 12 MACS HE donors inhibit HIV replication more efficiently than normal after IFN-beta treatment

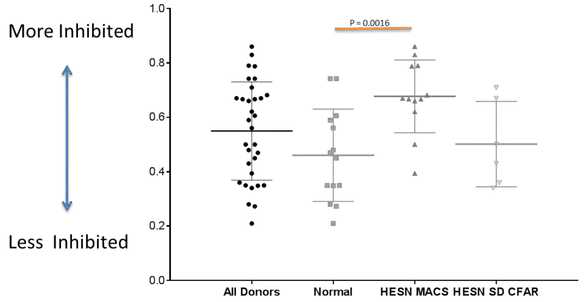


Figure 5 All samples were ranked for the inhibition observed with IFN-beta treatment. Samples receiving a rank of 1 exhibited the greatest inhibition. Rankings were generated for each virus and averaged by the number of viruses.

6 of 9 HESN with more efficient IFN-beta inhibition have variations in HIV binding genes.

	Donor	Avg Rank	Gene				Role/Pathway			HIV Interaction			
Average Normal Response	NR1	0.39	AIMP1	RTN3	LDLR	tRNA synth	beta-amyloid regulator	cholesterol					
	NR2	0.50											
	NR3	0.62											
Heightened Normal Response	HR1	0.66											
	HR2	0.67	ALMS1	GEMIN4		microtubule organization	splicing			PR	VPR		
	HR3	0.67											
	HR4	0.67	DIAPH1	ANKRD13A		deafness/actin	EGFR			PR	VPR		
	HR5	0.68											
	HR6	0.79	IFT122	ARFGEF1	NUP98	IPO4	cilia formation (microtubules)	vessicle trafficking/ EGFR	nuclear pore	nuclear import	PR	VPU	-	IN
Above Normal Response	HR7	0.79	PSMB6			proteasome				GP120			
	HR8	0.83	FUCA1	TPR	RNF213	glycosylation	nuclear export	EGFR/polycomb gene repression		-	-	VPU	
	HR9	0.86	SMG6			non-sense mediated mRNA decay/ telomere replication				PR			

Figure 6 Gene association analyses via SKAT-O were conducted by a combination of targeted gene-exome 454 sequencing and variant calling using an in-house pipeline based on 1000G and GATK best-practices protocols

Rare variants from HESN with more efficient HIV IFN-beta inhibition are hits in global HIV datasets.

Entrez Gene ID 23293	Symbol SMG6	APMS siRNA screen (Pache)	Host Factor (König)	MDDC-Phospho_ hit	MDDC-Ub_hit	cGAS target gene	Ub hit (up)	Ub hit (down)	Impens PR screen	ISGome	Schoggins +ssRNA	Wagner integration site associated with persistence X	TF Virus UB > Chronic UB
1729	DIAPH1								X				
4928	NUP98	Host	X		X			X					
5694	PSMB6	Host	X							X			
7840	ALMS1	Weak Restriction											
10565	ARFGEF1			X									
50628	GEMIN4						X						
55764	IFT122						X						
57674	RNF213				X	X					X		X
79711	IPO4						X						

X = meets criteria to be scored a hit in assay.
APMS siRNA screen = siRNA transfection of 293T cells infected with HIV for genes found to be HIV binding partners by Affinity Purified Mass Spec (Jager et al, Nature, 2011)
Host factor = siRNA knock-down of gene inhibited viral replication (Konig, Cell, 2008)
Phospho-hit = HIV infection of dendritic cells led to phosphorylation of protein
Ub Hit = HIV infection of dendritic cells led to ubiquitination of protein
cGAS target gene = gene was upregulated by transfecting HT DNA, a ligand for the HIV sensor cGAS
UB hit up = protein was ubiquitinated by HIV in HIV inducible cell line (Mary Lewinski, Guatelli Lab, UCSD)
UB hit down = protein lost ubiquitination after HIV induction in HIV inducible cell line (Mary Lewinski, Guatelli Lab, UCSD)
PR screen = protein was digested by HIV protease (Impens, Biol Chem, 2012)
ISG ome = gene is induced by Type I Interferon (Goujon, Nature, 2012)
+ssRNA inhibitor = was found to inhibit replication of some +ssRNA viruses when overexpressed (Schoggins, Nature, 2014)
Wagner = HIV integration sites that were found in multiple patients and cells (Wagner, Science, 2011)
TF virus UB > Chronic UB = Transmitted founder HIV infection led to increased ubiquitination compared to a chronic virus from the same individual (Jeffery Johnson, Krogan Lab, UCSF)

Donor	Gene Name	mRNA	NT Change	Amino Acid Change	Variant ID	Frequency
NR1	AIMP1	NM_001142415	c.A359G	p.E120G		
NR1	RTN3	NM_001265590	c.A954C	p.E318D		
NR1	LDLR	NM_001195800	c.G1530T	p.Q510H		
HR2	ALMS1	NM_015120	c.A9457T	p.T3153S		
HR2	GEMIN4	NM_015721	c.C2281T	p.R761C		
HR4	DIAPH1	NM_001079812	c.C2917T	p.L973F		
HR4	ANKRD13A	NM_033121	c.A1319G	p.Q440R		
HR6	ARFGEF1	NM_006421	c.A941G	p.Y314C	rs76811394	0.1 to 0.8%
HR6	NUP98	NM_005387	c.G2549A	p.G850E		
HR6	IPO4	NM_024658	c.G1311A	p.M437I		
HR6	IFT122	NM_052990	c.A1521T	p.X507C	rs146874343	0.001 to 0.0001%
HR7	PSMB6	NM_001270481	c.G425C	p.G142A		
HR8	FUCA1	NM_000147	c.T1016C	p.I339T		
HR8	TPR	NM_003292	c.A5128G	p.T1710A		
HR8	RNF213	NM_001256071	c.C3269T	p.T1090M		
HR9	SMG6	NM_017575	c.A2395G	p.I799V		

Conclusion PBMC from HESN respond to IFN in a manner that more efficiently inhibits some viruses, including two TFV. The ability to restrict TFV is of particular interest as these are the viruses which may be responsible for converting an HIV exposure to an HIV transmission resulting in disease. Genomic sequencing data indicate the HESN possess rare variants in HIV host factors. It is possible that these variant host factors additively interact with IFN-induced antiviral factors in order to confer a more efficient restriction of viral replication. Determining the mechanism of resistance will uncover modes of HIV resistance relevant to in vivo transmission events. Harnessing these modes of resistance will improve current microbicide, small molecule, and vaccine approaches to controlling HIV infection