TLR9 Variant is Associated with Earlier HIV, EBV, and CMV Acquisition in Infants

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Background

Herpesviruses are often acquired in infancy and may affect HIIV disease progression. Understanding host and virus factors associated with virus acquisition and containment can inform the development of interventions.

Cytomegalovirus (CMV)

- CMV can be transmitted *in utero, intrapartum,* via breast milk, or saliva.
- >80% of Kenyan HIV infected children acquire CMV in infancy.
- CMV is an important opportunistic infection at low CD4 counts (retinitis, CNS).
- CMV co-infection is associated with increased risk of death, neurologic disease, and non-AIDS defining deaths in HIV-infected individuals.

Epstein-Barr Virus (EBV)

- EBV is primarily transmitted via saliva.
- >80% of HIV-infected Kenyan infants acquire EBV before the age of 2.
- EBV is an important cause of lymphomas in HIV-infected individuals, including Burkitt Lymphoma in children.
- Poor containment of EBV may affect cancer risk.

Toll-like Receptors (TLRs) and Viral Infections

- Host genetic variations influence disease acquisition and progression.
- Toll-like receptors (TLRs) are critical proteins of the innate immune system that are responsible for initial pathogen recognition, induction of downstream signaling cascades, and initiation of adaptive defense mechanisms. 10 human TLRs have been identified.
- TLR9 is expressed primarily on endosomal membranes of monocytes and B, NK, and dendritic cells. TLR9 recognizes and binds to unmethylated CpG motifs in viral DNA.
- Our previous study showed that the TLR9 1635A/G variant (rs352140) was significantly associated with time to HIV acquisition and HIV disease progression in a perinatal African cohort.[1]

Rationale and Research Objectives

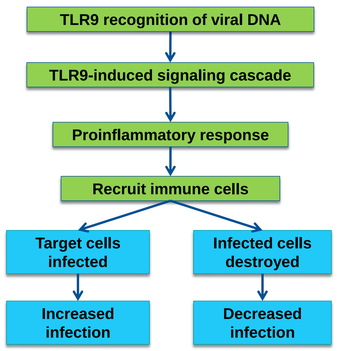
Given the role of TLR9 in recognition and response to viral infection, we hypothesized that *TLR9* 1635A/G variant may influence the ability of the innate immune system to respond to other viruses, altering the likelihood and impact of viral infections in exposed infants.

The aims of this project were:

1 – To determine the association between *TLR9* 1635A/G and timing of EBV acquisition, peak viral levels and viral suppression.

2 – To determine the association between *TLR9* 1635A/G and timing of CMV acquisition and peak viral levels.

Potential Infection Pathways



Study Design and Methods

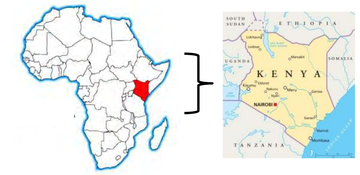
Population

Archived specimens and data were used from a cohort of mother-infant pairs from antenatal clinics in Nairobi, Kenya that were accrued and followed between 1999 and 2005. HIV, CMV and EBV outcomes were assessed longitudinally from birth to 1 year of age among HIV- exposed infants and to 2 years of age among HIV-infected infants.

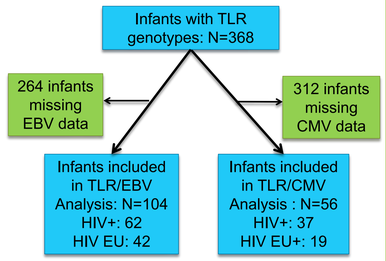
Data Collection and Analysis

HIV infection was diagnosed using PCR for HIV DNA or the GenProbe Assay for HIV RNA. CMV and EBV DNA were measured from plasma specimens using real-time PCR, with serologic confirmation of DNA-negatives. *TLR9* 1635A/G variation was genotyped using a custom microarray using the BeadArray platform from Illumina (Illumina, Inc., San Diego, CA). DNA samples were available for 398 infants from the initial cohort; of infants with available DNA, CMV data was available for 56 infants and EBV data was available for 104 infants.

