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IN VITRO STUDY OF LYMPHOTROPIC AND IMMUNOMODULATORY PROPERTIES OF THE PESTE DES PETITS RUMINANTS VIRUS (PPRV)

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ABSTRACT

The Peste des petits ruminants (PPR) is a viral disease which affects sheep and goats and even the wild ruminants. About a billion small ruminants are found in the PPR enzootic areas. Morbilliviruses are primarily lymphotropic and secondarily epitheliotropic. Viral infection and interaction of viral proteins with lymphatic tissues are partly responsible for immune suppression. Transient immunosuppression is also observed after vaccination with attenuated vaccines. Viral isolation is traditionally done on Vero but newer recombinant cell lines are also used. In this work, sensitivity of three cell lines i.e. SLAM-ve-Vero, Vero.DogSLAMtag and B95a having Marmoset SLAM have been compared for PPRV titration. Moreover, infectivity of phytohemagglutinin (PHA) stimulated peripheral blood mononuclear cells (PBMCs) with PPR virus (PPRV) has been tested. Finally, live PPRV, recombinant nucleoprotein of PPR Virus (NPPRV) and truncated Δ420-525 NPPRV have been used to demonstrate *in vitro* inhibition of PHA stimulated lymphoproliferation of PBMCs of naïve goats. The presence of canine SLAM (Signaling lymphocyte activation molecule) receptor but not the marmoset SLAM, enhances infectivity of cell lines by up to 0.8 $\log_{10}$. At multiplicity of infection (MOI) of 0.1, 83.6 percent of PBMCs can be infected with PPRV. PPRV could completely inhibit lymphoproliferation at MOI of 0.75 while NPPRV and Δ420-525 NPPRV could suppress lymphoproliferation by 7.4 % and 17.6 %, respectively. PPRV is lymphotropic as PPRV (Nigeria 75/1) is capable of infecting mitogen activated PBMCs and that presence of canine SLAM in Vero cell line greatly enhances infectivity of PPRV as compared to SLAM negative Vero. Vero.DogSLAMtag is more sensitive cell line for PPRV titration and PPRV isolation. PPRV (Nigeria 75/1) and recombinant N-proteins induce *in vitro* inhibition of cell proliferation of mitogen stimulated PBMCs of naive goats. Using deleted mutant of NPPRV expressed in baculovirus system, amino acid sequence of NPPRV between 1-420 was found responsible for inducing immune suppression.

Keywords: Immunomodulation, Immune suppression, lymphoproliferation, Nucleoprotein, SLAM, Morbillivirus, PPRV

INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious acute viral disease of small ruminants clinically characterized by fever, occulo-nasal discharges, stomatitis, diarrhea and pneumonia with foul offensive breath (Griffin and Pan, 2009). It is caused by the virus of *Peste des Petits Ruminants* (PPRV), which belongs to the genus *Morbillivirus* and family *Paramyxoviridae*. Morbidity rate of 90% and mortality rate of 50–80% may occur in susceptible populations. Like other viruses in the *Morbillivirus* genus, PPRV is also primarily lymphotropic and secondarily epitheliotropic (Birch *et al*., 2013; Pope *et al*., 2013). The viral tropism is also manifested by the clinical signs like occulo-nasal discharges, diarrhea, oral erosive lesions, marked immuno-suppression, and leucopenia; while viral antigen can be found in lymphoid organs and epithelial tissues of the affected animals (Kumar *et al*., 2004; Birch *et al*., 2013; Pope *et al*., 2013). The signaling lymphocyte activation molecule (SLAM) or CD150, is the primary receptor for PPRV (Pawar *et al*., 2008), which is found on activated T cells, B cells, thymocytes, macrophages and dendritic cells while an epithelial Nectin-4 protein receptor has recently been discovered (Birch *et al*., 2013; Pope *et al*., 2013).

Although Morbillivirus infections generate strong immune responses with resulting long term immunity, paradoxically, during temporary phase post-infection they also produce an immunosuppression with the emergence of secondary infections (Beckford *et al*., 1985; Griffin, 2010). PPRV infection in small ruminants also produces an impairment of cellular and humoral immune functions, resulting in frequent opportunistic infections (Mondal *et al*., 2001; Kumar *et al*., 2004). The immune suppression is not only observed during natural infections but also after vaccination with attenuated strains of Morbilliviruses (Hussey *et al*., 1996; Stevenson *et al*., 1999; Lund *et al*., 2000). The leucopenia and immunosuppression result from a combination of factors like cell death caused by infection of lymphocytes and apoptosis, and the cell cycle arrest, caused by exposure to the viral haemagglutinin and fusion surface glycoproteins as well as viral nucleoprotein, expressed from the

damaged cells (Mondal *et al*., 2001; Kumar *et al.*, 2004; Griffin 2010).

