

***In-vitro* Immune Response of FMD Virus Serotype Asia1 Vaccine Strain (IND 491/97) to the Antibodies Raised in Bovine and Guineapigs**

Manoj Kumar, R., A. Sanyal and D. Hemadri
 Indian Veterinary Research Institute Campus, Mukteswar-Kumaon,
 Nainital-263 138, Uttaranchal, India

Abstract: In this study, the response of Foot-and-Mouth Disease serotype Asia1 vaccine strain IND 491/97 to the immune pressure [antibodies raised in Bovine against IND 63/72 and in Guineapigs against IND 63/72 and IND 491/97] exerted *in-vitro* in BHK-21 cell culture system was analyzed in terms of genetic alterations at the nucleotide level. The Nr (neutralization resistant) viruses which were selected by growing in the presence of antibodies (Bovine Vaccinate Serum, BVS and Guineapig Serum, GPS) showed certain individual and also common changes in the structural protein coding P1 region. This shows that the virus responds to the antibodies raised in both bovine (natural host) and guineapig (experimental host). The result herein further extends the role of the host antibody in the rapid evolution of FMD Virus.

Key words: FMD, FMD virus, Immune pressure, nucleotide alterations

INTRODUCTION

Foot-and-Mouth Disease (FMD) is a highly contagious disease affecting *artiodactylae*, mostly cattle, sheep, goats and several species of wild ungulates and elephants^[1] and is responsible for the most economically important viral disease of all cloven-hoofed animals. Foot-and-Mouth Disease Virus (FMDV) is the prototype member of the genus *Aphthovirus* in the family *Picornaviridae*^[2]. The virus exists in the form of seven different serotypes; O, A, C, Asia1 and South African Territories 1 (SAT1), SAT2 and SAT3 and a large number of subtypes have evolved within each serotype^[3,4].

All populations of RNA viral genomes examined to date exist not as molecules with identical sequences but as molecules exhibiting some microheterogeneity. This phenomenon, referred to as the quasispecies nature of RNA viral populations, is closely related to the error rate of the RNA polymerases, involved in replication of RNA genomes^[5,6]. This property endows RNA viruses with a high potential for viral variation and adaptation. FMDV has high mutation rate and spontaneous mutants may be readily isolated in the laboratory^[7]. Genetic and antigenic heterogeneity of FMDV populations, as well as high rates of fixation of mutation have been observed in populations derived from cloned viruses upon a limited number of acute and persistent infections in cell culture^[8]. It has been proposed that selection by antibodies or other immune mechanisms may play a major role in the rapid evolution of the virus^[8,9].

Neutralization resistant viruses can readily be obtained both *in-vivo* and *in-vitro* indicating that such a selection is a common and rapid response of FMDV to antibody pressure^[10]. Genetic and antigenic variations were reported earlier in the neutralization resistant variants of FMDV strain A24 Cruzeiro^[10], O1 Caseros^[11] and C3 Resende^[12] selected *in-vitro* in the presence of immune pressure. The present work reports the response of the neutralization resistant viruses of FMDV serotype Asia1 vaccine strain IND 491/97 selected in the presence of different Guineapig Serum (GPS) and Bovine Vaccinate Serum (BVS) and to compare their effect on the genetic characters at the nucleotide level.

MATERIALS AND METHODS

Cells, viruses and anti-sera: BHK-21 clone 13 cells and FMDV Asia1 vaccine strains IND 63/72 and IND 491/97 available at the Project Directorate on FMD were used in this study. The reference antisera against the purified and inactivated whole virus (146S) particles of IND 63/72 was raised in hill bulls^[13] and against IND 63/72 and IND 491/97 in guineapigs.

Selection of Nr viruses: Parental virus IND 491/97 (cloned P0) was serially passaged in the presence of independent serum (IND 63/72 GPS, IND 63/72 BVS and IND 491/97 GPS) starting from the highest serum dilution (i.e., the dilution which neutralize 100 TCID₅₀ of the virus). The Nr viruses resistant to different sera were selected as

Corresponding Author: R. Manoj Kumar, Department of Microbiology and Immunology, Basic Sciences Building, New York Medical College, Valhalla, NY-10595, New York, USA

reported earlier^[12]. The selective cycle was continued for several times under identical conditions (IND 491/97+IND 63/72 GPS P30; IND 491/97 + IND 63/72 BVS P20; IND 491/97 + IND 491/97 GPS P20) still it grows in the presence of undiluted serum. The Nr viruses thus selected were subjected to nucleotide sequence analysis.

