

Gene-Expression Profiling of HIV-1C Infection and Transmission in Botswana

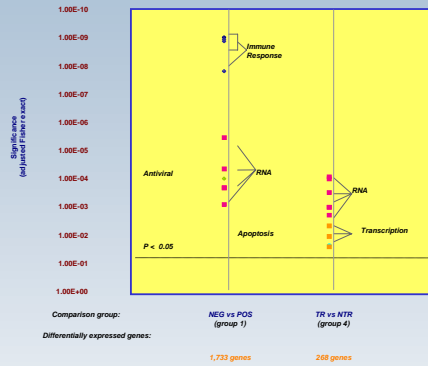
Monty Montano^{1,2}, Matthew Rarick¹, Paola Sebastiani¹, Patrick Brinkmann^{1,2},
Matthew Russell^{1,2}, Allison Navis¹, Carolyn Wester², Ibou Thior² and Max Essex²

¹Center for HIV-1 / AIDS Care and Research, Boston Medical Center; ²Botswana HSPH AIDS Initiative Partnership

ABSTRACT

Background The likelihood for perinatally acquired infection with the human immunodeficiency virus type 1 (HIV-1) is strongly associated with maternal viral load. However, we and others have observed infants born to drug naive women with high viral load but not infected and conversely, infants born to women with low viral load that were infected, suggesting that maternal cofactors may have an important influence on transmission outcome. The relation of maternal cofactors to mother-infant transmission outcome is largely unknown. **Methods** Prior to the availability of antiretroviral intervention (1999-2000), we collected PBMCs from 20 seronegative and 25 drug naive HIV-1C positive mothers in Botswana that included 14 mothers who did not transmit HIV-1C to their infants (NTRs) and 11 mothers who did transmit HIV-1C (TRs). The cells were then subjected to an assessment of gene expression with the use of 23,000 gene-probes to identify differentially expressed genes associated with maternal infection and transmission. **Results** We identified highly significant sets of differentially expressed genes in PBMCs that were associated with infection status and transmission outcome. Maternal HIV-1C infection was represented by increases of innate immune response genes, implicating toll-like receptor (TLR), interferon-stimulated pathways and RNA editing genes engaged as part of a broad antiviral response in these mothers. HIV-1C RNA processing gene expression was largely reduced, with the notable exception of genes associated with antiviral RNA editing / response. NTR mothers displayed an over-representation of immune response genes when compared with TR mothers and had viral load associated increases in RNA splicing factor expression. **Conclusions** Differential expression of genes associated with broad innate immune response, RNA processing, RNA editing and differential splicing were associated with maternal HIV-1C infection and perinatal transmission outcome.

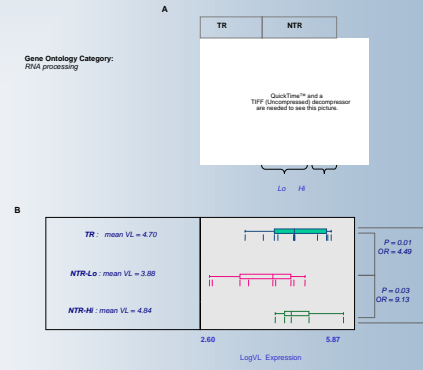
RESULTS



Summary of enriched gene categories. Shown are the adjusted Fisher's exact probabilities for the top 10 prominently ranked enriched biological categories within each comparison of mothers in Botswana. Note that all categories had significance values at $p < 0.05$.

PBMC (set 1), HIV	PBMC (set 2), HIV	PBMC (set 3), SARS	
Gene Category	Probability	Gene Category	Probability
defense response	7.05E-12	response to biotic stimulus	8.89E-11
immune response	7.08E-12	immune response	1.02E-09
response to biotic stimulus	2.28E-11	defense response	1.16E-09
response to stimulus	5.14E-09	response to stimulus	4.94E-08
antiviral response protein activ	6.09E-06	heat shock protein activity	4.95E-08
intracellular	8.88E-06	response to pest, pathogen	1.55E-05
cytoplasm	1.29E-05	response to stress	1.59E-05
regulation of cell cycle	4.66E-05	response to external biotic	1.81E-05
GTP binding	8.67E-05	response to wounding	1.97E-05
cell	0.000104	physiological process	7.12E-05
guanyl nucleotide binding	0.000147	inflammatory response	8.26E-05
interferon	0.000276	MHC class II receptor activi	0.000293
nucleotide binding	0.000467	response to external stimuli	0.0003
purine nucleotide binding	0.000578	antigen presentation, exog	0.000806
organismal physiological proces	0.000718	vacuole organization and bi	0.000806
response to pest, pathogen (c	0.001024	antigen processing, exog	0.000806
response to external biotic stim	0.001145	protein folding	0.000924
cell cycle	0.001463	death	0.001308
cytokine binding	0.001562	TslmUp	0.001465
laxis	0.001748	regulation of CDK activity	0.001782
		spliceosome complex	5.31E-06

Enriched category validation in an independent HIV dataset, but not in SARS. Shown are the top 20 biological categories enriched in two independent HIV infection datasets and in SARS infection dataset. Overlapping gene-probes (8,793) were identified for our HIV-positive/negative Botswana dataset, a US Army HIV-positive/negative dataset and a SARS-positive/negative dataset. The top 20 categories representing enriched gene sets are shown for the Botswana dataset (set 1: 25 HIV positive vs. 20 negative controls), the US Army dataset (set 2: 22 HIV positive vs. 12 negative controls, GEO series2171) and the SARS dataset (set 3: 8 SARS positive vs. 4 negative controls, GEO series GSE1739).



