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Early IgM Antibody Response in Chandipura Virus Infection: T cell-Independent Activation of B-cells

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Abstract

The T cell-independent B-cells activation by Chandipura virus is not known. In the current study, T cell-independent activation of B-cells by Chandipura virus was studied. The activation of B-cells was studied in the experiments determining the membrane expression of CD25 and CD69 markers, proliferation and virus specific IgM antibody secretion. Activation and proliferation of B-cells, and IgM secretions could be observed from the cells exposed to live virus. In case of B-cells treated with inactivated virus proliferation and IgM secretion was not observed. In order to determine the role of TLR4 in B-cell activation, the membrane TLR4 on B-cells were blocked with anti TLR4/MD2 antibody and exposed to live virus. Like inactivated virus, the antibody blocked cells were not showed proliferation and IgM secretion. In *in vivo* mice experiment, the T cell deficient nude mice were exposed to Chandipura virus. No significant difference could be observed in the level of IgM secretions between virus exposed nude and wild type mice. However, virus specific IgG was detected in wild type mice but not in nude mice. All the nude mice infected with Chandipura virus through intra-cerebral route were succumbed to infection. No clinical signs or death could be observed in wild type mice. In conclusion, we demonstrated that CHPV directly activates the B-cells. However, for proliferation and secretion of virus specific IgM, live virus and/or signalling via TLR4 is necessary. IgM antibody alone may not be sufficient to protect the mice from the infection and the CMI and/or virus specific IgG is necessary to confer protection from disease.

Keywords: Chandipura virus; VSV; T-independent antigen; B-cells

Introduction

Chandipura virus (CHPV) belongs to *Rhabdovirdae* family; genus *vesiculovirus* is associated with acute encephalitis and severe fatality in young children [1]. Upon experimental infection, CHPV causes acute infection in young susceptible mice. Antigen specific IgM is present in sera samples as early as 48 h post infection (PI). Despite the presence of virus specific IgM, 100% mortality observed in young mice [2]. Children under 15 years of age are vulnerable to natural infection, whereas adults are refractory. Similarly, susceptibility studies in mice showed that CHPV is lethal to young mice, but adults are susceptible only inoculated via intra-cerebral route [3]. This age-dependent susceptibility is also apparent in nude mice [4].

In normal course of immune response, the antigen presenting cells (APCs) such as dendritic cells and macrophages present the antigen to T-cells, resulting in T-cell activation. The antigen specific B-cells migrate to the T-cell rich areas of the lymphoid organs where cognate T-B cell interaction occurs [5,6]. The interaction occurs via co stimulatory molecules viz., C28 and CD40 ligand on activated T-cells and B7-1 and B7-2, CD40 on B cells and cytokines secreted by activated cells [7]. This interaction leads to differentiation of B cells into plasma cells and memory cells. A second encounter with the same antigen leads to a much quicker activation of memory B-cells and secretion of IgG antibody within few days [8]. In secondary immune response, T-cell help is not necessary to activate the memory cells [9].

Some virus particularly lytic virus (CPE producing viruses) by-pass this step and directly interact with B-cells either through their polymerised antigenic structure or through toll like receptors (TLRs). The early immune response is essential to limit the spread of virus and to reduce the tissue damage [10]. Therefore, lytic viruses elicit early antibody response without T-cell help [11,12]. Commonly, the antigens that do not need T-cell help are called as T-independent (TI) antigens. TI antigens allow B-lymphocytes to mount an antibody without the requirement of interaction with T-helper lymphocytes. The resulting antibody molecules are generally of the IgM isotype and do not give rise to a memory response. There are two basic types of TI antigens: TI-1 and TI-2 [13]. TI-1 antigens include lipopolysaccaride (LPS) which activates B-lymphocytes by binding to their specific TLRs rather than to B-cell receptors. T2 antigens bind to B-cell receptors. Involvement of TLRs in B cell activation has been reviewed elsewhere [14].

Innate immunity is mediated by several mechanisms. Among them TLRs is one of the key host molecules involved in innate immune responses during infections [15,16]. TLRs were first identified in Drosophila species. Till date, thirteen mammalian TLRs have been identified [17]. Toll pathways were known to respond to bacterial and fungal infections, and anti viral immunity [18]. In our earlier study, we demonstrated that Chandipura virus regulates TLR4 in infected RAW cells [19].

Studies on polyoma virus infection in SCID mice reconstituted with splenic B- cells showed that a virus can behave as a TI antigen and synthesis antibodies without T-cell help [20]. In rota virus infection in mice, it was noticed that activated B cell but not T cells as early as 2 days post infection in Peyer's patches. The activated B-cells also demonstrated in rota virus infected T-cell deficient mice [21]. In another experiment, it was shown that VP7 protein is responsible

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for polyclonal activation of B-cells [22]. In hepatitis B infection, core protein acts both T independent and dependent manner [23]. It was shown that UV-inactivated Influenza A virus subtypes H1, H2, H3 and H6 are mitogenic for unprimed splenic B lymphocytes [24]. Lymphocytic choriomeningitis virus produces antiviral IgM response in CD40 ligand deficient mice in $CD4^+$ T independent manner [25]. Vesicular stomatitis virus (VSV) generates IgM response in T-cell deficient mice [26].