The nucleocapsid (N) protein of morbilliviruses is a major viral protein both in the virion and in the infected cell (Rima, 1983). NPPRV consists of 525 amino acid residues and has a molecular weight of about 60 kDa (Diallo *et al*., 1987; Diallo *et al*., 1994). It is a structural protein which encapsidates the viral genome into an RNAse-resistant nucleocapsid (the template for RNA synthesis) and together with phosphoprotein forms the ribonucleoprotein, which constitutes the minimum essential for transcription and replication of viral genome in the cell cytoplasm (Knipe and Howley, 2001). Morbillivirus N protein consists of two domains; i.e. a conserved N-terminal moiety (N_{CORE}, aa 1–400) and a variable C-terminal moiety (N_{TAIL}, aa 401–525) (Diallo *et al*., 1994). The region involved in nucleoprotein receptor (NR) binding is localized within the hyper variable N-terminal region of Measles virus (MeV) N. N_{TAIL} binds with the NR found on both T and B lymphocytes to induce a cell cycle arrest and suppression of cell proliferation (Laine *et al*., 2007). The NR specifically binds with aa 401-420, that is well conserved among Morbilliviruses (Diallo *et al*., 1994).

In this study, infectability of goat peripheral blood mononuclear cells (PBMCs) with PPRV has been evaluated *in vitro*. Moreover, susceptibility of one SLAM-ve cell line (Vero) along with two cell lines (Vero.DogSLAMtag and B95a) having Marmoset and canine SLAM receptors, respectively, have been compared through viral titration. Finally, the immunosuppressive property of PPRV, NPPRV and the truncated Δ420-525 NPPRV has been studied *in vitro*.

MATERIALS AND METHODS

Cell culture: Peripheral blood mononuclear cells (PBMCs) of naive goats were isolated on Histopaque®-1077 (Sigma- Aldrich, Inc., USA) density gradient and cultured in RPMI medium supplemented with 0.5% gentamycin, 1% glutamine, 0.1% mercaptoethanol and 10% fetal calf serum (FCS). The viability of cells was assessed through trypan blue dye exclusion. B95a cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 1% Glutamine, and 10% (v/v) FCS. Vero cells were maintained in Eagle's minimum essential medium (EMEM) supplemented with 1% Glutamine, and 10% (v/v) FCS. Vero.DogSLAMtag were cultured in RPMI 1640 containing 10% FCS. SF21 (*Spodoptera frugiperda* passage 21) cells were cultured in Grace's culture medium supplemented with 10% (v/v) FCS. All cells were incubated in a humidified incubator at 37^0C with 5% CO_2 except SF21 cells which were incubated at 27^0C without CO_2.

PPRV production, titration and immunofluorescence staining: PPRV (Nigeria-75/1), used for infecting PBMCs and lympho proliferation studies, was produced and titrated using Vero cells. Part of produced PPRV was 6-fold concentrated using the Centricon plus-70 filter, (Millipore Corp., Bedford, MA). Moreover, viral titrations on three cell lines were performed under level-3 biosecurity conditions. Calculations were done in accordance with Reed-Muench method (Reed and Muench, 1938). The PPRV strains Nigeria-75/1 and Ethiopia-95/1 were serially 10 fold diluted in DMEM and each dilution was transferred to 96 well cell culture plates already having $2.5x\ 10^4$ Vero, Vero.DogSLAMtag or B95a cells /well. Viral infection was evaluated by observation of cytopathic effect as well as immuno-staining on day-9. Briefly, media were removed and 150 µl cold acetone 80% was added to each well and incubated at -20°C for 30 minutes. Then, acetone was removed and wells were washed thrice with PBS. 100 µl 1% Paraformaldehyde (PFA) was added to each well and kept at room temperature for 30 minutes. The plates were then moved from level-3 biosecurity laboratory to the level-2 biosecurity facility. PFA was removed and wells were washed thrice with PBS. Anti-PPRV mab (38-4) diluted to 1:450 in PBS-1X was added to all wells. Plates were washed with PBS thrice and the conjugate anti-mouse diluted to 1:100 in PBS-1X was added to all wells. Finally, plates were washed thrice with PBS and 50µl of PBS was added to each well. Plates were observed under inverted fluorescent microscope to calculate PPRV titers.

Baculoviral clone selection and expression recombinant entire (N-PPRV) and truncated nucleoprotein of PPRV (Δ420-525N-PPRV): Recombinant baculoviruses expressing NPPRV and Δ420-525 NPPRV were kindly gifted by Dr. Charles Bojo. Clones of the two recombinant baculoviruses expressing NPPRV and Δ420-525NPPRV proteins were separately purified by plaque assay using agarose overlay in 6-well cell culture plates. These were amplified initially in confluent 6-well cell culture plates and verified by immunoflourescence staining. Briefly, infected SF21 cells were scrapped and transfered to 96-well culture plates. These were fixed in 80% acetone at room temperature for 30 minutes and were then rinced in PBS. These were first reacted with anti-NPPRV monoclonal antibody (MAB) 38.4 (IgG1 isotype) (Libeau *et al*., 1995) for 30 minutes and washed twice with PBS. Then, these were stained with FITC labeled anti-mouse-IgG cojugate (dilution 1/80, Biorad). After washing, these were observed under fluoresecnet microscope to verify positive clones. These clones were then used to infect confluent T-75 flasks at MOI of 10. Four days later when cytopathic effects were evident, supernatents were discarded as nucleoprotein is intra-cytoplasmic. The cell layer was washed twice with PBS, the monolayer was

scrapped and resuspended in 15 ml of PBS. The cell suspensions were freeze-thawed (-80^{0}C and 37^{0}C) thrice. The lysates were 6-fold concentrated using the Centricon plus-70 filter, (Millipore Corp., Bedford, MA) having capacity to concentrate particles above 300 kDa and aliquotes were frozen at -80^{0}C.

