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Comparative Analysis of Cellular Immunogenicity between Japanese Encephalitis (JE) Vaccine Non Responder and High Antibody Titer Group in JE Endemic Area of Northern India

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Abstract

Background: Japanese Encephalitis (JE) is the leading cause of viral encephalitis in Asia. Vaccination is the most effective countermeasure for protecting individuals from Japanese Encephalitis virus (JEV) infection. Vaccine-mediated immunity is often multifactorial and the best protection is likely to be elicited by the combination of strong humoral and cell-mediated immune responses. Role of T cell in protection against JEV infection were previously reported. However, neutralizing antibodies and its persistence after JEV vaccination is considered as important correlate of protection following JEV infection. Despite the excessive research covering the humoral response after Japanese encephalitis infection or vaccination, role played by cellular immunity is largely unknown. Thus this study was undertaken to investigate contribution of T cell response in antibody generation.

Methods: Children of 1-15 years of age received single dose of live attenuated SA-14-14-2 JE vaccine under JE vaccination drive (April 2014 to May 2014) were enrolled for this study. Blood samples from 149 children were collected on day 0 (pre- vaccination) and day 28 post-vaccination. Neutralizing anti-JE virus antibody titer were assessed in serum by plaque reduction neutralization test (PRNT). Further percentage of T cell subset $CD3^+$, $CD8^+$, $CD4^+$ IFN- γ^+ , $CD4^+$ IL-4^+, $CD4^+$ IL-$17^+$and $CD4^+CD25^+$ Tregs in children belongs to non responders and high titer group were determined by flow cytometry. Plasma level of various cytokine IFN- γ, IL-4, IL-17, TGF-ß and IL-10 were measured by ELISA.

Results: 23(15.43 %) children were found to be non responder (PRNT <10) and 30 (20.13%) were found to be of high antibody titer group (PRNT>320). Higher percentage of Treg cell was found in non responder (2.44± 0.19%) when compare to high titer group (0.812± 0.14%) (p<0.001). Moreover significant increased expression of TGF-ß was also found in non responder than high titer group. (p=0.002).

Conclusion: This study indicates that Treg cells expansion have role in down regulating the antibody response to Japanese Encephalitis vaccination. Increased expression of TGF-ß in non responder seems to stimulate additional Treg production. Further studies are needed to evaluate if removing dominant Treg epitopes could improve the immunogenicity and developing an effective JE vaccine strategy in future.

Aim of Study

To perform T cell subsets analysis in JE vaccine non responder and High titre group.

Material and Methods

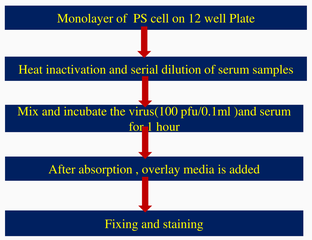
Vaccination Protocol: Single dose of live attenuated JE vaccine SA-14-14-2 (0.5mlwas injected) in children after basic investigation with age and sex matched control, under JE vaccination Drive.

Blood sample collection :2 ml blood collected in edta vial and 1 ml blood collected in plain vial prevaccination (day 0) and day 28 post vaccination.

Cell line :Porcine stable cell line was used and maintained in 10% MEM.

Virus Propogation and it's quantification : GP78 stain of Japanese Encephalitis virus (isolated from JE Endemic region of Northen India) propagated in mice model. Virus is quantified by plaque assay and will used for PRNT assay.

PRNT50 Assay



PRNT50 Antibody Titer = $\frac{1}{\text{Dilution of serum reduces the plaque num by 50\%}}$

Overview of Work Done

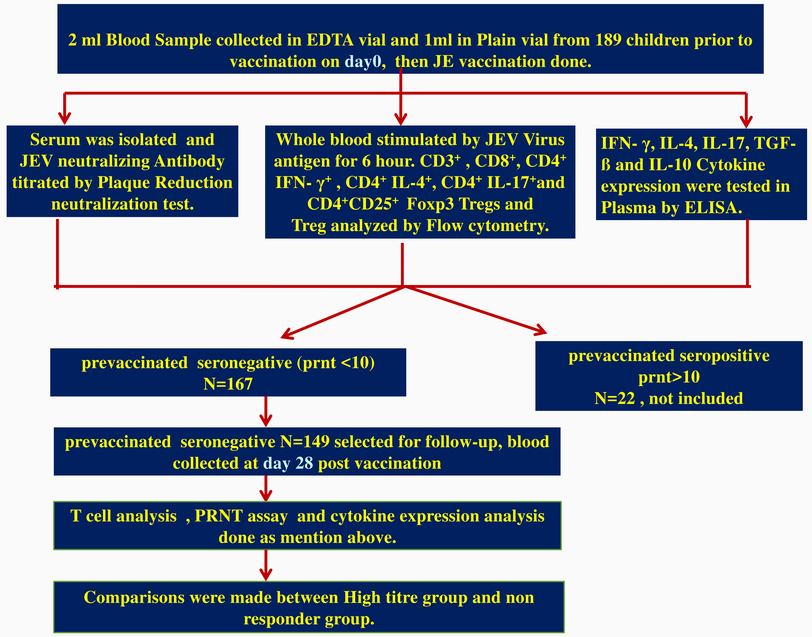
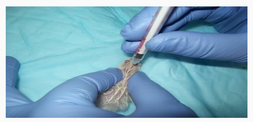
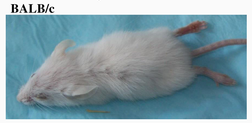