RNA extraction and reverse transcription-polymerase chain reaction: RNA was extracted from the infected cell culture fluids by guanidine isothiocyanate method using Rneasy Total RNA kit (Qiagen) following the manufacturer's recommendations. The structural protein-coding region (P1) was amplified using primers L01F (5' -GTGCCCCAGTTTAAAAAGCTT,^[14]) and NK61 (5'-GACATGTCCTCCTGCATCTG,^[15]) lying on 5'UTR and 2B gene, respectively using Superscript™ One-Step RT-PCR system (Life Technologies). The thermal conditions used for amplification: 1 cycle at 48°C for 30 min, 40 cycles at 94°C for 20 s, 53°C for 30 s and 68°C for 3 min, followed by 1 cycle at 68°C for 10 min (Hybaid, UK). The amplified PCR products of ~3 kb size were identified by 1% agarose gel electrophoresis and ethidium bromide staining.

Nucleotide sequencing: Prior to sequencing the PCR products were purified using QIAquick gel extraction kit (Qiagen) following the recommendations of the suppliers. The nucleotide sequences were determined by direct sequencing of the PCR products using fmol DNA sequencing kit (Promega) using the Cy-5 labeled primers^[16]. The sequencing reaction was resolved on an ALF Express II DNA analysis system (Amersham Pharmacia Biotech). Nucleotide and amino acids sequences were aligned using CLUSTAL W algorithm^[17] available in OMIGA 2.0 package (Oxford Molecular Ltd., UK).

RESULTS AND DISCUSSION

Selection of FMDV variants by circulating antibodies in FMD infected animals is a common feature and the variants are selected upon replication in immune or partially immune hosts^[8,9]. In a country where the disease is endemic, it is expected that majority of the FMD susceptible animals will have antibodies against this virus and it is likely that the viruses circulating in this region are under immune pressure in some part of their evolutionary path^[18]. This could lead to the selection and emergence of genetic as well as antigenic variants during field outbreaks^[19-21] as well as when passaging in the experimental hosts or cell culture system.

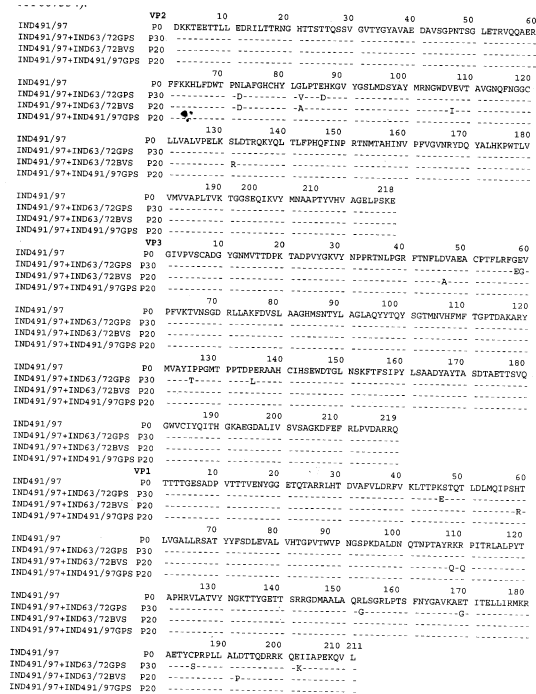


Fig. 1: Amino acid substitution in the Nr viruses of the vaccine strain IND 491/97 selected with antibodies raised both in bovine and guineapig. P0 is the consensus sequence (Gen/Bank Ac. No. AY687334)

During the process of selecting Nr viruses, after a certain number of passages under immune pressure a constant increase in the amount of undiluted serum was required to suppress the infectivity, thus suggesting that Nr viruses had been selected. To study the response to antibodies raised in Bovine and Guineapigs at the nucleotide level, the structural protein coding P1 region was sequenced for all the Nr viruses selected from the vaccine strain IND 491/97 with different GPS and BVS. The effect of FMDV serotype Asia1 vaccine strain IND 63/72 with different GPS and BVS had been compared and reported earlier^[22]. The antigenic sites and the residues involved in the neutralization of Asia1 viruses have not been studied in detail. The response of FMD virus to immunological pressure exerted *in-vitro* by antiviral polyclonal sera has been reported for the other serotypes like, O, A and C by various researchers^[10-12].