Pearson's Chi squared tests, Cox proportional hazards, and linear regression were performed to assess the association of the TLR9 variant with acquisition, peak viral levels, and viral suppression. All analysis were stratified by HIV status and assumed a dominant model of inheritance.



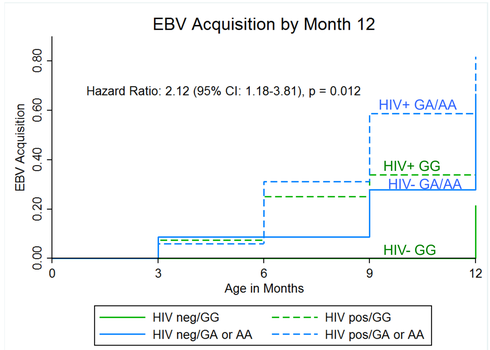
Study Population



Population Characteristics

Characteristic	EBV Cohort		CMV Cohort	
	N	Median (IQR) or Number (%)	N	Median (IQR) or Number (%)
Female	104	44 (42)	56	24 (43)
HIV Positive	104	62 (60)	56	37 (66)
Log peak HIV-1 plasma RNA level	62	6.7 (6.4-7.4)	37	6.9 (6.4-7.4)
EBV or CMV Positive	104	46 (44)	56	53 (95)
Log peak EBV or CMV plasma RNA level	46	2.5 (2.1-2.9)	53	3.0 (2.4-3.3)
Completed 12 Months of Follow-up	104	79 (76)	56	45 (80)
Completed 24 Months of Follow-up	62	26 (42)	37	17 (46)
Infant deaths (12 months)	104	19 (18)	56	10 (18)
Infant deaths (24 months)	62	29 (47)	37	17 (46)

EBV and *TLR9*



EBV and *TLR9* genotype data were available in 104 HIV-exposed and infected infants. The probability of EBV infection at 12 months was 61% among HIV-infected and 19% among exposed infants.

Infants with one or more copies of the 1635A allele showed an increased risk of EBV acquisition during the first year of life, adjusting for HIV status (HR=1.98, 95%CI=1.11, 3.54).

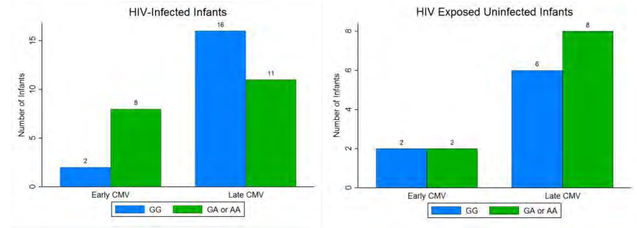
No associations were found between *TLR9* 1635A/G and EBV peak viral loads, or with time to EBV suppression (p>0.05 for all).

CMV and *TLR9*

CMV and *TLR9* genotype data were available in 37 HIV-infected infants. More than 80% of CMV infections occurred before 3 months of age.

At 1 month of age, 42% of HIV-infected infants with one or more copies of *TLR9* 1635A, and 11% of infants with 1635 GG genotype had acquired CMV (p=0.03).

No associations were found between *TLR9* 1635A/G and CMV peak viral loads, or with time to CMV suppression (p>0.05 for all).



Conclusions and Future Directions

- This study is the first to evaluate the association between the *TLR9* 1635A/G polymorphism and EBV- and CMV-related outcomes in a perinatal HIV-exposed African cohort.
- Having one or more copies of the *TLR9* 1635A variant was associated with earlier acquisition of CMV and EBV, similar to a prior analysis which noted that TLR9 1635A was associated with earlier acquisition of HIV, suggesting a key role for this locus in defense against viral infection.
- Further defining the functional phenotype of this *TLR9* variant may inform the development of novel therapeutic or vaccination strategies that exploit the innate immune response.

References

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Acknowledgements

This research was supported by US National Institutes of Health (NIH) research grants R21 AI073115, TL1 TR 000422, R01 HD3412, K01AI087369, K24 HD054314-07, and R24 TW007988 and a CFAR developmental grant from the FHCRC (P30 CA 015704-35S3). **We gratefully acknowledge and appreciate the participation of patients and study**

CROI 2016
POSTER #780

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