In the current study, the role of CHPV to activate the B-cells of Swiss albino mice and *in vivo* in T-cell deficient nude mice in TI manner were examined. The results indicate that CHPV interacts with B-cells in TI manner and cell mediated immune response is essential to protect the mice from disease pathogenesis.

Materials and Methods

Cells and virus

Vero E6 cell line was obtained from the National Centre for Cell Science, Pune, India. The cell line was cultured and maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS, Gibco) and antibiotics. The Chandipura virus strain (034267) was originally isolated from the Andhra Pradesh outbreak in 2003 [2]. The virus was propagated and titrated in vero E6 cells.

Mononuclear cells preparation and B-cell purification

The splenocytes were isolated from 30-days-old Swiss albino mice after euthanized by CO_2 asphyxiation. The intact body was submerged into 70% ethanol. In the laminar flow, the skin was excised and the spleen was carefully removed. The spleen was placed on a Petri plate and minced with scissors. The spleen was teased against 100 mm cell strainer (Sigma) placed in a Petri plate which containing 5 mL of RPMI 1640 medium supplemented with 10% FBS, 25 mM hepes buffer and $5x10^{-5}$ M β-mercapto ethanol and antibiotics (growth medium). The splenocytes were washed thrice and resuspended in growth medium. The splenocytes were layered on to a density gradient medium (HISTOPAQUE, 1.083 gm/mL, Sigma) with a ratio of 3:2 and centrifuged at 400 xg for 30 min at room temperature. Mononuclear cells from plasma-HISTOPAQE interface were recovered. The cells were washed thrice and suspended in MACS buffer (Miltenyi Biotec, USA). Naïve untouched B-cells were isolated from mouse mononuclear cells by negative selection using an immune-magnetic bead B-cell isolation kit (Miltenyi Biotec, USA) according to the manufacturer's instructions. Following isolation, a small aliquot of cells was phenotyped by flow cytometry to assess B-cell purity.

Flow cytometry

One million cells were suspended in 100 µL of FACS staining buffer (PBS pH 7.2, 2% FBS, 0.09% sodium azide, 1% mouse serum) and stained with an anti-mouse CD19 antibody conjugated to phycoerythrin (CD19-PE) (eBioscience, USA). Appropriate isotype controls were included to set the base line data. After 30 min incubation at 4 °C, the cells were washed thrice with staining buffer and the pellet was resuspended in 500 µL of 1% paraformaldehyde. The cells were analyzed using a FACSCalibur flow cytometer and Cell Quest Pro softwares (BD bioscience, USA).

Virus exposure

One million purified B-cells were exposed with 10^7 50% tissue culture infective dose ($TCID_{50}$) of CHPV. The cells were incubated for 1h at 4°C to allow virus adsorption. The cells were then washed with RPMI 1640 medium. In order to neutralize residual virus, the cells were suspended in RPMI 1640 medium containing rabbit anti-CHPV polyclonal sera. The cells were allowed in ice for 30 min. The cells were then washed thrice with RPMI 1640 medium and the final pellet was suspended in growth medium. Unexposed cells were kept as a control. In order to block membrane TLR4, before exposure to virus, the B-cells were incubated with 20 µg/mL of anti mouse TLR4/MD2 complex antibody (clone MTS510, BioLegend, USA) for 45 min at 4°C.

Inactivation of CHPV

CHPV was purified from infected vero E6 cell culture supernatant as previously described [27] and inactivated using BEI as described by Bahnemann, 1990 [28]. Based on the inactivation kinetics of CHPV (data not shown), the virus was inactivated with 2 mM BEI. The innocuity of the inactivated virus suspension was tested in vero E6 cells for up to third passage and stored at -80°C. For various assays, 10^6 purified B-cells in 100 µL of RPMI 1640 medium was exposed to 1 µg of inactivated virus.

Infectious centre assay

Virus replication in B-cells was checked by infectious centre assay. The B-cells were exposed with CHPV as described above. The virus exposed cells were serially diluted between one million cells/mL and 10 cells/mL in methyl cellulose overlay medium (0.5% carboxymethyl cellulose dissolved in DMEM with 2% FBS). Six well culture plate containing confluent monolayer of vero E6 cells was layered with 1 mL per well of diluted cells. The plate was centrifuged at low speed in a centrifuge fitted rotor with plate adapter to sediment the B-cells on to the vero E6 cells monolayer. The plate was incubated for 72 h at 37°C with 5% CO_2 and stained with 0.1% crystal violet dissolved in 10% formal saline. The plaques were counted and expressed in number of plaque forming unit per number of B-cells seeded.

Virus specific IgM secretion

The CHPV exposed and unexposed cells were adjusted to one million cells per mL and seeded into 96 well tissue culture plate at the rate of 100 µL per well. The plate was incubated at 37°C with 5% CO_2. The cells with medium were collected from three wells of both virus exposed and unexposed at 24, 48, 72 and 96 h post exposure. The cells were separated by centrifugation and the supernatant was tested for the presence of virus specific IgM capture ELISA.