The amino acid alignment of the variants selected from IND 491/97 is shown in Fig. 1. The variants showed a total of 21 changes throughout the P1 region. The variants generated with IND 63/72 GPS showed 12 changes. These are in VP2 [N₇₂-D, G₈₂-V, E₈₆-D], VP3

[G₅₈→E, E₅₉→G, I₁₂₅→T, P₁₃₅→L] and VP1 [K₄₆→E, R₁₅₂→G, E₁₆₉→G, C₁₈₅→S, E₂₀₂→K] and the variants generated with IND 63/72 BVS showed 9 changes and they are at residues, VP2 [N₇₂→D, G₈₂→A, V₁₀₇→I, S₁₃₁→R], VP3 [D₅₆→A] and VP1 [H₅₉→R, R₁₀₈→Q, R₁₁₀→Q, L₁₉₂→P]. The virus showed no characteristic change when passaged in the presence of its homologous GPS. Hence the passage was continued further in the presence of IND 491/97 GPS up to 30 passages and at this level the residue 72 of VP2 protein was found to be changed with another residue.

A constant change at residue 72 of VP2 protein of IND 491/97 variants may be critical for the property of their increased resistance to neutralization. The area of VP2 protein consisting of residues 70 to 100 is highly variable and some of the residues of this region were predicted to lie adjacent to the major antigenic loop of VP1^[23]. This region is conformation dependent and some of the critical residues play important role in the biological properties and survival of the variants^[24,25].

The same residue 72 of VP2 protein is also being substituted by S instead of N in the variants of another vaccine strain IND 63/72^[22]. The residue 58 and 59 of VP3 protein is highly variable and they are said to be under positive selection^[18]. The VP1 protein of IND 491/97 variants showed maximum changes at its carboxy terminal region as compared to IND 63/72 variants. They also showed changes at the residues 46 and 59 (40-60 region) of VP1 protein when passaged in the presence of IND 63/72 GPS and IND 63/72. Some of the other changes observed in IND 63/72 variants^[22] are not observed in IND 491/97, which may be said as their strain characters.

CONCLUSION

The results obtained suggested that the whole structural protein coding P1 region of the vaccine strain participated in response to the *in-vitro* exerted immunological pressure by the antibodies raised in both the bovine and guinea pig. Changes were observed in some of the critical residues like VP2 (72), VP3 (58 and 59) and VP1 (46 and 59) whereas the other changes are different between the variants selected with GPS and BVS in comparison to the same study conducted with another vaccine strain IND 63/72 indicates the strain differences which may be necessary for their evolution and to resist against the antibodies circulating in the animal host system which are produced against some other strain during the previous exposure to FMD virus.

ACKNOWLEDGEMENT

We are thankful to Indian Council of Agricultural Research, New Delhi for providing necessary facilities to

carry out this work. R.M.K also thanks IVRI for providing financial assistance in the form of fellowship during this study.

REFERENCES

1. Brooksby, J.B., 1982. Portraits of viruses: Foot-and-mouth disease virus. *Intervirology*, 18: 1-10.
2. Racaniello, V.R., 2001. Picomaviridae: the viruses and their Replication. In: Knipe DM, Howley PM, 4th Edn., *Fields virology*, Lippincott Williams and Wilkins, Philadelphia, 1: 685-722.
3. Domingo, E., C. Escarmis, E. Baranowski, C.M. Ruiz-Jarabo, E. Carrillo, J.I. Nunez and F. Sobrino, 2003. Evolution of foot-and-mouth disease virus. *Virus Res.*, 91: 47-63.
4. Knowles, N.J. and A.R. Samuel, 2003. Molecular epidemiology of foot-and-mouth disease virus. *Virus Res.*, 91: 65-80.
5. Domingo, E., E. Martinez-salas, F. Sorbino, J.C. De La Torre, A. Prtela and J. Ortin *et al.*, 1985. The quasispecies (extremely heterogeneous) nature of viral RNA populations: Biological relevance- *A Rev. Gene.*, 40: 1-8.
6. Steinhauer, D.A. and J.J. Holland, 1987. Rapid evolution of RNA viruses. *Annu. Rev. Microbiol.*, 41: 409-433.
7. McCahon, D., W.R. Slade, R.A.J. Priston and J.R. Lake, 1977. An extended genetic recombination map of foot-and-mouth disease virus. *J. Gen. Virol.*, 35: 555-565.
8. Domingo, E., M. Davila and J. Ortin, 1980. Nucleotide sequence heterogeneity of the RNA from a natural population of foot-and-mouth disease virus. *Gene.*, 11: 333-346.
9. Sobrino, F., M. Davila, J. Ortin and E. Domingo, 1983. Multiple genetic variants arise in the course of replication of foot-and-mouth disease virus in cell culture. *Virology*, 128: 310-318.
10. Carrillo, E.C., E. Rieder Rojas, L. Cavallaro, M. Schiappacassi and R. Campos, 1989. Modification of foot-and-mouth disease virus after serial passages in the presence of antiviral polyclonal sera. *Virology*, 171: 599-601.
11. Rieder Rojas, E., E.C. Carrillo, M. Schiappacassi and R. Campos, 1992. Modification of foot-and-mouth disease virus O1 Caseros after serial passages in the presence of antiviral polyclonal sera. *J. Virol.*, 66: 3368-3372.

