Treg cells regulates antibody response to Japanese Encephalitis vaccination.

- Amreen Zia*, Dharamveer Singh, Swati Saxena, Dr Vikas Agarwal, Dr T.N Dhole

- Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh

INTRODUCTION

- Japanese Encephalitis :Most recognized cause of childhood viral encephalitis in Asia

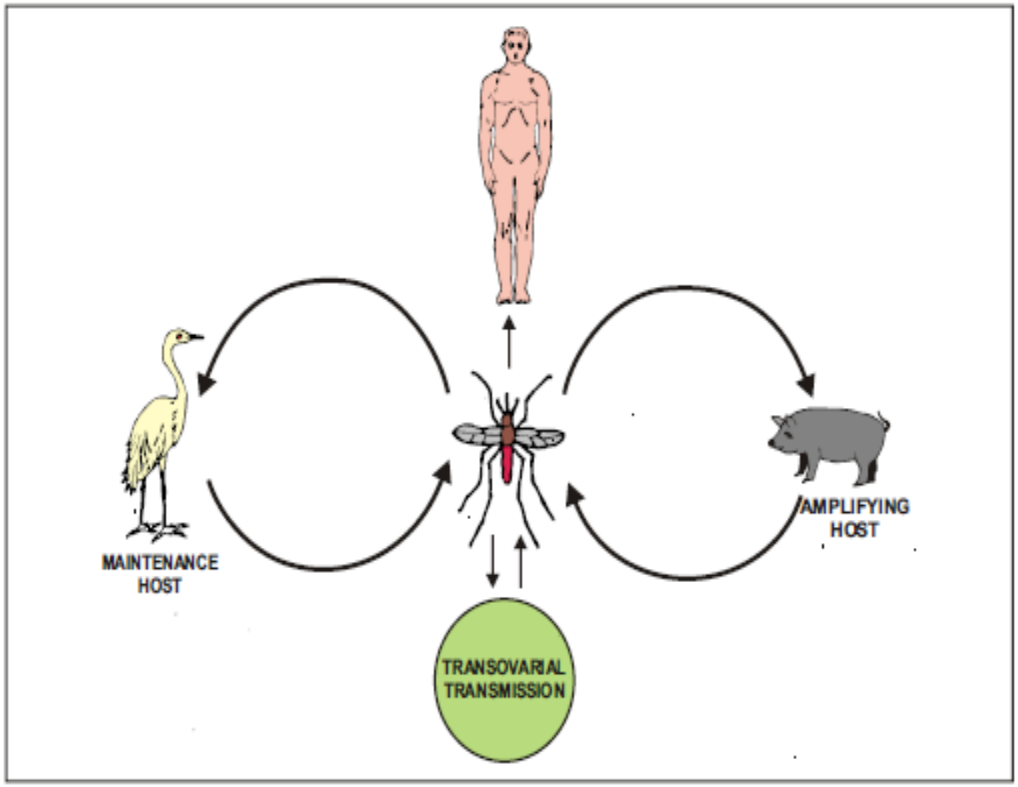
Vector-borne

- ❑ Transmitted by biting C*ulex* mosquitoes that breed in paddy field , ditches, and ground pools
- ❑ Pigs –Amplifying Hosts
- ❑ Birds - Reservoirs.

Humans are not infectious reservoirs.

- ❑ There is no human to human transmission.

- Vaccine SA-14-14-2 only effective preventive strategy in India



Zoonotic transmission cycle of JEV in nature

Immune response to Japanese Encephalitis infection and vaccination

Humoral Immune Response

- Disappearance of neurological signs has been noted in the presence of **IgM antibodies** during JE infection. (Burke *et al* Am J Trop Med Hyg. 1985 Nov;34(6):1203-10.)

Protective Role of T cell

- Adoptive transfer of **JEV-immune T cells** protected mice from subsequent virus challenge (Mathur et al., 1983; Murali-Krishna et al., 1996).

