



University of Puerto Rico Medical Sciences Campus
Caribbean Primate Research Center



Secondary Zika virus infection do not support evidences of Antibody-Dependent Enhancement *in vivo* in dengue pre-exposed rhesus macaques

Zika virus WORK IN PROGRESS AT Caribbean Primate Research Center

Version 1.1, September 5, 2016

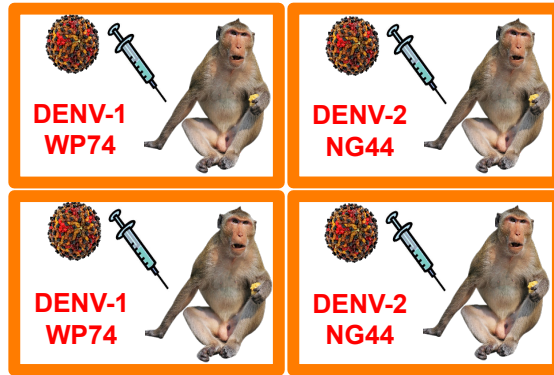
Experimental Overview

Cohort 1
DENV infected
 5×10^5 pfu by s.c.

October 2013



N=4



ZIKV Infection
FP/H/2013
 1×10^6 pfu by s.c.

Bled



August 1st 2016



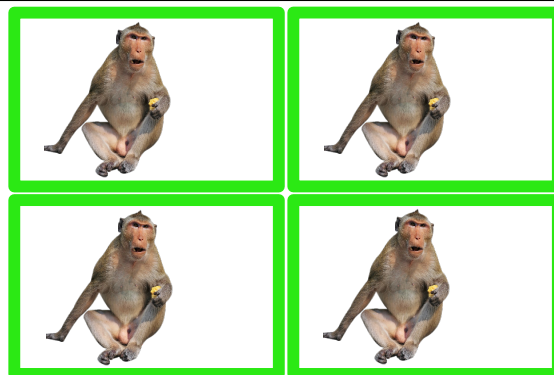
Rhesus Macaques

Time after DENV Infection: Months 12 | 18 | 30 ZIKV: Month 34

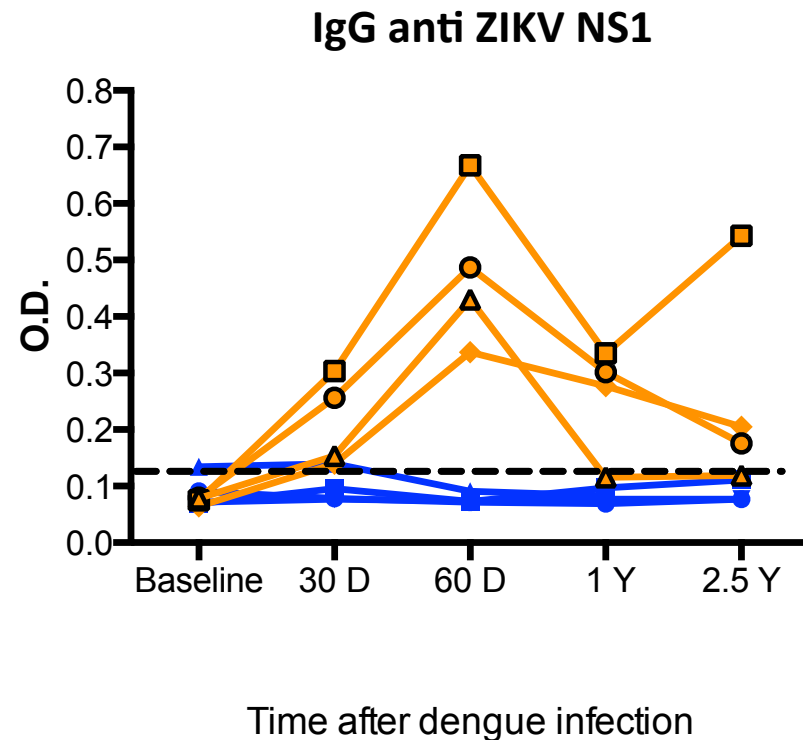
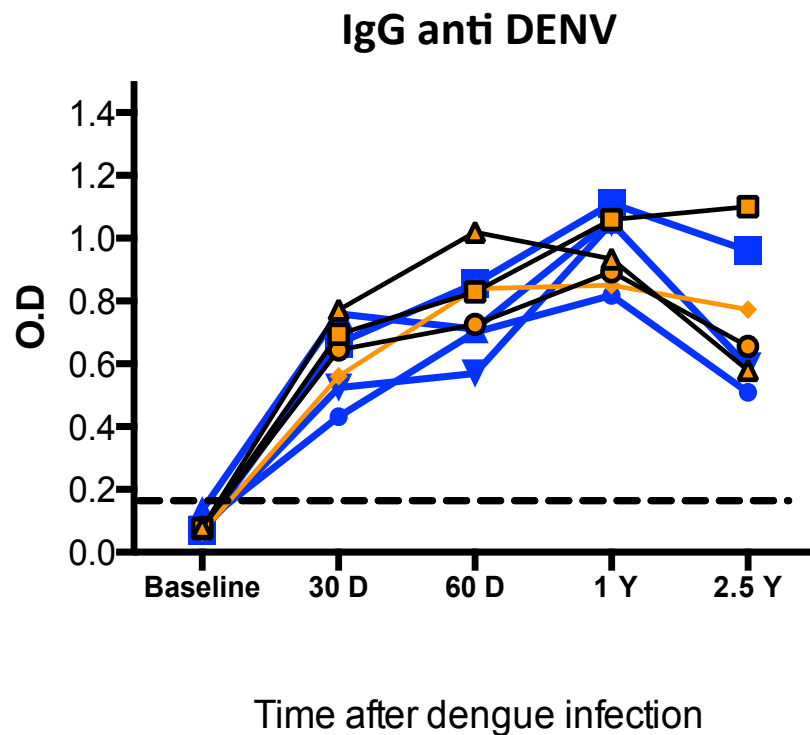
Cohort 2
DENV-Naive



N=4



Cross reactivity in ELISA to DENV and ZIKV before ZIKV infection*



DENV1 and **DENV2** exposed animals.

*Samples collected up to 1 year after DENV infection, were collected in year 2014, before ZIKV was introduced in LA region.

Observations

- ELISA results include four animals per DENV group (DENV1 and 2). However only two per group were ZIKV-infected.
- All exposed animals showed an increase of DENV antibody titers after DENV infection.
- Only animals exposed to DENV2 showed cross-reactivity to ZIKV-NS1 after 30 days of DENV infection. Cross-reactivity persist in most of the animals up to 2.5 years later.