IgM capture ELISA

IgM capture ELISA was done following procedure described for human [2] with a modification that coating was done with anti-mouse IgM as a capture antibody. The level of IgM was determined from undiluted samples. The cut-off value was set by average plus 3 standard deviation of optical density (OD) from control.

B-cell proliferation assay

The purified B-cells were stained with carboxy fluorescein succinimidyl ester (CFSE; Sigma, USA). Briefly, the B-cells were exposed to CHPV. The cells were then washed thrice with CFSE staining buffer (DPBS, pH 7.2, 0.1% BSA). The cell concentration was adjusted to 10^7 cells per mL in CFSE staining buffer and labelled with CFSE at 5 µM final concentration. The cells were mixed well and incubated at room temperature (RT) for 15 min in dark with gentle shaking. The dye uptake was stopped by adding equal volume of FCS and incubated for one min. The cells were then washed and cultured in growth medium

for 72 h at 37°C with 5% CO_2. At the end of incubation, the cells were washed thrice with FACS staining buffer and stained with anti mouse CD19-PE. The cells were acquired and analyzed using a FACSCalibur flow cytometer and CellQuest Pro softwares (BD bioscience, USA). The proliferation of cells were analysed in FlowJo software (Tree Star, USA).

B-cell activation assay

B-cell activation assay was carried out using mouse B-lymphocyte activation antibody cocktail (BD Pharmingen; USA). The kit contains cocktail of mouse anti CD25-PE-Cy7, CD69-PE and CD19-FITC and cocktail of respective isotype controls. The purified B-cells were exposed to virus and unexposed cells were kept as a control. At 24 h post exposure, both the virus exposed and unexposed cells were stained with antibody cocktail. A small aliquot of unexposed cells were stained with cocktail of isotype antibodies. The stained cells were incubated for 30 min at 4°C after incubation, the cells were washed thrice with staining buffer and the pellet suspended in 500 µL of 1% paraformaldehyde. The cells were analysed in flow cytometer (FACSCalibur) using Cell Quest pro software. The activation of cells were analysed by FlowJo software. The percentage of CD25 or CD69 positive $CD19^+$ cells was calculated by following formula.

% of CD25or CD69 positive $CD19^+$ cells=number of CD25or CD69 positive CD19 + cells in analysed sample X 100/ number of total $CD19^+$ cells in analysed sample.

In vivo study in nude mice

NIH nude (Swiss) and wild (Swiss albino) mice (30-days-old) were used in this experiment (n=60). The study was carried out in strict accordance with the recommendations in the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCESA), India and the protocol was approved by the Institute Animal Ethics Committee (IAEC), National Institute of Virology, Pune. The mice were divided into four groups (n=15). Two groups (one each from nude and wild type mice) were inoculated with 100 µL (10^5 $TCID_{50}$/mL) of live CHPV through subcutaneous (sc) route. The virus does not kill the adult mice through peripheral route of infection but kills through intra-cerebral (ic) route. The remaining two groups were inoculated with 100 µL of PBS. Mice were bled through intra-orbital plexus at weekly intervals and the sera were separated and frozen for further use. On 21 days PI, all the mice were infected with 20 µL of live CHPV (10^5 $TCID_{50}$/mL) through ic route and the mortality pattern was recorded. The collected sera were used for virus specific IgM and IgG detection. The IgM and IgG capture ELISA was done following procedure described for human [2] with an only modification that coating was done with anti-mouse IgM or IgG as a capture antibody. The level of antibodies were determined from 1/50 diluted samples. The cut-off value was set by average plus 3 standard deviation of O.D from uninfected negative control mice.

Statistical analysis

The Student t test was used to compare the means values for the treated and control groups. A P value < 0.05 was considered significant. The percentage survival was calculated using Kaplan Meier statistics for survival function (GraphPad Prism 5 software).

Results

The purity of B-cells from Swiss albino mice was 96% (Figure 1). The replication of CHPV in B-cells was studied by infectious centre assay and no infectious virus particle could be detected. The activation of B-cells with CHPV was confirmed by membrane expression of CD25 and CD69 markers. CD25, the low affinity IL-2 receptor expresses on activated T and B lymphocytes. CD69, very early activation antigen expresses rapidly upon activation of lymphocytes. No significant difference in expression of CD69 membrane receptor was noticed between virus exposed and un-exposed B-cells (Figure 2b). However, greater percentage (36%) of virus exposed B-cells expressed CD25 than those in unexposed B-cells (25%) (Figure 2a).