Lymphoproliferation and infection of PBMCs with PPRV: For studying immunosuppressive effect of PPRV, 4x10^6 PBMCs/well were cultured in 24 well cell culture plates and were infected with MOIs of 0.016, 0.1, 0.25, 0.5, 0.75 and 1. Moreover, 99 µl of 6-fold concentrated PPRV (MOI 0.016), NPPRV and Δ420-525NPPRV were added to duplicate wells with PBMCs. Further, PPRV (unconcentrated) was added at MOIs of 0.1, 0.25, 0.5, 0.75 and 1. Similar volumes of lysates of Vero (concentrated or unconcentrated) and SF21 (concentrated) cells were also added to control wells. PBMC were stimulated with Phytohaemagglutinin from *Phaseolus vulgaris* (Sigma-Aldrich, Inc., USA) at final concentration of 3.5 µg/ml and incubated. Lymphoproliferation was evaluated 72 hours later by flow cytometry. Whereas, for infection of PBMCs with PPRV, 1x10^6 PBMCs/well were cultured in 48 well cell culture plates, stimulated with PHA at the concentration of 5 µg/ml and infected four hours later with PPRV (unconcentrated) at MOIs of 0.1, 0.5, and 1.

Flow Cytometry: The relative expression of PPRV nucleoprotein was used as a measure of infection of PBMCs with PPRV. The expression of the protein was quantified by immunofluorescence staining and reading by flow cytometry. Seventy-eight hours post infection; the cells were plated in 96-well cell culture plate. They were permeabilized with 3% saponine (w/v) in PBS 0.1% Sodium azide and 5% horse serum. The cells were first reacted with anti-NPPRV MAB 38.4 (Libeau *et al*., 1995) and later with anti-mouse fluorescein-conjugated secondary antibody IgG (Bio-Rad). The plates were agitated for 30 minutes at 4°C and were washed twice at the end of each incubation. Finally, cells were fixed with 1% paraformaldehyde. The expression of viral nucleoprotein expression was measured by flow cytometry using a FACSort (Becton Dickinson). The analysis was done using the CELLQuestTM software (BD Bioscience).

For lymphoproliferation studies, lymphocyte population in the PBMCs was characterized by using FSC (Forward scatter) and SSC (Side scatter) parameters of size and granularity of cells. The cell population was separated by R1 gate representing the debris and R2 gate representing lymphocytes. Later, proportion of lymphocytes undergoing blastogenesis in the "lower right" window, were used for calculation of percentage of inhibition by using following formula: [1-Test/control x 100].

RESULTS

PPRV infects PBMCs of goat: In order to evaluate ability of Vero cell adapted Nigeria 75/1 strain of PPRV to infect lymphocytes of naïve goats *in vitro*, PBMCs, with or without mitogen stimulation, were put in contact with PPRV. Moreover, lysates of Vero and SF21 cell lines were also added as controls and both did not have any negative effect upon lymphoproliferation. It was found that unstimulated PBMCs are less susceptible to PPRV infection than PHA activated lymphocytes. A dose dependent increase in proportion of infected PBMCs is seen in non-stimulated PBMCs. At the highest MOI used, virus could infect only 32 % of the non-stimulated cells. At the lowest MOI of 0.1, PPRV could infect 83.6% of PHA stimulated PBMCs (Fig. 1). Using higher MOIs did not improve infectivity. Infection after 24 and 72 hours after mitogen stimulation also did not improve infectivity of PBMCs (data not shown).