12. Schiappacassi, M., E. Rieder Rojas, E. Carrillo and R. Campos, 1995. Response of foot-and-mouth disease virus C3 Resende to immunological pressure exerted *in-vitro* by antiviral polyclonal sera. *Virus Res.*, 36: 77-85.
13. Sanyal, A., C.B. Gurumurthy, R. Venkataramanan, D. Hemadri and C. Tosh, 2003. Antigenic characterization of foot-and-mouth disease virus serotype Asia1 field isolates using polyclonal and monoclonal antibodies. *Vet. Microbiol.*, 93: 1-11.
14. Roberts, P.J. and G.J. Belsham, 1995. Identification of critical amino acids within the foot-and-mouth disease virus leader protein a cysteine protease. *Virology*, 213: 140-146.
15. Knowles, N.J. and A.R. Samuel, 1995. Polymerase chain reaction amplification and cycle sequencing of the 1D (VP1) gene of foot-and-mouth disease virus. Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Vienna, Austria, Rome: FAO, pp: 45-53.
16. Mohapatra, J.K., A. Sanyal, D. Hemadri, C. Tosh, G.P. Sabarinath and R. Venkataramanan, 2002. Sequence and phylogenetic analysis of the L and VP1 genes of foot-and-mouth disease virus serotype Asia1. *Virus Res.*, 87: 107-118.
17. Thompson, J.D., Higgins, D.G. and T.J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22: 4673-4680.
18. Tosh, C., D. Hemadri, A. Sanyal and S.K. Bandyopadhyay, 2003. Genetic and antigenic analysis of two recently circulating genotypes of type A foot-and-mouth disease virus in India: evidence for positive selection in the capsid-coding genes. *Arch. Virol.*, 148: 853-869.
19. Martinez, M.A., C. Carrillo, J. Palma, R. Mascarella, J. Bergada, E. Palma, E. Domingo and F. Sobrino, 1988. Genetic and immunogenic variations among closely related isolates of foot-and-mouth disease virus. *Gene.*, 62: 75-84.
20. Mateu, M.G., E. Rocha, O. Vicente, F. Vayreda, C. Navalpotro and A. Andreu *et al.*, 1988. Reactivity with monoclonal antibodies of viruses from an episode of foot-and-mouth disease. *Virus Res.*, 8: 261-274.
21. Mateu, M.G., M.A. Martinez, L. Capucci, D. Andrew, E. Giralt and F. Sorbino *et al.*, 1990. A single amino acid substitution affects multiple overlapping epitopes in the major antigenic site of foot-and-mouth disease virus serotype C. *J. Gen. Virol.*, 71: 629-637.
22. Manoj Kumar, R., A. Sanyal and D. Hemadri, 2006. Effect of antibodies raised in bovine and guinea pigs on the genetic characters of serotype Asia1 FMD virus. *J. Anim. Vet. Adv.*, 5: 294-297.
23. Martinez, M.A., J. Dopazo, J. Hernandez, M.G. Mateu, F. Sorbino, E. Domingo and N.J. Knowles, 1992. Evolution of the capsid protein genes of foot-and-mouth disease virus. Antigenic variation without accumulation of amino acid substitutions over six decades. *J. Virol.*, 66: 3557-3565.
24. Acharya, R., E. Fry, D. Stuart, G. Fox, D. Rowlands and F. Brown, 1989. The three dimensional structure of foot-and-mouth disease virus at 2.9 Å resolution. *Nature*, 337: 709-716.
25. Bolwell, C., B.E. Clarke, N.R. Parry, E.J. Ouldrige, F. Brown and D.J. Rowlands, 1989. Epitope mapping of foot-and-mouth disease virus with neutralizing monoclonal antibodies. *J. Gen. Virol.*, 70: 59-68.