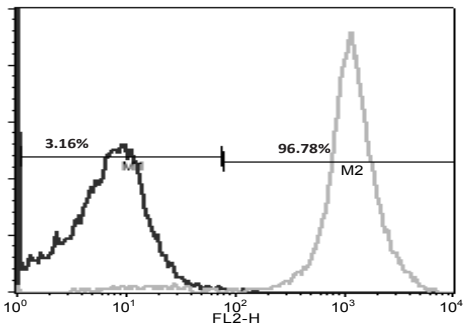

Figure 1: Purity of CD19 positive B-cells isolated from mouse splenocytes
CD19 positive B-cells were purified from mouse splenocytes using an immune-magnetic bead B-cell isolation method (negative selection). The untouched cells eluted from magnetic column were stained with anti mouse CD19 antibody conjugated with PE and analyzed in flowcytometer. In histogram, bold line represent CD19 negative and grey line represent CD19 positive population of cells.

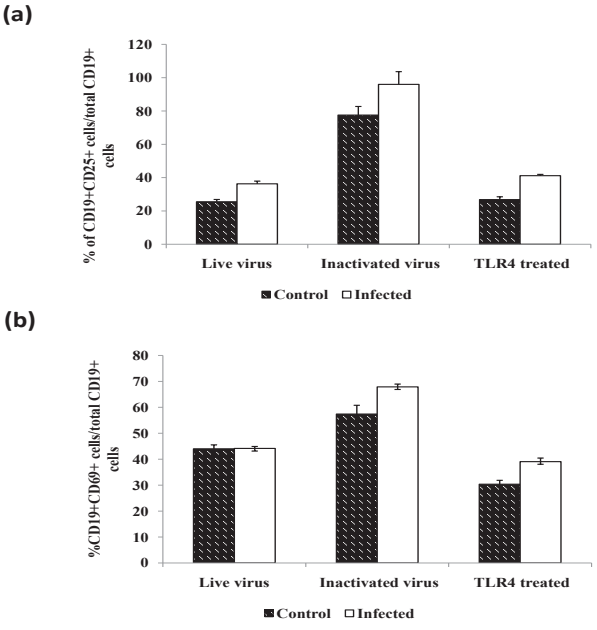

Figure 2: Percentage positivity of $CD19^+CD25^+$and $CD19^+CD69^+$cells in CHPV exposed $CD19^+$ cells
The B cells were purified from Swiss albino mice and exposed to live and inactivated CHPV. In another experiment, the B-cells were treated with anti mouse TLR4/MD2 antibody and then exposed to live virus. The unexposed B-cells were used as a control. At 24 h post infection, the cells were stained with B lymphocyte activation antibody cocktail which containing anti mouse CD19-FITC, CD69-PE and CD25-PE-Cy7 antibodies. The cells were acquired in FACSCalibur using cell quest pro software. a) Percentage of $CD19^+CD25^+$ cells b) Percentage of $CD19^+CD69^+$ cells out of total number of $CD19^+$ cells plus standard deviation. The data represents the average of three experiments.

Proliferation of activated B-cell was quantified using cell tracking dye CFSE by flow cytometer. The method relies on the ability of CFSE to covalently label intracellular molecules and emit green fluorescent. Upon proliferation, the fluorescent molecules equally distribute to daughter cells following each cell division. The level of CFSE dye gets decrease upon cell division. It was noticed that the proliferation in virus exposed B-cells *in vitro* because their level of the CFSE dye, which was divided equally among daughter cells upon cell division, has decreased. A total of 20% cells was divided and contributed to daughter population (Figure 3a). The proliferated B-cells secreted virus specific IgM at 96 h post exposure and the level was significantly higher than those in unexposed B-cells (Figure 4)

To determine whether infectious virus is required for B-cell activation, purified B-cells were exposed with BEI inactivated CHPV. The treated B-cells expressed higher percentage of CD25 (95% *vs.* 77%) and CD69 (67% *vs.* 57%) receptors on the membrane than those in B cells from control experiment (Figure 2a and 2b). Inactivated virus treatment did not result in significant cell proliferation (Figure 3b). No