Tcell influencing Antibody

- **CD4+T** helper cell ,played an essential part in the maintenance of an effective antibody response necessary to combat the infection.(*larena et al .2012).*

Cnt ▪ ▪ ▪ ▪

- ***Humoral immune response to vaccination is well characterized in human and Animal Model***
- ***Protective efficacy of this vaccine is conferred by antibody titre genrated after vaccination i.e (Antibody titre > 10 is protective antibody titre)***
- ***But cellular immune response to vaccination are less well known.***

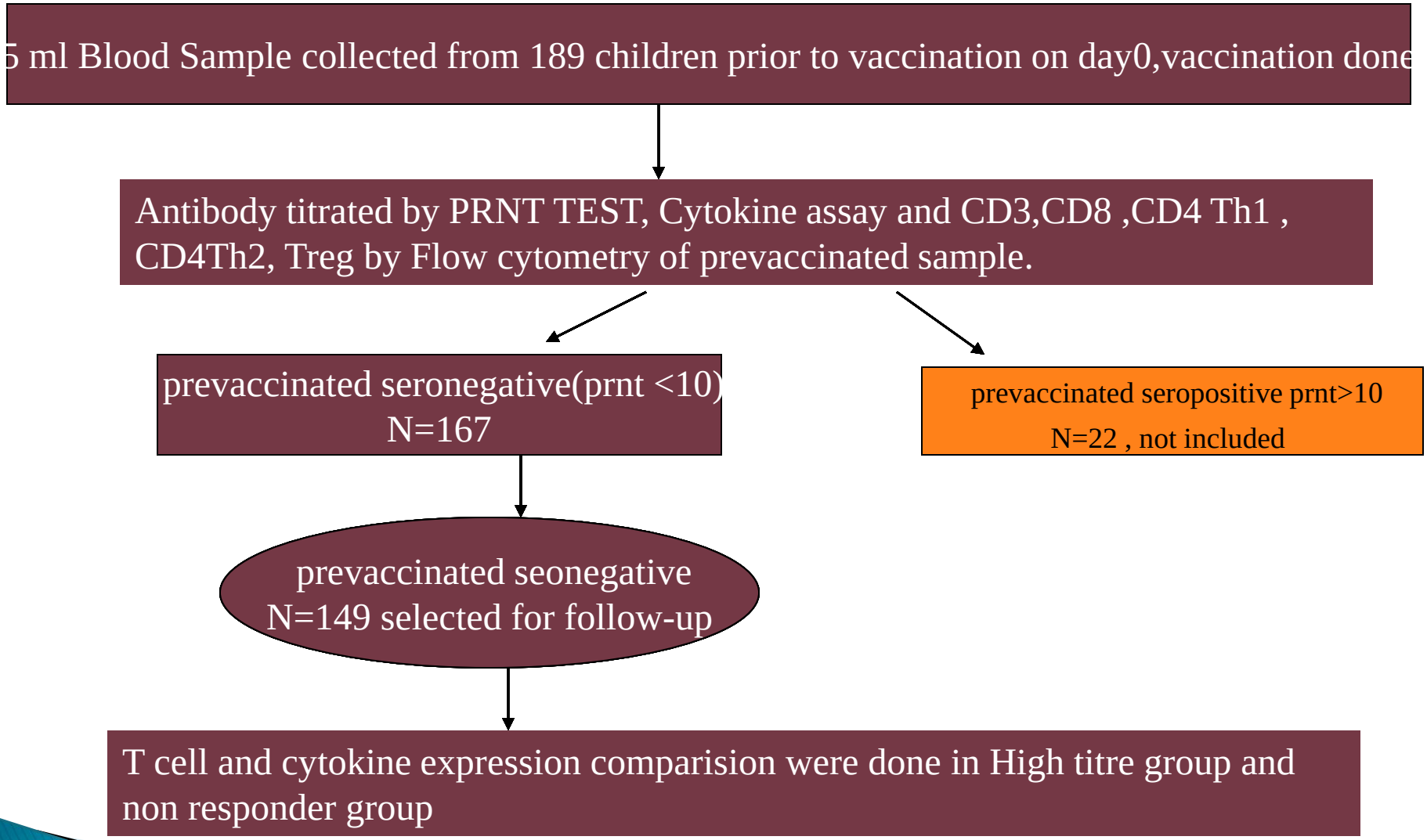
Key Question?

- Is there any participation of cellular immune response to JE vaccination in human ?