Pre-existing immunity to DENV before ZIKV infection

ID (infecting serotype)	SAMPLE DATE	TIME POINT	Dengue IgG	ZIKV IgG NS1	DENV-Neut titer	ZIKV-Neut titer
			RESULT	RESULT	50/60/80%	50/60/80%
RM1 (D1)	9/24/13	BASELINE	NEG	NEG	D1 <20/<20/<20	<20/<20/<20
RM2 (D1)	8/1/13	BASELINE	NEG	NEG	D1 <20/<20/<20	<20/<20/<20
RM3 (D2)	8/14/13	BASELINE	NEG	NEG	D2 <20/<20/<20	<20/<20/<20
RM4 (D2)	8/14/13	BASELINE	NEG	NEG	D2 <20/<20/<20	<20/<20/<20
RM1 (D1)	11/4/13	DAY 30	POS	NEG	320/320/320	<20/<20/<20
RM2 (D1)	11/4/13	DAY 30	POS	NEG	640/640/320	<20/<20/<20
RM3 (D2)	11/20/13	DAY 30	POS	POS	1280/1280/1280	<20/<20/<20
RM4 (D2)	11/20/13	DAY 30	POS	POS	1280/1280/1280	<20/<20/<20
RM1 (D1)	12/3/13	DAY 60	POS	NEG	D1 in Progress	In Progress
RM2 (D1)	12/3/13	DAY 60	POS	NEG	D1 in Progress	In Progress
RM3 (D2)	12/17/13	DAY 60	POS	POS	D2 in Progress	In Progress
RM4 (D2)	12/17/13	DAY 60	POS	POS	D2 in Progress	In Progress
RM1 (D1)	10/22/14	1 YEAR	POS	NEG	1280/1280/640	<20/<20/<20
RM2 (D1)	10/22/14	1 YEAR	POS	NEG	320/160/160	<20/<20/<20
RM3 (D2)	10/22/14	1 YEAR	POS	POS	1280/1280/1280	<20/<20/<20
RM4 (D2)	10/22/14	1 YEAR	POS	NEG	1280/1280/640	<20/<20/<20
RM1 (D1)	4/1/16	2.5 YEAR	POS	NEG	160/160/80	<20/<20/<20
RM2 (D1)	4/1/16	2.5 YEAR	POS	NEG	1280/640/640	<20/<20/<20
RM3 (D2)	4/1/16	2.5 YEAR	POS	POS	1280/1280/640	<20/<20/<20
RM4 (D2)	4/1/16	2.5 YEAR	POS	NEG	320/320/160	<20/<20/<20
RM5 (Naïve)	7/5/16	BASE L Before ZIKA	NEG	NEG	<20/<20/<20	<20/<20/<20
RM6 (Naïve)	7/5/16	BASE L Before ZIKA	NEG	NEG	<20/<20/<20	<20/<20/<20
RM7 (Naïve)	7/5/16	BASE L Before ZIKA	NEG	NEG	<20/<20/<20	<20/<20/<20
RM8 (Naïve)	7/5/16	BASE L Before ZIKA	NEG	NEG	<20/<20/<20	<20/<20/<20