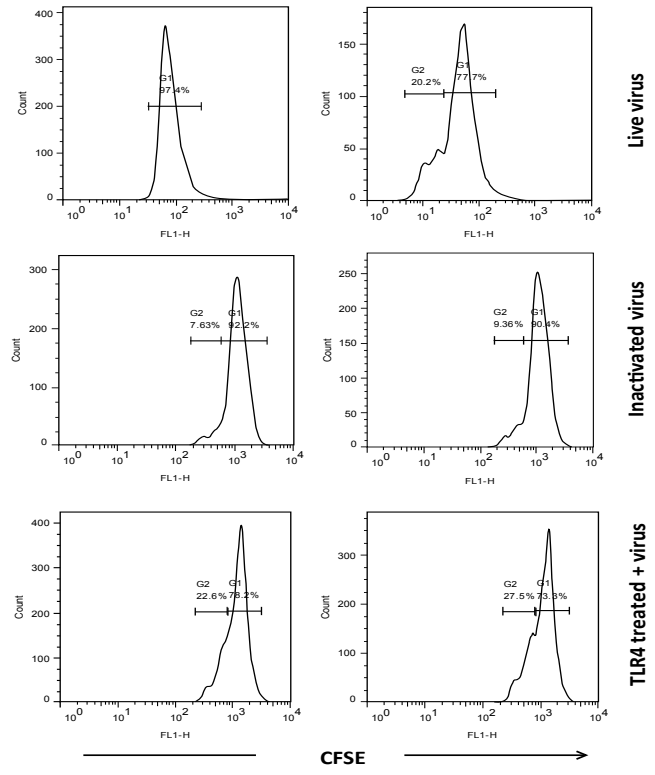

Figure 3: *In vitro* proliferation B-cells exposed to CHPV
The B-cells were exposed to live and inactivated virus. In another experiment, the B-cells were treated with anti mouse TLR4/MD2 antibody and then exposed to live virus. The exposed cells were stained with CFSE. The stained cells were incubated for 72 h at 37 °C. The CFSE stained, virus unexposed cells were kept as a control. At the end of incubation, the cells were then stained with anti mouse CD19-PE. The stained cells were acquired in FACSCalibur using cell quest pro software. The proliferation of cells was analyzed using Flow Jo software. During analysis, the CD19 positive cells were gated first for analysis of CFSE positive cells. In the histogram, CFSE positive cell population is located near to 10^2 (live virus) and 10^3 (Inactivated and TLR4 treated) in X axis (parent population) and other peaks which fall left to parent population are daughter population. The percentage of cells positive for CFSE was calculated and mentioned inside the histogram. These are representative picture of one of three experiments.

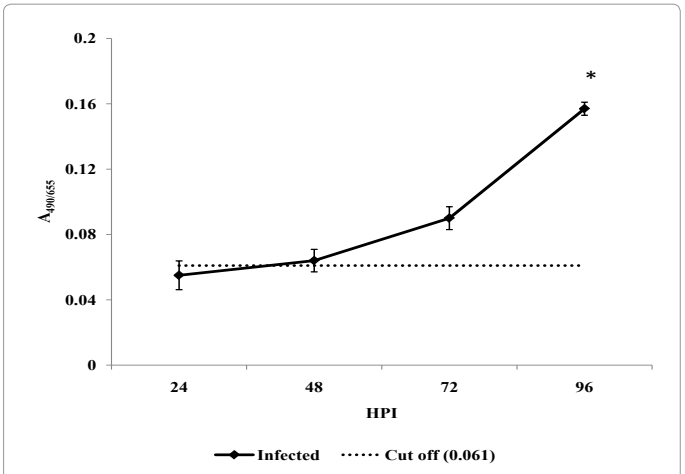

Figure 4: *In vitro* IgM secretion by live CHPV exposed B-cells
The B-cells were exposed to live virus and the exposed cells were incubated up to 96 h post exposure. The culture supernatants were collected at 24 h intervals and tested for CHPV specific IgM using mouse IgM capture ELISA. The mean OD from unexposed B-cell supernatants plus 3 standard deviation (SD) was set as a cut off value.

virus specific IgM could be detected in culture supernatant of B- cells treated with inactivated CHPV.

Membrane TLR4 of B-cells was blocked with anti mouse TLR4/MD2 antibody before live virus exposure. The membrane expression of CD25 and CD69 from antibody blocked cells indicates that the cells were activated. The percentage expression of CD25 (41% *vs.* 26%) and CD69 (39% *vs.* 30%) was higher in virus exposed B-cells than those in control cells (Figure 2a and 2b). TLR4 antibody treatment did not result in significant cell proliferation (Figure 3c).The culture supernatant was negative for virus specific IgM in all time points tested.

In order to find out the virus specific IgM secretion without T-cell help, the T cell deficient (nude) mice were exposed to CHPV. The presence of virus specific IgM and IgG in sera collected at different time points were tested by capture ELISA. No significant difference could be observed in level of IgM between virus exposed nude and wild type mice (Figure 5a). However, virus specific IgG was detected in wild type mice but not in nude mice (Figure 5b). In *in vivo*, all the nude mice infected with CHPV through intra-cerebral route were succumbed to infection. No clinical signs or death could be observed in wild type mice (Figure 6).

Discussion

Although, it is well established that antibodies played pivotal role in protection against VSV infection [29], no information is available for CHPV - the same genus of VSV. The current study is based on our earlier observation that rapid IgM response in CHPV infected susceptible young mice [2]. There is lack of evidence that mechanism that leads to the generation of virus specific IgM antibody in CHPV infection. We used purified B- cells from normal healthy mice splenocytes and T-cell deficient nude mice to demonstrate that live CHPV is required for activation of B-cells.