- Does it influence humoral (Ab) immune response?

- **Aim of study**
- To perform T cell subset analysis in JE vaccine non responder and High titre group.

DIAGRAMATIC REPRESENTATION OF WORK PLANNED



Material and Method

Method continue…..

Vaccination protocol

- Single dose of vaccine (0.5ml) in children was injected after basic investigation with age and sex matched control. Under JE vaccination programme

- Blood sample collection - prevaccination (Day 0) and 28 days post vaccination

Method continue….

- **Blood collection:** 5mL (3 ml for PBMC isolation and 2 ml for sera)

- **PBMC Isolation :** Peripheral blood mononuclear cells was prepared by using **cpt tubes (cell preperation tube)**

Cell line and Virus Preparation

- **Cell line** :Porcine stable cell line (10% MEM)
- **Virus** : GP78 stain (isolated from Gorakhpur Region) propagated in mice model.
- **Quantification of virus** : By Plaque assay

JEV Infection to the mice

Intracerebral injection of JEV in BALB/c mice

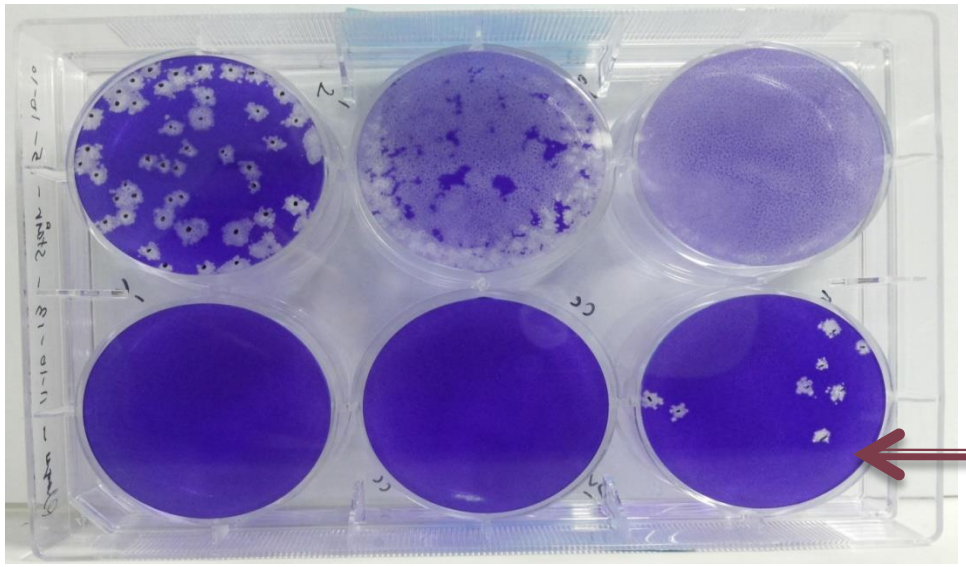
Mice showing Hunching back

Mice showing paralysis in hind limb

Isolation of brain

Plaque Assay

10^{-4} 10^{-3} 10^{-2}



Plaque

CC CC 10^{-5}

Figure: Plaque assay on PS cells for JEV

Plaque forming unit /ml(pfu/ml)= **No. of Plaques / (D x V)**

D = Dilution

V = Volume of diluted virus/well(ml)

Method continue…..

PRNT Assay

Monolayer of PS cell on 12 well Plate

↓

Heat inactivation and serial dilution of serum samples

↓

Mix and incubate the virus(100 pfu/0.1ml)and serum for 1 hour

↓

After absorption overlay media is added

↓

Fixing and staining

Antibody Titer - $\frac{1}{\text{Dilution of serum reduces the plaque num by50\%}}$