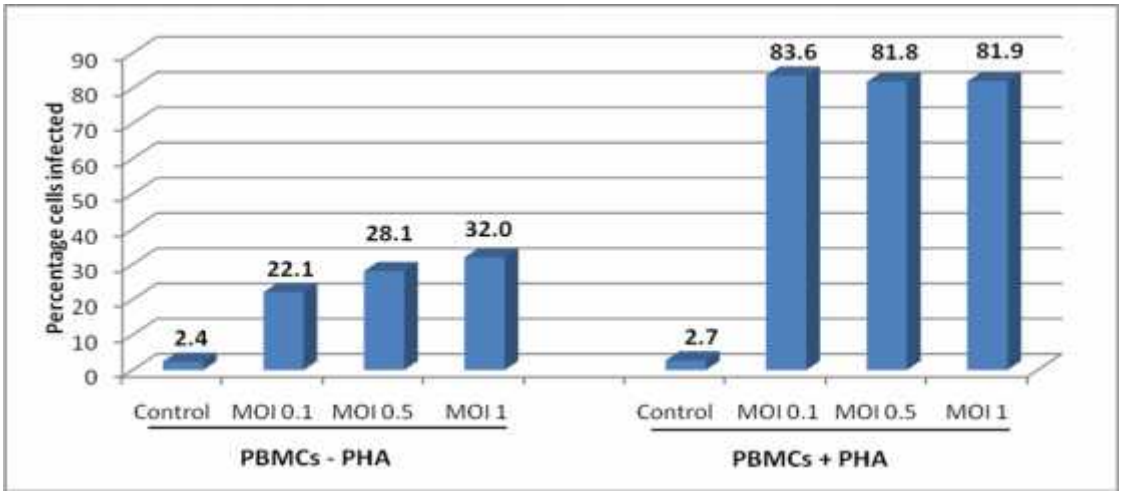

Figure 1: Infection of PHA stimulated and non-stimulated PBMCs at various MOIs of PPRV

Presence of SLAM or CD150 receptor enhances infectivity of cells by PPRV: Next, infectivity of Vero (without SLAM), B95a (having Marmoset SLAM) and Vero.DogSLAMtag (having canine SLAM) with wild-type Ethiopia-94/1 and attenuated Nigeria 75/1 strains of PPRV was tested. Both strains were serially ten-fold diluted in DMEM and transferred to plates having the three cell lines. B95a was the least sensitive, Vero was moderately sensitive and Vero.DogSLAMtag was most sensitive to infection by both strains of PPRV. Vero.DogSLAMtag showed 0.8 $\log_{10}$ higher virus titer than the Vero cell line. The B95a showed viral 3.6 and 3.9 $\log_{10}$ lesser titers than Vero. Dog SLAMtag cell line for Ethiopia-94/1 and Nigeria 75/1 strains of PPRV, respectively (Fig. 2).

Expression and relative quantification of recombinent NPPRV and Δ420-525NPPRV proteins: Relative quantities of two recombinant nucleoproteins and NPPRV in PPR virus solutions were measured by immunocapture ELISA (IC-ELISA). For the purpose, equal volumes (100 µl) of 6-fold concentrated lysates of SF21 infected with recombinent baculoviruses expressing two proteins, along with equally concentrated lysates of vero cells infected with PPRV were used and compared by IC-ELISA as described elsewhere (Libeau *et al*., 1994). It can be seen that Δ420-525NPPRV was much better expressed as compared to NPPRV (Fig. 3). The concentration of Δ420-525NPPRV is almost equivalent to PPRV MOI of 0.001. Difference in expression level of two recombinant proteins is probably due to culture conditions.

PPRV and NPPRV inhibit the proliferation of PHA stimulated PBMCs of goats: PPRV (unconcentrated) could completely inhibit blastogenesis by lymphocytes under the influence of PHA (Fig. 4). In fact, inhibition at MOIs 0.75 and 1 was greater than what was found in negative control i.e. PBMCs not under the influence of mitogen but having low level blastogenesis due to auto-stimulation. Both NPPRV and Δ420-525NPPRV proteins could inhibit lymphoproliferation *in vitro*. However, inhibition was 2.36 times higher by Δ420-525NPPRV protein. An equal volume of 5-fold concentrated PPRV solution (MOI 0.016) could inhibit lymphoproliferation to 17.6 % which almost equal to what was found for Δ420-525NPPRV.

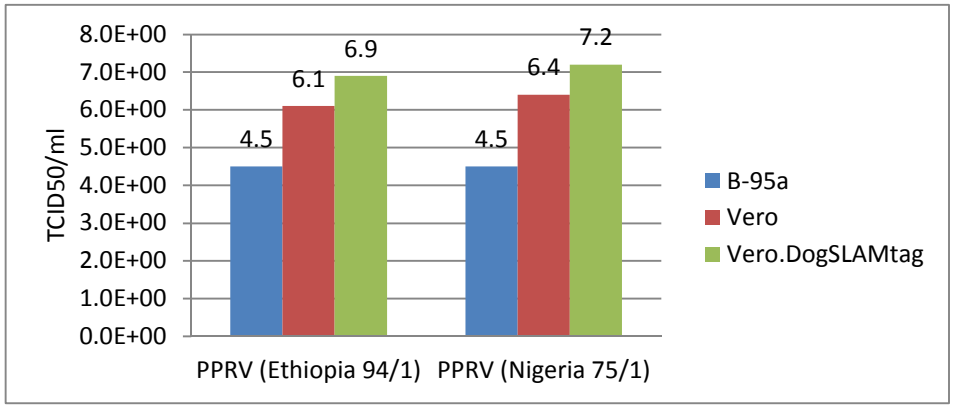

Figure 2: PPRV (Ethiopia 94/1 and Nigeria 75/1) titration using B-95a, Vero, and Vero. DogSLAMtag cell lines.