Many viruses cause polyclonal activation of B-cells [30-35]. Some viruses, for example mouse mammary tumor virus (MMTV), are thought to induce B-cell activation by directly infecting B-cell [36] while the glycoproteins of some virus, such as VSV, induce T cell independent B-cell activation [37]. Earlier works on VSV demonstrate

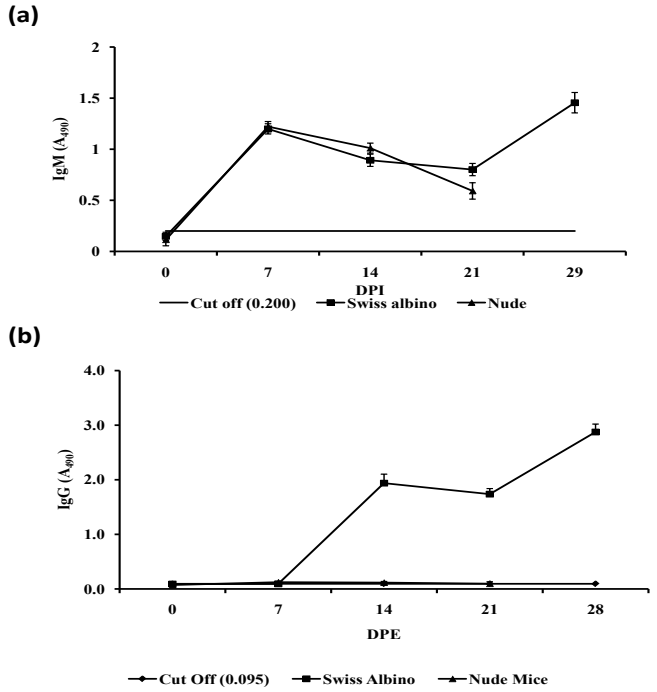

Figure 5: CHPV specific IgM and IgG level in sera of CHPV exposed mice
The virus exposed and unexposed Swiss albino (SA) and nude mice were bled at different time points. The mice were infected with live CHPV through intra-cerebral route on 21st day post exposure (DPE). The CHPV specific IgM and IgG in the sera was detected by mouse IgM and IgG capture ELISA. The cut off values are calculated by Mean +3 SD of OD from virus unexposed control mice. All the nude mice were died after intra-cerebral infection.

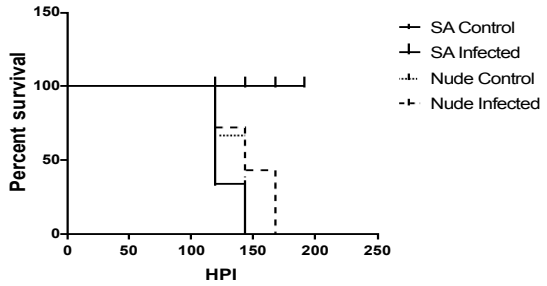

Figure 6: Percentage survival of CHPV exposed mice after intra-cerebral infection
The virus exposed and unexposed Swiss albino (SA) and nude mice were infected with CHPV through intra-cerebral route on 21st day post exposure. The mortality was recorded up to 10 days post infection. The percent survival was calculated using Kaplan-Meier statistics and GraphPad Prism 5 software.

that the virus induce neutralizing IgM response without T cell help as early as 4 days PI in mice [38]. It was reported that VSV glycoprotein act as a T cell dependent and independent antigen [39]. The repetitive nature of glycoprotein epitope in virus envelop act as polyclonal activator of B-cells and induce T independent IgM response. Loosely distributed G protein in infected cells induces both T dependent and independent IgM response [30]. Like VSV, Chandipura virus also induces IgM response as early as 2 days PI [2]. Activation and proliferation of B- cells was noticed only from cells treated with live CHPV. The absence of membrane CD69 expression in live virus exposed B-cells might be due to the clonally proliferated cells loses the early activation marker (CD69) to become plasma cells [40]. In order to confirm the role of infectious CHPV in activation, B-cells were treated with live virus or BEI inactivated viruses and the results were compared. BEI has less effect on antigenic structure of virus [28,41]. Hence the antigenic structure is protected for stimulation of B-cells. We observed that inactivated CHPV induced B-cell activation but no proliferation. In many instances, activation occurs prior to proliferation. However, proliferation is not always a consequence of activation [36,42-45]. The similar observation was also reported in lymphocytes treated with inactivated rota-virus [17]. We earlier reported that CHPV interacts with TLR4 and activation leads increase the severity of disease in mice [26]. Therefore in the current study, the role of TLR4 in B-cell activation was examined. The assay was performed using anti TLR4/MD2 blocking antibody (clone MTS510). This clone of antibody only blocks the receptor without stimulating the B-cells. The results of activation and proliferation of B cells blocked with TLR4 antibody and exposed with live CHPV or B-cells directly (without blocking) exposed to inactivated CHPV was similar. The above finding indicates that live CHPV and/or signalling through TLR4 by is necessary for cell proliferation and secretion of virus specific IgM. TLR along with engagement of B-cell receptor (BCR) is necessary for activation and proliferation of naïve B-cells [46-48]. Our study shows that like VSV [49], the B-cells are non permissive to CHPV.

The IgM antibody level against live CHPV was studied in athymic nude mice (deficient of T lymphocytes). The mice secreted virus specific IgM but not IgG. The nude mice were succumbed to disease after intra-cerebral infection. In contrast, CHPV inoculated wild type mice produced virus specific IgM as well as IgG and no clinical signs or death was observed. This experiment suggests that both cell mediated immune response and/or virus specific IgG is essential for protection from infection. In VSV infection, mice with different specific immune defects showed that antibodies are pivotal for survival in early phase of infection and T cells are required for long term survival [29]. We earlier reported that CHPV produces acute infection in young mice and infected mice succumbed to infection within 4 days PI. The infected mice produce virus specific IgM and effectively clear the virus from circulation [2]. The possible explanations of the death of young mice could be due to the immature cell mediated immune system or not having enough time to activate the cell mediated immune response and IgG secretion during CHPV infection.