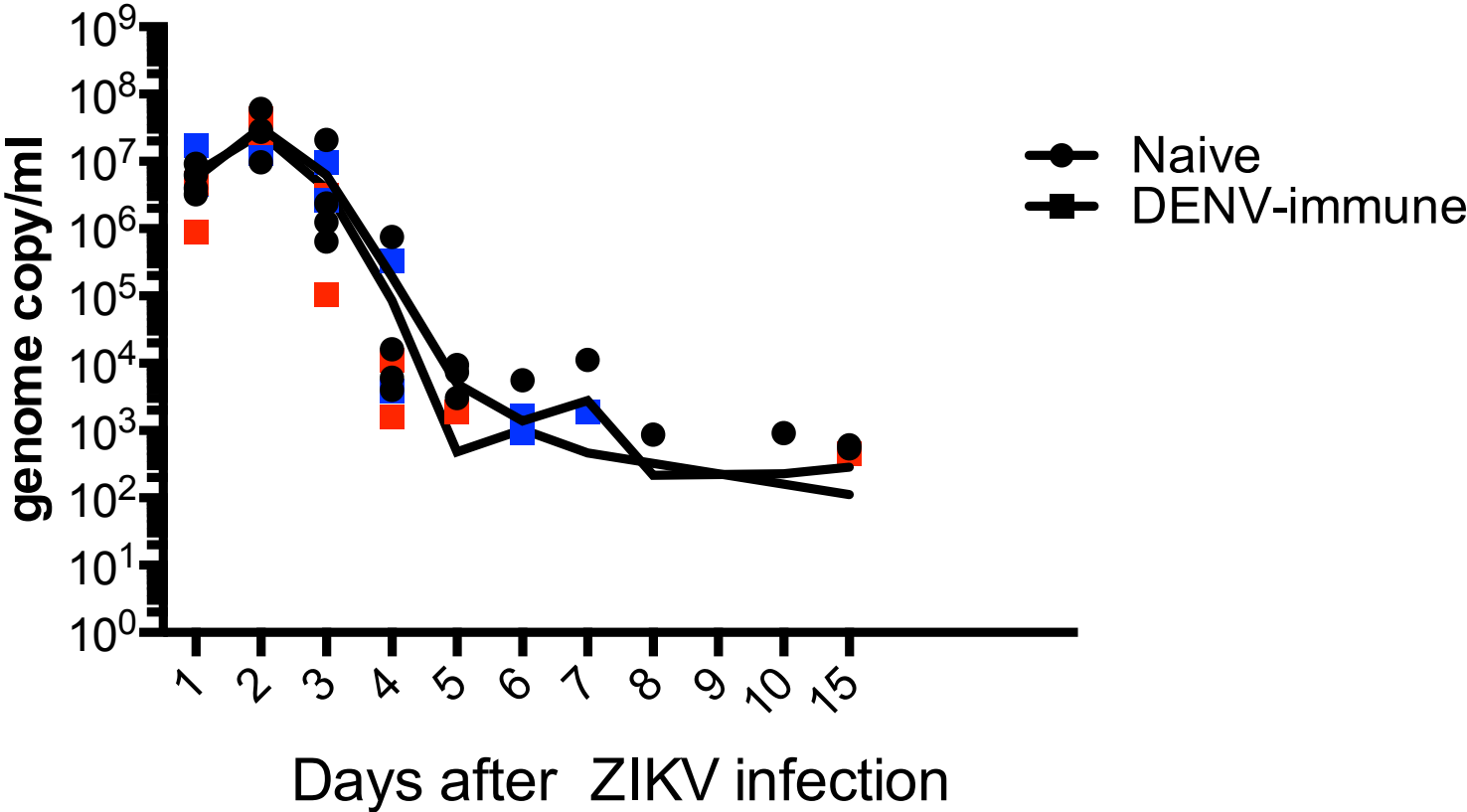
*Challenged on August 1st, 2016

Observations

- Acute or convalescent samples after DENV infection do not neutralize ZIKV *in vitro*.

Serum Viremia

Viremia



DENV1 and DENV2 pre-exposed animals.

Observations

- ZIKV peak viremia is not modified by pre-existing immunity to DENV.
- In general, animals pre-exposed to DENV showed shorter ZIKV viremia compared to DENV-naïve animals.