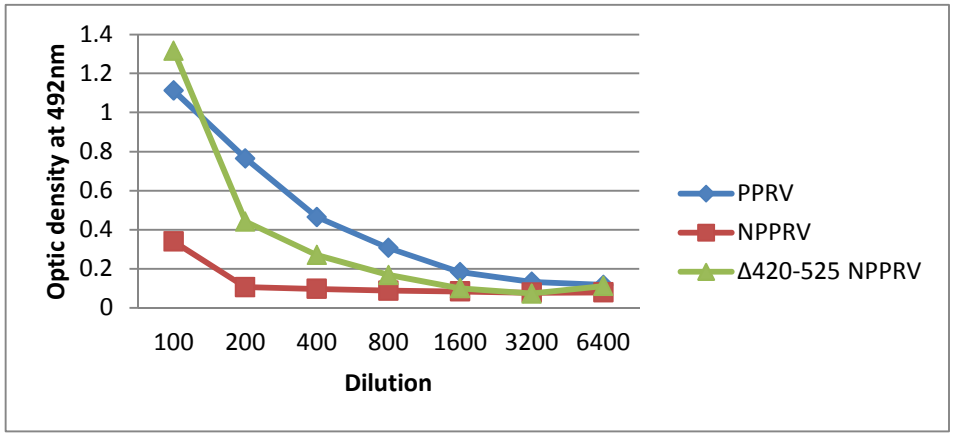

Figure 3: Concentrations of nucleoproteins in Vero and SF21 lysates having live PPRV, N-PPRV andΔ420-525N-PPRV proteins, respectively.

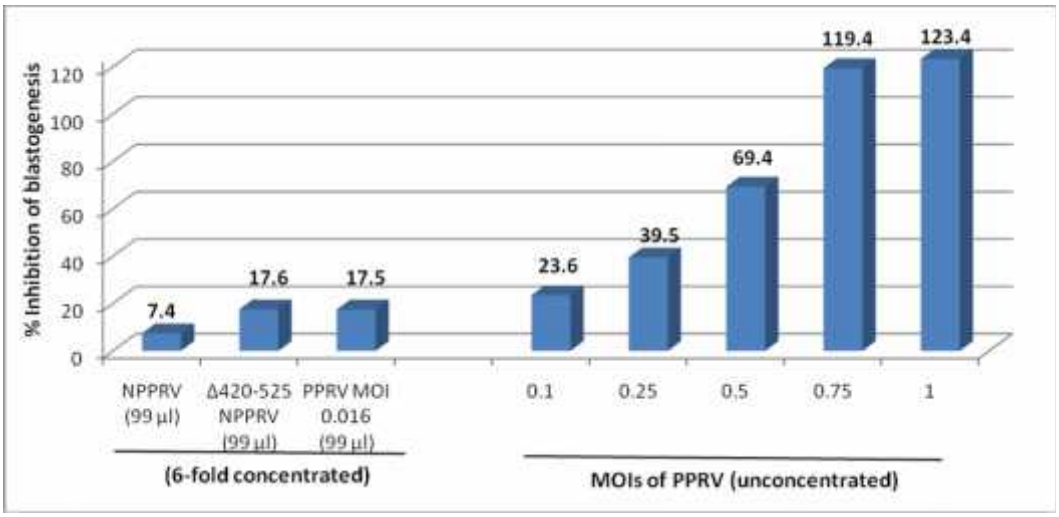

Figure 4: Inhibition of PHA induced blastogenesis in PBMCs by N-PPRV, Δ420-525N-PPRV and various multiplicities of infection (MOIs) of PPRV.

DISCUSSION

In this study, it has been shown that the vaccine strain of PPRV (Nigeria 75/1) is capable of efficiently infecting mitogen activated goat lymphocytes *in vitro*. PPRV infects PHA activated lymphocytes more efficiently than the unstimulated ones. PHA stimulation is known to increase SLAM expression by lymphocytes however, SLAM is also expressed, at lower levels, in unstimulated cultured lymphocytes (Hamalainen *et al*., 2000; Erlenhoefer *et al*., 2001). Lower SLAM expression explains the lower the infectivity of unstimulated PBMCs. The lymphocyte subpopulations have not been studied in the present study, but morbilliviruses are known to infect both T and B cells while PHA activates both B and T lymphocytes (Hamalainen *et al*., 2000; Erlenhoefer *et al*., 2001). Interestingly, although Nigeria 75/1 strain of PPRV was attenuated by blind passages on epithelial Vero cell line (Diallo *et al*., 1989) but has still retained its ability to use original SLAM receptor and infect lymphocytes as lymphatic tissues of small ruminants do not express the second PPRV receptor; i.e. Nectin-4 (Birch *et al*., 2013). Among Morbilliviruses, the vaccine strain of measles virus (MV) is also known to be capable of using CD46 along with SLAM receptor (Tatsuo *et al*., 2000).