Identification of genes associated with transmitter status and viral load in Botswana. A) Heatmap for RNA processing gene set, with the HIV NTR viral load subsets (Lo, Hi) indicated below. B) Plasma viral load boxplots for HIV TR subjects, the NTR-Hi, and the NTR-Lo clusters. Plasma viral load is log transformed to base 10. P-values are shown for both a t-test and odds-ratio with equal variance.

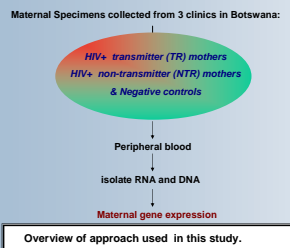
METHODS

Human Subjects. The study population consisted of a cross-sectional group of 20 HIV negative Botswana mothers with a mean age = 27 (ranging from 18 to 38) and 25 drug naive HIV-1 positive Botswana mothers with a mean age = 25 (ranging from 17 to 44) living within 3 different study sites. We have previously described viral burden in relation to transmission outcome (Montano et al, JID, 2003).

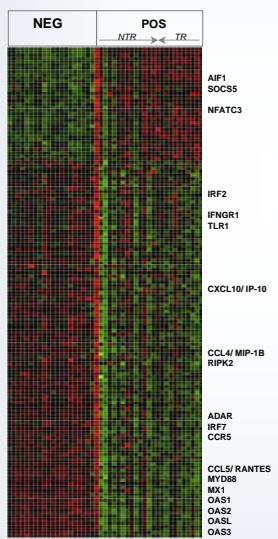
Microarray Processing. Total RNA was extracted from peripheral blood. Hybridization to U133A 2.0 chips (Affymetrix (Santa Clara, CA) were conducted. The 22,777 gene-probes were filtered based on presence/absence resulting in data for 11,705 gene-probes.

Analytic methodology. The arrays data sets were analyzed for differential expression based on HIV status (seronegative, seropositive) and transmitter status (transmitter; TR; nontransmitter; NTR) using BADGE version 1.0, a computer program implementing a Bayesian approach to identify differentially expressed genes (Sebastiani et al, Nat.Gen, 2005).

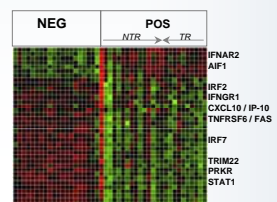
Once differentially expressed genes were identified by BADGE, the biologically enriched categories were identified, as recently described (Montano et al, Int Immunol, 2006), by implementing a stand-alone version of the EASE statistical software (Hosack et al, Genome Biol., 2003). Selected genes were validated using RT-PCR, data not shown.



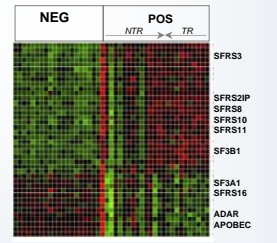
A. Gene Ontology Category: Immune response



B. Gene Ontology Category: Interferon



C. Gene Ontology Category: mRNA metabolism



Gene profile comparison for HIV- (NEG) with HIV+ (POS) women in Botswana. Heatmaps for Gene Ontology (GO) categories: A) immune response; B) interferon; C) mRNA metabolism, in association with HIV infection (NEG, POS) and transmission (NTR, TR) among mothers in Botswana, as indicated. Color code: red is downregulated and green is upregulated.

DISCUSSION

Overall, the findings in this study document that HIV-1C infection and transmission status were associated with the expression of different functional groups of genes that form a bridge between the innate and adaptive immune response. Our findings point to specific gene-sets associated with innate immune response, RNA processing and splicing, antiviral RNA response. Furthermore, the presence of common features of host response induced during HIV-1 infection in different settings, despite ethnographic, gender and viral subtype differences, and in contradistinction with other infections. Also implicated in these profiles were a subset of genes coding for SR proteins associated with RNA splicing, notably in the TR subset compared with HIV- and in the two subsets of NTR subjects. The relative abundance of SR proteins may influence the pattern of viral and host gene expression to influence local viral production and potentially transmission likelihood.

ACKNOWLEDGEMENTS

This work was supported by Grant R01AI1183 (M.M.) from the NIAID.