Serum Viremia vs. Neut Antibodies

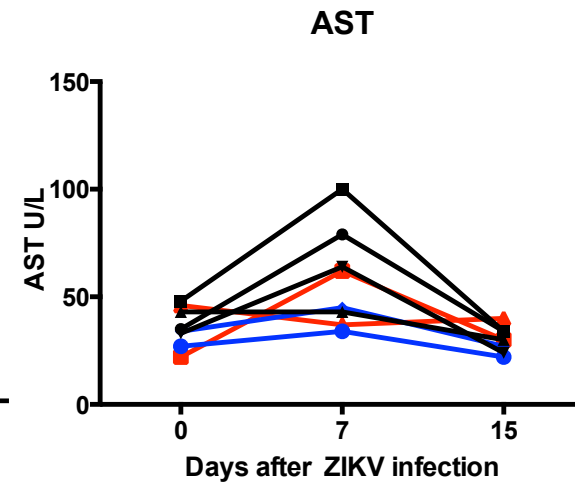
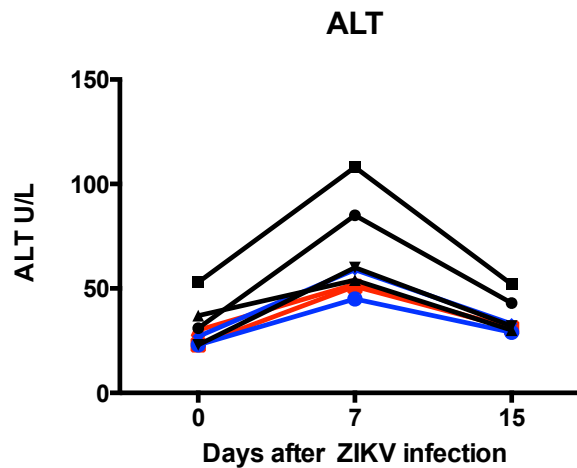
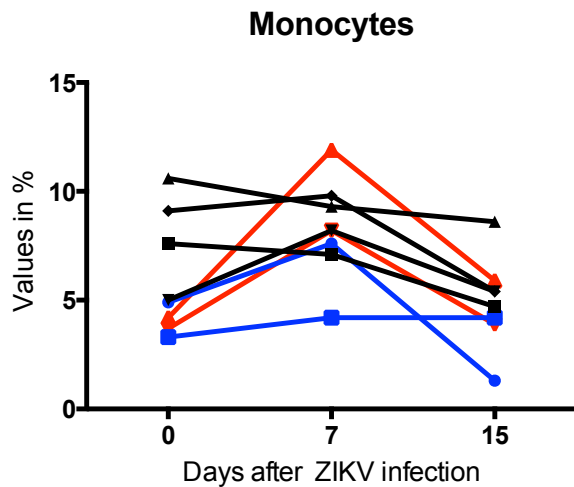
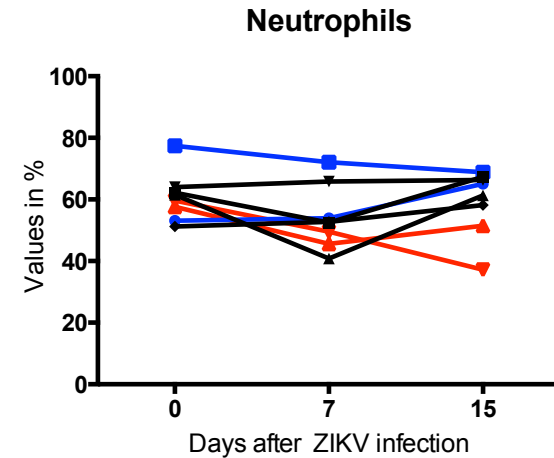
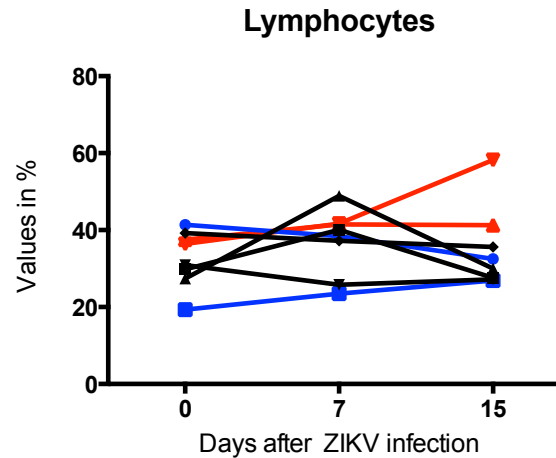
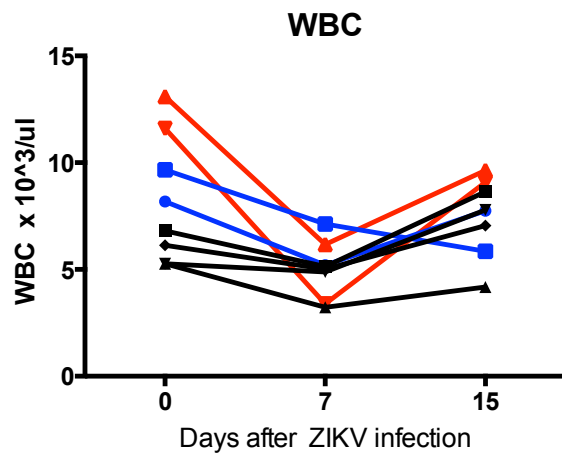
Group	Monkey ID	Viremia ^a (log ₁₀ genome equivalent/ml) during days 1-10 post Zika infection											Average Duration (days)	Pre challenge anti-DENV1 Neut ₅₀	Pre challenge anti-DENV2 Neut ₅₀	Pre challenge anti-ZIKV Neut ₅₀	30 days Post challenge anti-DENV1 Neut ₅₀	30 days Post challenge anti-DENV2 Neut ₅₀	30 days Post challenge anti-ZIKV Neut ₅₀
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 15							
Uninfected controls	RM5	+	+	+	+	+	-	-	-	-	-	+	6.5	<20	<20	<20	P	P	P
	RM6	+	+	+	+	+	-	-	-	-	-	+		<20	<20	<20	P	P	P
	RM7	+	+	+	+	+	+	+	-	-	+	-		<20	<20	<20	P	P	P
	RM8	+	+	+	+	-	-	-	+	-	-	-		<20	<20	<20	P	P	P
DENV1 Infected	RM1	+	+	+	+	-	-	-	-	-	-	-	5.5	160	ND	<20	P	P	P
	RM2	+	+	+	+	-	+	+	-	-	-	-		1280	ND	<20	P	P	P
DENV2 infected	RM3	+	+	+	+	+	+	-	-	-	-	+	5.5	ND	1280	<20	P	P	P
	RM4	+	+	+	+	-	+	-	-	-	-	-		ND	320	<20	P	P	P