In conclusion, we demonstrated that CHPV directly activates the B-cells. However, for proliferation and secretion of specific IgM, live virus and/or signalling via TLR4 is necessary. IgM antibody alone may not be sufficient to protect the mice from the infection and the CMI and/or virus specific IgG is necessary to confer protection from disease.

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References

1. Rao BL, Basu A, Wairagkar NS, Gore MM, Arankalle VA, et al. (2004) A large outbreak of acute encephalitis with high fatality rate in children in Andrapradesh, India, in 2003, associated with Chandipura virus. Lancet 364: 869-874.
2. Balakrishnan A, Mishra AC (2008) Immune response during acute Chandipura viral infection in experimentally infected susceptible mice. Virol J 5: 1-11.
3. Bhatt PN, Rodrigues FM (1967) Chandipura: a new arbovirus isolated in India from patients with febrile illness. Indian J Med Res 55: 1295-1305.
4. Jortner BS, Bhatt PN, Solitare GB (1973) Experimental Chandipura virus infection in mice. I.Virus assay and light microscopic studies with emphasis on neuropathologic observations. Acta Neuropathol 23: 320-325.

5. Garside P, Ingulli E, Merica RR, Johnson JG, Noelle RJ, et al. (1998) Visualization of specific B and T lymphocyte interactions in the lymph node. Science 281: 96-99.

6. Cyster JG (1999) Chemokines and cell migration in secondary lymphoid organs. Science 286: 2098-2102.

7. Kehry MR, Hodgkin PD (1993) Helper T cells: delivery of cell contact and lymphokine-dependent signals to B cells. Semin Immunol 5: 393-400.

8. Ahmed R, Gray D (1996) Immunological memory and protective immunity: understanding their relation. Science 272: 54-60.

9. Hebeis BJ, Klenovsek K, Rohwer P, Ritter U, Schneider A, et al. (2004) Activation of virus-specific memory B cells in the absence of T cell help. J Exp Med 199: 593-602.

10. Griffin DE (2003) Immune responses to RNA-virus infections of the CNS. Nat Rev Immunol 3: 493-502.

11. Bachmann MF, Zinkernagel RM (1997) Neutralizing antiviral B cell responses. Annu Rev Immunol 15: 235-270.

12. Zinkernagel RM, LaMarre A, Ciurea A, Hunziker L, Ochsenbein AF, et al. (2001) Neutralizing antiviral antibody responses. Adv Immunol 79:1-53.

13. Vos Q, Lees A, Wu ZQ, Snapper CM, Mond JJ (2000) B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms. Immunol Rev 176: 154-170.

14. Lanzavecchia A, Sallusto F (2007) Toll like receptors and innate immunity in B cell activation and antibody responses. Curr Opin Immunol 19: 268-274.

15. Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124: 783-801.

16. Gray D, Gray M, Barr T (2007) Innate responses of B cells. Eur J Immunol 37: 3304-3310.

17. Beutler B (2004) Innate immunity: an overview. Mol Immunol 40: 845-859.

18. Zambon RA, Nandakumar M, Vakharia VN, Wu LP (2005) The Toll pathway is important for an anti-viral response in Drosophila. Proc Natl Acad Sci USA 102: 7257-7262.

19. Anukumar B, Prajakta shahir (2012) Chandipura Virus infection in mice: the role of toll like receptor 4 in pathogenesis. BMC Infect Dis 12: 125.

20. Szomolanyi-Tsuda E, Welsh RM (1996) T cell-independent antibody-mediated clearance of polyoma virus in T cell-deficient mice. J Exp Med 183: 403-411.

21. Blutt SE, Warfield KL, Lewis DE, Conner ME (2002) Early response to rotavirus infection involves massive B cell activation. J Immunol 168: 5716-5721.

22. Blutt SE, Crawford SE, Warfield KL, Lewis DE, Estes MK, et al. (2004) The VP7 outer capsid protein of rotavirus induces polyclonal B-cell activation. J Virol 78: 6974-6981.

23. Milich DR, McLachlan A (1986) The nucleocapsid of hepatitis B virus is both a T-cell-independent and a T-cell-dependent antigen. Science 234: 1398-1401.

24. Andres EM, Scalzo AA, White DO (1984) Influenza viruses are T cell-independent B cell mitogens. J Virol 50: 960-963.

25. Borrow P, Tishon A, Lee S, Xu J, Grewal IS, et al. (1996) CD40L-deficient mice show deficits in antiviral immunity and have an impaired memory $CD8^+$ CTL response. J Exp Med 183: 2129-2142.

26. Freer G, Burkhart C, Ciernik I, Bachmann MF, Hengartner H, et al. (1994) Vesicular stomatitis virus Indiana glycoprotein as a T cell dependent and independent antigen. J Virol 68: 3650-3655.