Next, the efficiency three cell lines for titration of wild-type and vaccine strains of PPRV was compared. Despite using same viral solutions for titration of two PPRV strains, low, moderate and high titers were recorded on B95a, Vero and Vero.DogSLAMtag cell lines, respectively. B95a is marmoset B-lymphoblastoid cell line capable of attachment to a substrate surface. It is known to be 10,000-fold more sensitive to wild-type measles virus isolation than Vero cell line (Kobune *et al*., 1990) and has been used for culture of PPRV as well (Sreenivasa *et al*., 2006; Pawar *et al*., 2008). However, it was found that titration on B95a gave 2.4 $\log_{10}$ and 2.7 $\log_{10}$ lower titers for Nigeria 75/1 and Ethiopia 95/1 strains respectively as compared to Vero.DogSLAMtag cell line. Vero.DogSLAMtag, expressing canine SLAM receptor, also showed 0.8 $\log_{10}$ higher titers for both virus strains than Vero cell line.

Several factors appear to contribute in the immunosuppression produced by Morbilliviruses. Various viral proteins play an important role in the immunopathogenicity. Immunosuppression is initiated either by direct contact of viral proteins with the cells of immune system, which may or may not be infected, as has been shown by Heaney *et al.* 2002 that the F-H complex of RPV and PPRV can inhibit bovine and caprine PBMCs. It is also reported that the nucleocapsids of Morbilliviruses attach with lymphocytes of their natural hosts and induce an immunosuppression (Kerdiles *et al*., 2006). The nucleoprotein of Measles virus (MeV) has been shown to suppress B and T cell proliferation by interacting with NR and FcγRIIB receptors (Laine *et al*., 2007). The region involved in NR binding is localized within the hyper variable C-terminal region of MeV N, N_{TAIL} (Laine *et al*., 2007). Specifically, NR binds with aa 401-420, which is well conserved among Morbillivirus members (Diallo *et al*., 1994). It is also well conserved among wild-type and vaccine MeV strains. MeV N binding to inhibitory receptors can inhibit both cell proliferation and antibody secretion by activated B cells N_{TAIL}.– NR interaction. Moreover, MeV N also binds to FcγRIIB via N_{CORE}. T cell proliferation is inhibited and/or modulated by MeV N in two ways. Firstly, N_{TAIL} may bind to NR expressed by activated T cells to suppress their activation and subsequent proliferation. Secondly, N_{CORE} can directly interact with both FcγRII and NR on DCs to inhibit their function, including IL-12 secretion,

thus precluding efficient activation of T lymphocytes (Laine *et al*., 2007).

In this study, PPRV has been used as a model for studying the immunosuppressive properties of the nucleoprotein. Complete NPPRV and a truncated NPPRV were also used to localize the region responsible for immune suppression. One can suppose that the biochemical and antigenic characteristics of both recombinant and viral nucleoproteins are identical as has been for nucleoproteins of Measles virus (Ravanel *et al*., 1997). Moreover, goat lymphocytes probably also possess NR receptor (Laine *et al*., 2003) and interaction of nucleoproteins with this receptor on T and B lymphocytes could be responsible for suppression of lymphoproliferation of naive PBMCs stimulated with mitogen.

The expression NPPRV and Δ420-525 NPPRV was measured by IC-ELISA and it was seen that the latter was better expressed and had concentration comparable to PPR virus (MOI of 0.016) after concentration. After expression, the truncated N protein was also tested to see if it had the immunosuppressive properties similar to the N-MeV. The immunosuppressive effect was observed and it was found that the anti-proliferative property lies in the amino acid sequence 1-420 of truncated nucleoprotein of PPRV as is the case for other Morbilliviruses. The variable region of C-terminal, contains highly immunogenic sites, amino acids 520-525 for Rinderpest virus and aa 457-472 for PPRV, which make possible the differentiation of two viruses by indirect ELISA and Western blot (Diallo *et al*., 1994; Choi *et al*., 2003; Choi *et al*., 2004; Dechamma *et al*., 2006). The recombinant Δ420-525 NPPRV has retained its immunosuppressive capacity even after deletion. This suggests that the epitopes of nucleoprotein of Morbilliviruses implicated for immune activation are different from those which recognize the cellular receptors responsible immune suppression. This may explain the coexistence of two mechanisms occurring during acute Morbillivirus infections. Thus after initial immune suppression the host develops a strong lifelong immunity against reinfection.

The immunosuppressive effect of Δ420-525 NPPRV was apparently greater than what was seen for the NPPRV. A better immune suppression may also have come from better expression of the Δ420-525 NPPRV and difference of molar quantities of two proteins which engage the host receptors. As the molar mass of Δ420-525 NPPRV is about 45 kDa where as that of NPPRV is around 60 kDa, which becomes a quantity about ¼ times greater. Therefore more NPPR has to be added to have an effect equal to truncated N. Interestingly, Morbilliviruses inactivated by ultraviolet rays, including PPRV, are known to be immune suppressive however in our study heat inactivated PPRV did not inhibit lymphoproliferation but in fact stimulated lymphoproliferation (data not shown).