P: In progress

Observations

- In animals pre-exposed to DENV, neutralization titers do not correlate with the peak or the magnitude of viremia after infection with ZIKV.

Laboratory Parameters After ZIKV infection



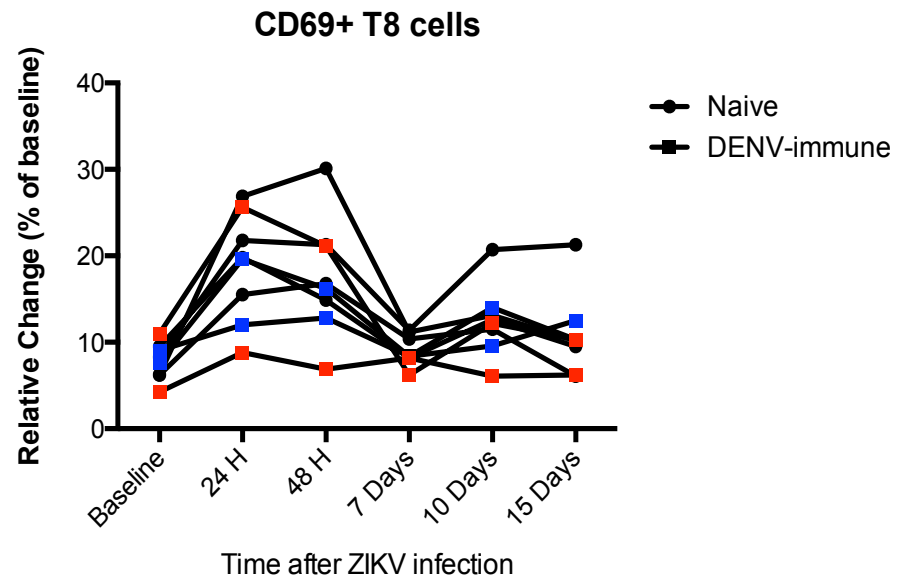
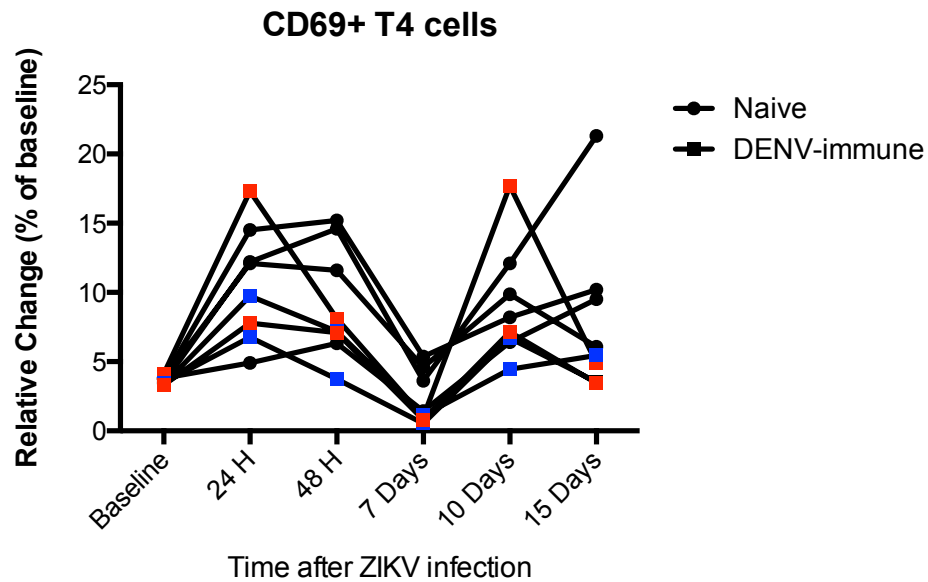
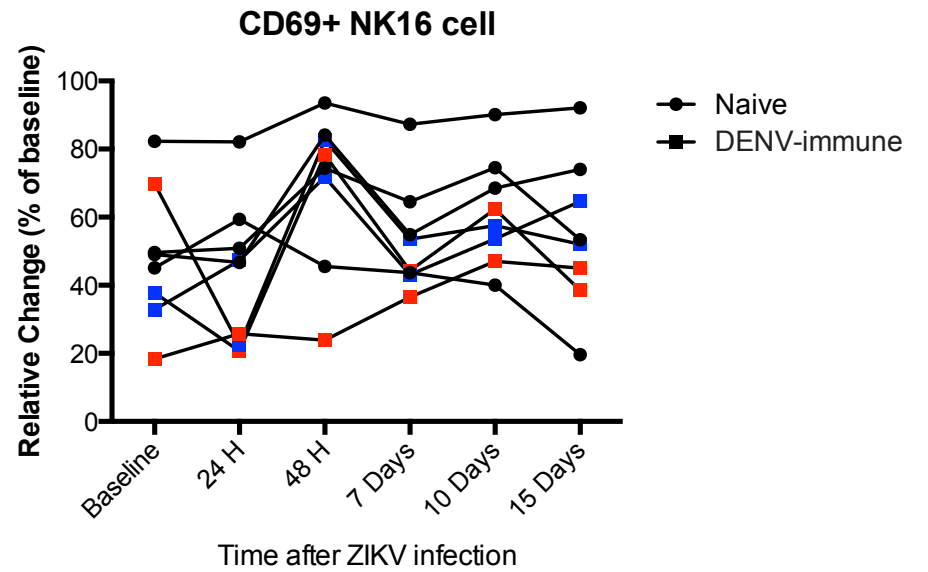
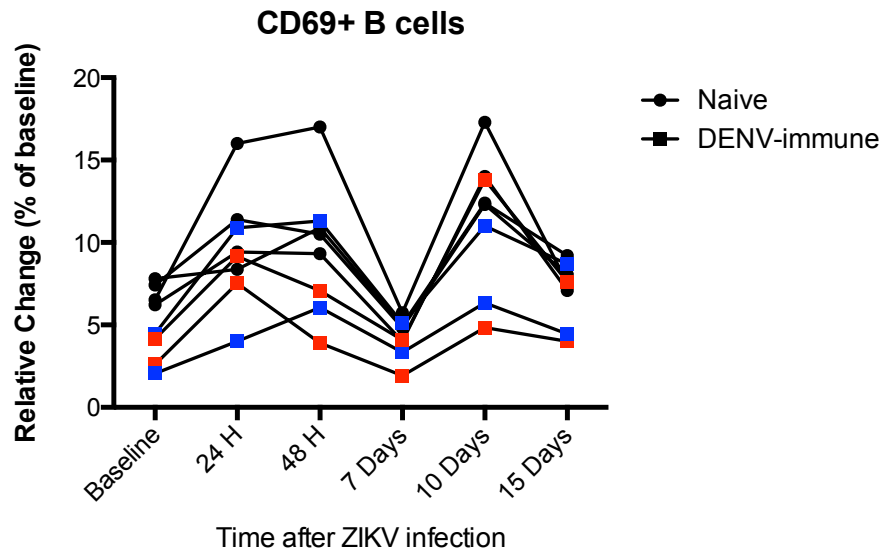
DENV2 and **DENV1**-exposed animals; **Naïve** animals.