27. Jadi RS, Sudeep AB, Barde PV, Arankalle VA, Mishra AC (2011) Development of an inactivated candidate vaccine against Chandipura virus (Rhabdoviridae: Vesiculovirus) Vaccine 29: 4613-4617.

28. Bahnemann HG (1990) Inactivation of viral antigens for vaccine preparation with particular reference to the application of binary ethylenimine. Vaccine 8: 299-303.

29. Thomsen AR, Nansen A, Andersen C, Johansen J, Marker O, et al. (1997) Cooperation of B cells and T cells is required for survival of mice infected with vesicular stomatitis virus. Int Immunol 9: 1757-1766.

30. Bachmann MF, Hengartner H, Zinkernagel RM (1995) T helper cell independent neutralizing B cell response against vesicular stomatitis virus: role of antigen patterns in B cell induction? Eur J Immunol 25: 3445-3451.

31. Coutelier JP, Coulie PG, Wauters P, Heremans H, van der Logt JT (1990) In vivo polyclonal B-lymphocyte activation elicited by murine viruses. J Virol 64: 5383-5388.

32. Karupiah G, Sacks TE, Klinman DM, Fredrickson TN, Hartley JW, et al. (1998) Murine cytomegalovirus infection induced polyclonal B cell activation is independent of CD4+ T cells and CD40. Virology 240: 12-26.

33. Rott O, Charreire J, Mignon-Godefroy K, Cash E (1995) B cell super stimulatory influenza virus activates peritoneal B cells. J Immunol 155: 134-142.

34. Scalzo AA, Anders EM (1985) Influenza viruses as lymphocyte mitogens. II. Role of I-E molecules in B cell mitogenesis by influenza A viruses of the H2 and H6 subtypes. J Immunol 135: 3524-3529.

35. Stevenson PG, Doherty PC (1999) Non-antigen-specific B-cell activation following murine gamma herpesvirus infection is CD4 independent in vitro but CD4 dependent in vivo. J Virol 73: 1075-1079.

36. Ardavin C, Luthi F, Andersson M, Scarpellino L, Martin P, et al. (1997) Retrovirus-induced target cell activation in the early phases of infection: the mouse mammary tumor virus model. J Virol 71:7295-7299.

37. Fehr T, Bachmann MF, Horst Bluethmann, Hitoshi Kikutani, Hans Hengartner, et al. (1996) T-independent activation of B cells by vesicular stomatitis virus: no evidence for the need of a second signal. Cell Immunol 168: 184-192.

38. Charan S, Zinkernagel RM (1986) Antibody mediated suppression of secondary IgM response in nude mice against vesicular stomatitis virus. J Immunol 136: 3057-3061.

39. Freer G, Burkhart C, Ciernik I, Bachmann MF, Hengartner H, et al. (1994) Vesicular stomatitis virus Indiana glycoprotein as a T cell dependent and -independent antigen. J Virol 68: 3650-3655.

40. Dörken B, Möller P, Pezzutto A, Schwartz-Albiez R, Mol denhauer G (1989) B-cell antigens: CD19. In: Knapp W (ed) Leucocyte Typing IV: White Cell Differentiation Antigens. Oxford University Press, New York, 34-36.

41. Ramakrishnan MA, Pandey AB, Singh KP, Singh R, Nandi S, et al. (2006) Immune responses and protective efficacy of binary ethylenimine (BEI)-inactivated bluetongue virus vaccines in sheep.Vet Res Commun 30: 873-880.

42. Melchers F,Corbel C (1983) Studies on B-cell activation in vitro. Ann Immunol (Paris) 134D: 63-73.

43. Muraguchi A, Kehrl JH, Butler JL, Fauci AS (1984) Regulation of human B-cell activation, proliferation, and differentiation by soluble factors. J Clin Immunol 4: 337-347.

44. Sacco RE, Hagen M, Donelson JE, Lynch RG (1994) B lymphocytes of mice display an aberrant activation phenotype and are cell cycle arrested in G0/G1A during acute infection with Trypanosoma brucei. J Immunol 153: 1714-1723.

45. Tough DF, Sun S, Sprent J (1997) T cell stimulation in vivo by lipopolysaccharide (LPS). J Exp Med 185: 2089-2094.

46. Ruprecht CR, Lanzavecchia A (2006) Toll-like receptor stimulation as a third signal required for activation of human naive B cells. Eur J Immunol 36: 810-816.

47. Fillatreau S, Manz RA (2006) Tolls for B cells. Eur J Immunol 36: 798-801.

48. Minguet S, Dopfer EP, Pollmer C, Freudenberg MA, Galanos C, et al. (2008) Enhanced B-cell activation mediated by TLR4 and BCR crosstalk. Eur J Immunol 38: 2475-2487.

49. Schmidt MR, Gravel KA, Woodland RT (1995) Progression of a vesicular stomatitis virus infection in primary lymphocytes is restricted at multiple levels during B cell activation. J Immunol 155: 2533-2544.