Fig1: Representative diagram of JEV Propagation in mice

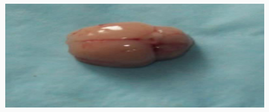


Intracerebral injection of JEV in BALB/c

Mice showing hunching back

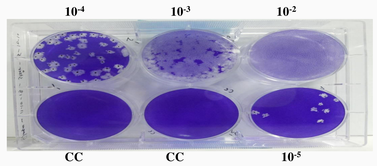


Mice showing paralysis in hind limb



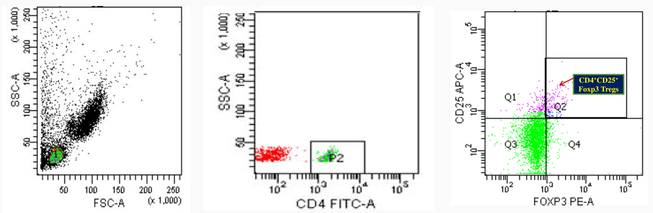
Mice brain isolates

Fig2: Representative diagram of Plaque assay



Plaque forming unit /ml(pfu/ml)= **No. of Plaques / (D x V)**
D = Dilution
V = Volume of diluted virus/well(ml)

Fig3: Representative Plot of Treg cells analysis



Results

Table 1: Antibody titer of different Groups

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

Table 2: Comparative frequency of T cells subsets in High titer Group and Non Responders

% Frequency of T cells	Pre Vaccination Mean ± SD			Post Vaccination Mean ± SD		
	Non Responder	High Titer group	P- value	Non Responder	High Titer group	P-value
CD3	30.06 ± 3.6	29 ± 2.7	NS	52.6 ± 4.3	51.1 ± 3.6	NS
CD4TH1	0.4 ± 0.04	0.38 ± 0.035	NS	1.8 ± 0.27	1.69 ± 0.25	NS
CD4TH2	0.13 ± 0.016	0.11 ± 0.02	NS	0.21± 0.06	0.18 ± 0.04	NS
T regs	0.85 ± 0.12	0.87 ± 0.16	NS	2.44± 0.19	0.812 ± 0.14	<0.001
CD8	16.75 ± 2.3	18.2 ± 2.7	NS	27.44 ± 3.1	26 ± 2.5	NS
CD4TH17	0.75±0.24	0.73±0.35	NS	0.76 ± 0.18	0.79 ± 0.12	NS

Table 3: Comparative cytokine expression in High titer Group and Non Responders

Cytokine Pg/ml	Pre Vaccination Mean (CI: 95%)			Post Vaccination Mean (CI: 95%)		
	Non Responder	High Titer group	P- value	Non Responder	High Titer group	P-value
IFN-γ	12.93(10.7-15)	13.32 (9.3-15.3)	NS	25.3 (20-29.1)	28.6 (19-33)	NS
IL-4	2.73 (2.4-3.3)	2.82 (2.5-2.7)	NS	7.15 (6-8.4)	6.54 (5.1-7.2)	NS
IL-10	7.8 (6.5-8.5)	7.14 (5.6-8.2)	NS	7.1 (5.8-7.5)	7.56 (6.45-8.2)	NS
IL-17	52.84 (47.04- 58.6)	56.36 (51.62-61.1)	NS	66.72 (62.12-71.3)	71.2 (64.29-78.11)	NS
TGF-ß	144 (139-151)	146 (141-157.1)	NS	287 (272-297)	151 (134-159)	0.002

Fig4: Treg frequency comparison

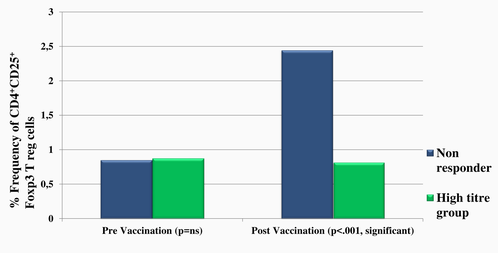
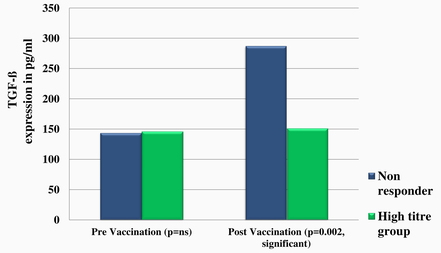


Fig5: TGF-ß cytokine expression comparison



Conclusion

- SA-14-14-2 Japanese Encephalitis vaccine is capable of inducing cellular immune response.
- Most vaccinee belongs to moderate titre group.
- Expansion of Treg inhibits humoral immune response in non responder.
- Increased expression of TGF-ß in non responder may stimulate additional Treg production.