Plaque Reduction Neutralization Assay

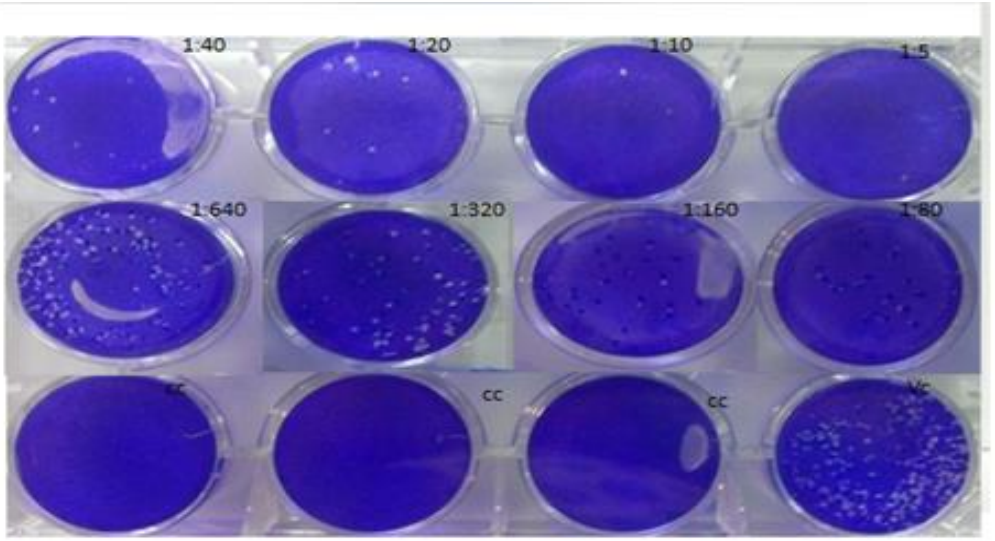


Table: PRNT Assay on PS cells for JEV

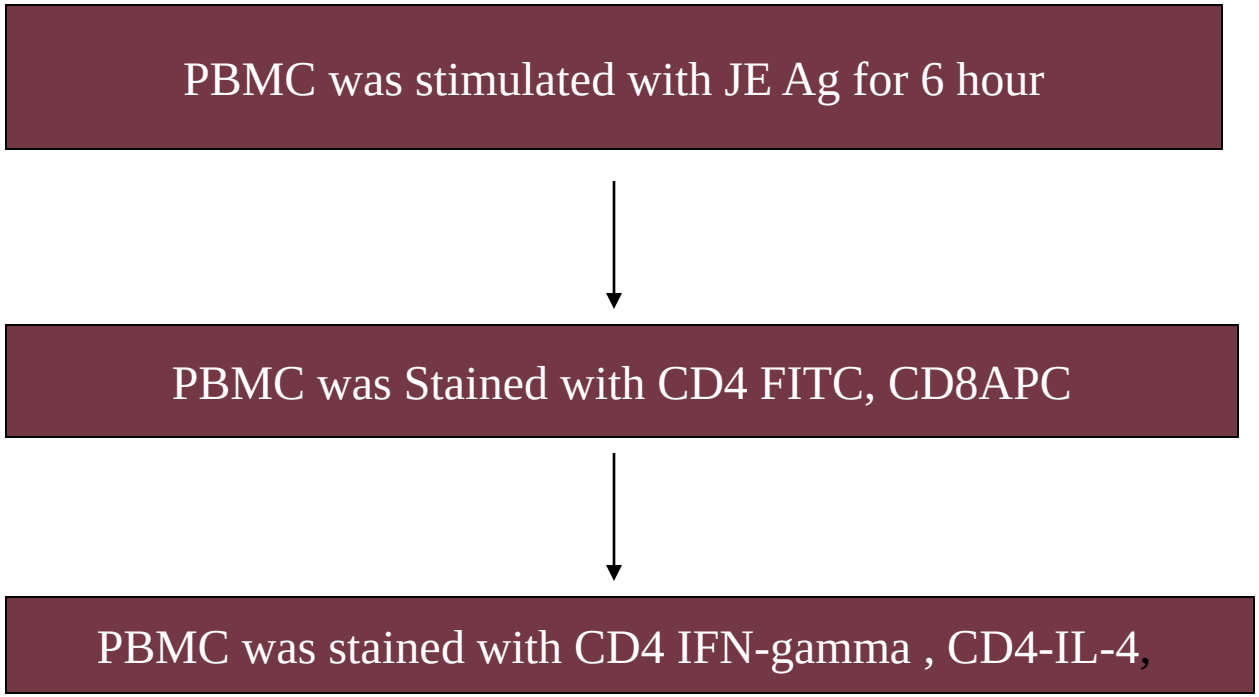
S.No	Dilution	No. of Plaque
1	1:5	0
2	1:10	2
3	1:20	6
4	1:40	10
5	1:80	15
6	1:160	35
7	**1:320**	**55**
8	1:640	89