Since both recombinent proteins were not HIS-Tagged therefore it was not possible to purify these proteins and lysates of SF21 infected with two baculoviruses were used. In future to identify exact zone on NPPRV which is immune suppressive, higher concentrations of viral protein purified by his-tag will be needed. Moreover, multiple deletions on various zones of NPPRV will have to be tested to specify exact zone of NPPRV which inhibits lymphoproliferation. However, it needs to be seen whether deletions in other parts may change the tertiary structure of the protein which may change its properties. However, after a single deletion in aa 420-525, truncated NPPRV retains its immunosuppressive properties. Preparation of recombinant PPRV with the deletion of immunosuppressive zone of NPPRV, will reduce the immunosuppresive nature of the current Nigeria 75/1 vaccine. The part of C-terminal part of N protein of Morbilliviruses in association with P protein, is needed during viral transcription (Harty and Palese, 1995; Bankamp *et al*., 1996; Liston *et al*., 1997). It may therefore be necessary to introduce a modified N gene by inverse genetics in the complete genome of PPRV and ensuring that it remains functional with the help of a minigenome having a reporter gene like eGFP to determine the effects of the deletion.

Conclusion: PPRV is lymphotropic as vaccine strain of PPRV (Nigeria 75/1) is capable of infecting mitogen activated PBMCs and that presence of canine SLAM in Vero cell line greatly enhances infectivity as compared to SLAM negative Vero. Vero.DogSLAMtag is more sensitive cell line for PPRV titration and PPRV isolation. Vaccine strain of PPRV and recombinant N-proteins induce *in vitro* inhibition of cell proliferation of PBMCs of naive goats. The deleted mutants of this protein expressed in baculovirus system, allowed us to localize the amino acid sequence of the truncated N-protein of PPRV between 1-420 to be responsible for inducing immune suppression, like N-protein of other *Morbilliviruses*. Further studies are needed using multiple deletions in the aa 1-420 of NPPRV to localize immunosuppressive fragment which may be deleted in future recombinant vaccines to avoid immunosuppressive effects produced by current vaccine strains of PPRV.

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REFERENCES

Bankamp, B., S.M. Horikami, P.D. Thompson, M. Huber, M. Billeter and S.A Moyer (1996).

Domains of the measles virus N protein required for binding to P protein and self-assembly. Virology 216, 272-7.

Beckford, A.P., R.O. Kaschula and C. Stephen (1985). Factors associated with fatal cases of measles. A retrospective autopsy study. S Afr Med J. 12, 858-63.

Birch, J., N. Juleff, M.P Heaton., T. Kalbfleisch, J. Kijas and D. Bailey (2013). Characterization of ovine Nectin-4, a novel Peste des petits ruminants virus receptor. J Virol 87, 4756-61.

Choi, K.S., J.J. Nah, Y.J. Ko, C.U. Choi, J.H. Kim, S.Y. Kang and Y.S. Joo (2003). Characterization of antigenic sites on the rinderpest virus N protein using monoclonal antibodies. J Vet Sci 4, 57-65.

Choi, K.S., J.J. Nah, Y.J. Ko, S.Y. Kang, K.J. Yoon and Y.S. Joo (2004). Characterization of immunodominant linear B-cell epitopes on the carboxy terminus of the rinderpest virus nucleocapsid protein. Clin Diagn Lab Immunol 11, 658-64.

Dechamma, H.J., V. Dighe, C.A. Kumar, R.P. Singh, M.J agadish and S. Kumar (2006). Identification of T-helper and linear B epitope in the hypervariable region of nucleocapsid protein of PPRV and its use in the development of specific antibodies to detect viral antigen. Vet Microbiol 118, 201-11.

Diallo, A., T. Barrett, M. Barbron, G. Meyer and P.C. Lefevre (1994). Cloning of the nucleocapsid protein gene of peste-des-petits-ruminants virus: relationship to other morbilliviruses. J. General Virology 75 (Pt 1), 233-7.

Diallo, A., T. Barrett, P.C. Lefevre and W.P. Taylor (1987). Comparison of proteins induced in cells infected with rinderpest and peste des petits ruminants viruses. J. General Virology 68 (Pt 7), 2033-8.

Diallo, A., W.P. Taylor, P.C. Lefevre and A. Provost (1989). [Attenuation of a strain of peste des petits ruminants virus: a potential candidate for live homologous vaccine]. Rev Elev Med Vet Pays Trop 42, 311-9.

Erlenhoefer, C., W.J. Wurzer, S. Löffler, S. Schneider-Schaulies and J. Schneider-Schaulies (2001). CD150 (SLAM) Is a Receptor for Measles Virus but Is Not Involved in Viral Contact-Mediated Proliferation Inhibition. J. Virology 75, 4499-505.

Griffin, D.E. (2010). Measles virus-induced suppression of immune responses. *Immunological Reviews* 236, 176-89.

Griffin, D.E. and C.H. Pan, (2009). Measles: old vaccines, new vaccines. Curr Top Microbiol Immunol 330, 191-212.

Hamalainen, H., S. Meissner and R. Lahesmaa (2000). Signaling lymphocytic activation molecule (SLAM) is differentially expressed in human Th1 and Th2 cells. J. Immunological Methods 242, 9-19.