Observations

- Similar to other viral infections, there was an initial decrease in the total WBC count at 7 days after infection in both cohorts of animals and returning to baseline levels by day 15.
- There is a slight increase in the % of lymphocytes that decrease at 7 days after infection both in naïve and pre exposed animals.
- There was an clear initial increase in the % of monocytes at 7 days after infection, more evident in cohort 1. This increase tends to be higher in the animals exposed to DENV2 compared to those exposed to DENV1.
- Both ALT and AST, but particularly ALT showed a strong trend ($p = 0.09$) to be higher in naïve animals exposed to ZIKV than in animals with previous immunity to any of the two dengue serotypes.
 - **These results suggest a protective role to the liver damage by the DENV pre-existing immunity.**

Observations

- Generalized Rash in RM1 (DENV1 pre-exposed macaque) seen by day 8 after infection. Rash was still present after 23 days of infection.
- We are evaluating the rash evolution and the meaning in the context of ZIKV infection and dengue-pre existing immunity.
- Other causes of rash are being ruling out .



Flow Cytometry Results. **Red** and **blue** squares: **DENV2** and **DENV1** pre-infected animals.

Observations

- The frequency of B cells activation was significant at 24 and 48 hours after infection compared to the basal level in both cohorts.
- The frequency of activated B cells was statistically higher in the naïve group at 48 hours and 10 days after the infection compared to the DENV pre-exposed animals.
- In both cohorts activated NK16 cells increased at 48 hours after the infection, with a decrease in the frequency of activation by day 7, returning to baseline levels by day 15. However, differences in the frequency of activation was not significant in any cohort.
- CD4+ T cells activation was significantly higher in both cohorts at 24, 48 hours and by day 10. However the frequency and magnitude of CD4+/CD69+ cells showed a trend to be higher in the naïve animals.
- There is a significant increase in the frequency of CD8+/CD69+ cells at 24 and 48 hours after infection in both cohorts. However the magnitude of activation trend to be higher in naïve animals.

Upcoming Results

- ZIKV in Saliva: In Progress
- ZIKV in urine: In Progress
- Serum Cytokines
- Quality and magnitude of ZIKV and DENV Neutralizing Antibodies several times after ZIKV infection
- More Clinical data: Weight, Temperature
- More immunological data: DC, MO, NK8 cells
- *In vitro* ADE using samples before and after ZIKV infection
- Characterization of the epitopes linked to ZIKV vs. DENV neutralization
- Dengue infection of ZIKV-exposed animals

This work is being performed by

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<http://nprcresearch.org/primate/hot-topics/CPRC-Zika-Virus-Research-Page.pdf>

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