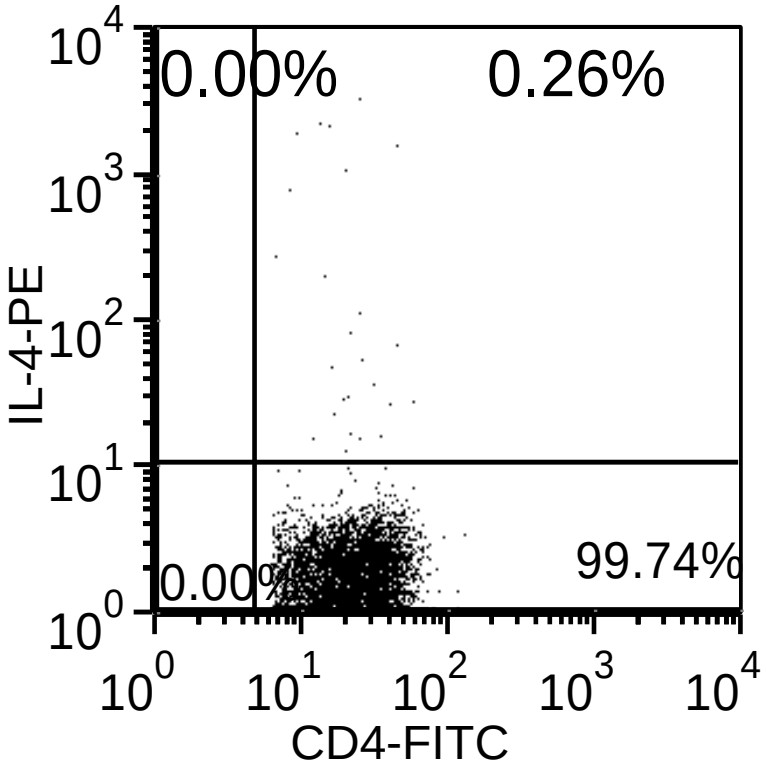
Total number of plaque in virus control well= 106