Harty, R.N. and P. Palese (1995). Measles virus phosphoprotein (P) requires the NH2- and COOH-terminal domains for interactions with the nucleoprotein (N) but only the COOH terminus for interactions with itself. J. General Virology 76 (Pt 11), 2863-7.

Heaney, J., T. Barrett and S.L. Cosby (2002). Inhibition of in vitro leukocyte proliferation by morbilliviruses. J Virol 76, 3579-84.

Hussey, G.D., E.A. Goddard, J. Hughes, J.J. Ryon, M. Kerran, E. Carelse, P.M. Strebel, L.E. Markowitz, J. Moodie, P. Barron, Z. Latief, R. Sayed, D. Beatty and D.E. Griffin (1996). The effect of Edmonston-Zagreb and Schwarz measles vaccines on immune responses in infants. J. Infectious Diseases 173, 1320-6.

Kerdiles, Y.M., C.I. Sellin, J. Druelle and B. Horvat (2006). Immunosuppression caused by measles virus: role of viral proteins. Rev Med Virol 16, 49-63.

Knipe, D.M. and P.M. Howley (2001). *Fundamental Virology*. Lippencott Williams and Wilkins, Philadelphia.

Kobune, F., H. Sakata and A. Sugiura (1990). Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. J. Virology 64, 700-5.

Kumar, P., B.N. Tripathi, A.K. Sharma, R. Kumar, B.P. Sreenivasa, R.P. Singh, P. Dhar and S.K. Bandyopadhyay (2004). Pathological and immunohistochemical study of experimental Peste des petits ruminants virus infection in goats. J. Vet. Med. Series B-Infectious Diseases and Veterinary Public Health 51, 153-9.

Laine, D., M.C. Trescol-Biemont, S. Longhi, G. Libeau, J.C. Marie, P.O. Vidalain, O. Azocar, A. Diallo, B. Canard, C. Rabourdin-Combe and H. Valentin (2003). Measles virus (MV) nucleoprotein binds to a novel cell surface receptor distinct from FcgammaRII via its C-terminal domain: role in MV-induced immunosuppression. J Virol 77, 11332-46.

Laine, D., P. Vidalain and A. Gahnnam (2007). Interaction of measles virus nucleoprotein with cell surface receptors: impact on cell biology and immune response. In: Measles virus nucleoprotein (ed. by Longhi S), pp. 113-52 Nova Science, New York.

Libeau, G., A. Diallo, F. Colas and L. Guerre (1994). Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. Vet Rec 134, 300-4.

Libeau, G., C. Prehaud, R. Lancelot, F. Colas, L. Guerre, D.H. Bishop and A. Diallo (1995). Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. Res Vet Sci 58, 50-5.

Liston, P., R. Batal, C. DiFlumeri and D.J. Briedis (1997). Protein interaction domains of the measles virus nucleocapsid protein (NP). Arch Virol 142, 305-21.

Lund, B.T., A. Tiwari, S. Galbraith, M.D. Baron, W.I. Morrison and T. Barrett (2000). Vaccination of cattle with attenuated rinderpest virus stimulates CD4(+) T cell responses with broad viral antigen specificity. J. General Virology 81, 2137-46.

Mondal, B., B.P. Sreenivasa, P. Dhar, R.P. Singh and S.K. Bandyopadhyay (2001). Apoptosis induced by peste des petits ruminants virus in goat peripheral blood mononuclear cells. Virus Research 73, 113-9.

Pawar, R.M., G.D. Raj, T.M. Kumar, A. Raja and C. Balachandran (2008). Effect of siRNA mediated suppression of signaling lymphocyte activation molecule on replication of peste des petits ruminants virus in vitro. Virus Res 136, 118-23.

Pope, R.A., S. Parida, D. Bailey, J. Brownlie, T. Barrett and A.C. Banyard (2013). Early events following experimental infection with Peste-Des-Petits ruminants virus suggest immune cell targeting. PLoS ONE 8, e55830.

Ravanel, K., C. Castelle, T. Defrance, T.F. Wild, D. Charron, V. Lotteau, and C. Rabourdin-Combe (1997). Measles virus nucleocapsid protein binds to FcgammaRII and inhibits human B cell antibody production. J Exp Med 186, 269-78.

Reed, L.J. and H. Muench (1938). A simple method of estimating fifty per cent endpoints. American J. Epidemiology 27, 493-7.

Rima, B.K. (1983). The proteins of morbilliviruses. J. General Virology 64 (Pt 6), 1205-19.

Sreenivasa, B.P., R.P. Singh, B. Mondal, P. Dhar and S. K. Bandyopadhyay (2006). Marmoset B95a cells: a sensitive system for cultivation of Peste des petits ruminants (PPR) virus. Vet Res Commun 30, 103-8.

Stevenson, P., P.B. Rossiter, L. Munga, E.K. Ndung'u and R.B. Dolan (1999). Rinderpest vaccination and the incidence and development of trypanosomosis in cattle. Tropical Animal Health and Production 31, 65-73.

Tatsuo, H., N. Ono, K. Tanaka and Y. Yanagi (2000). SLAM (CDw150) is a cellular receptor for measles virus. Nature 406, 893-7.