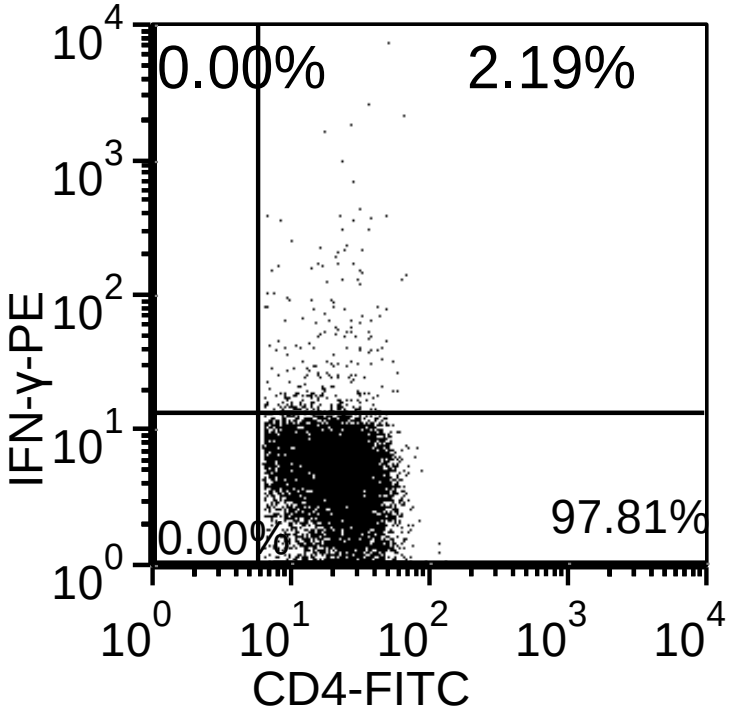
PRNT $_{50}$= 320

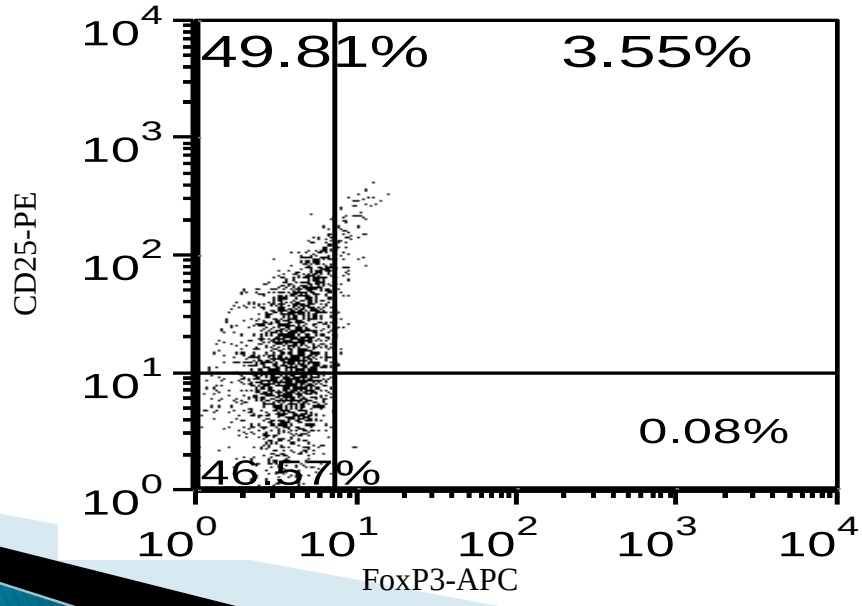
Method continue…..

FLOW CYTOMETRY




0.00%
0.26%
0.00%
99.74%
IL-4-PE
CD4-FITC


0.00%
2.19%
0.00%
97.81%
IFN-γ-PE
CD4-FITC


49.81%
3.55%
0.08%
46.57%
CD25-PE
FoxP3-APC

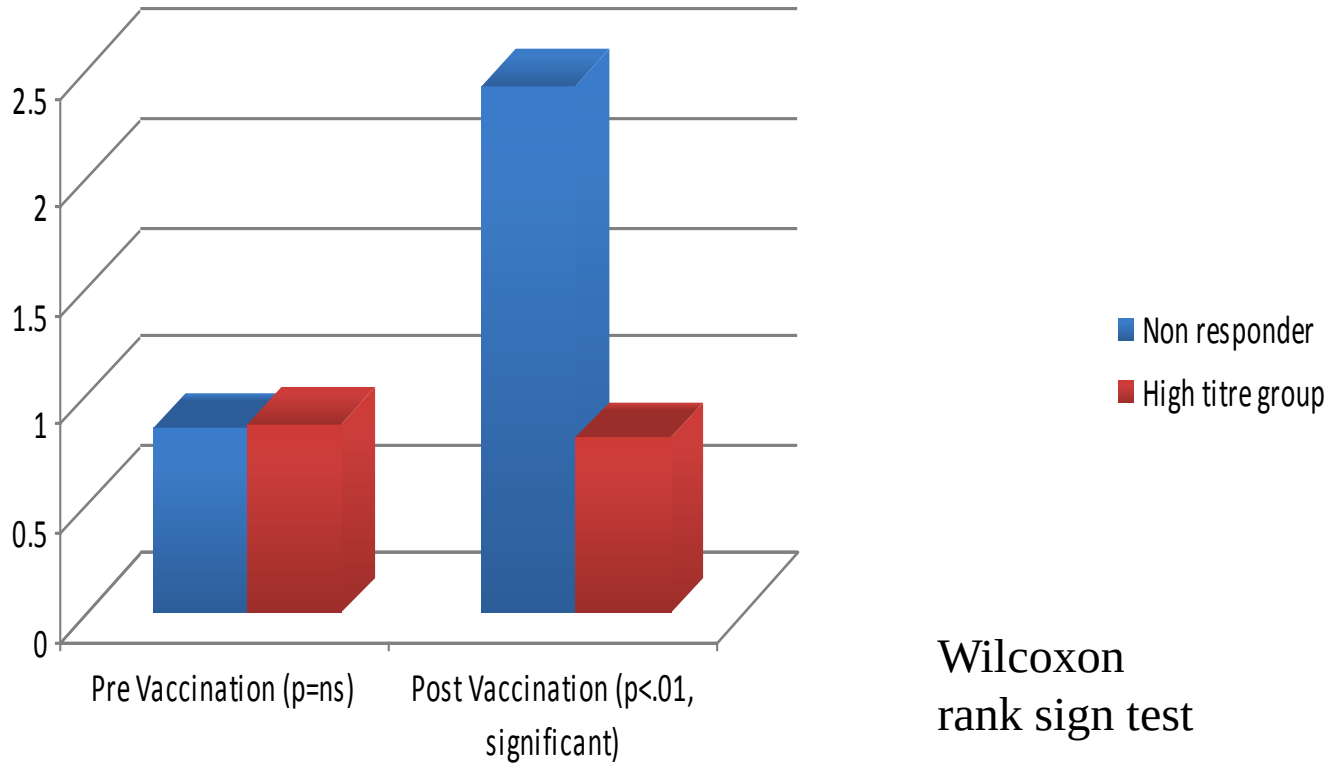
RESULT

Antibody Titre Range	Type of Responder	N= 149	Geometric antibody titre
<10	Non Responder	23(15.43 %)	5
10-40	Low titre group	14 (9.4%)	23.2 (16.23- 33.16)
80-160	**Moderate titre group**	**82 (55.06%)**	**120.5(112- 129.6)**
>320	High titre group	30 (20.13%)	463.1(406.1-528)

Table: Vaccine responder and their distribution of antibody titer

% frequency of T cell	Pre vaccination (Mean ± SD)		Post vaccination(Mean ± SD)	
	Non Responder	High titre group	Non responder	High titre group
CD3	30.06±3.6	29± 2.7	52.63±4.3	51.11±3.6
CD4TH1	0.4±0.04	0.38±0.035	1.8±0.27	1.69±0.25
CD4TH2	0.13±0.016	0.11±0.021	0.21 ±0.06	0.18±0.043
Treg	0.854±0.12	0.873±0.16	2.44±0.19	0.812±0.14
CD8	16.75±2.3	18.21± 2.7	27.44±3.1	26±2.5

Treg frequency pre and post vaccination



Wilcoxon rank sign test

Cytokine Expression

Cytokine (pg/ml)	Pre vaccination Mean (CI : 95%)			Post vaccination Mean(CI: 95%)		
	High titre group	Non Responder	P value	High titre group	Non responder	P value
IL-2	3.2(2.76-3.89	2.6 (2.3- 3.1)	ns	7.22 (6.3-8.2)	6.9(5.6- 8)	ns
IL-4	2.82 (2.5-2.76)	2.73 (2.4- 3.3)	ns	6.54(5.1-7.2)	7.15(6-8.4)	ns
IL-10	7.14(5.6-8.2)	7.8(6.5-8.5)	ns	7.56(6.45-8.2)	7.11(5.8-7.5)	ns
IFN-γ	13.32 (9.3-15.3)	12.93(10.7- 15)	ns	28.6(19-33)	25.3(20-29.1)	ns
TGF-β	146 (141-157.1)	144 (139-151)	ns	151(134-159)	287 (272-297)	P=0.002

Discussion

.

- **Treg expansion leads to the poor immunogenceity to JE vaccine in mice model (*Jiequiong et al* : Vaccine 2013)**
- **Role of TGFβ**
- **Polymorphism in Dendritic cell receptor (moderate responsiveness to vaccine).**
- **Development of Antagonist to surface receptor of Treg prevent expansion. (PNAS, 2012).**
- **Further studies are needed to evaluate if removing dominant Treg epitopes could increase the chances of developing successful vaccine in future.**
- **Increase in foxP3 mRNA was also observe in non Responder(Data not Presented)**

Conclusion

- SA-14-14-2 is capable of inducing humoral and cellular immune response
- Most vaccinee belongs to moderate titre group
- Expansion of Treg inhibits humoral immune response

THANK YOU