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

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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 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 Cruziero[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 $TCID_{50}$ of the virus). The Nr viruses resistant to different sera were selected as

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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 isothiocynate 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.

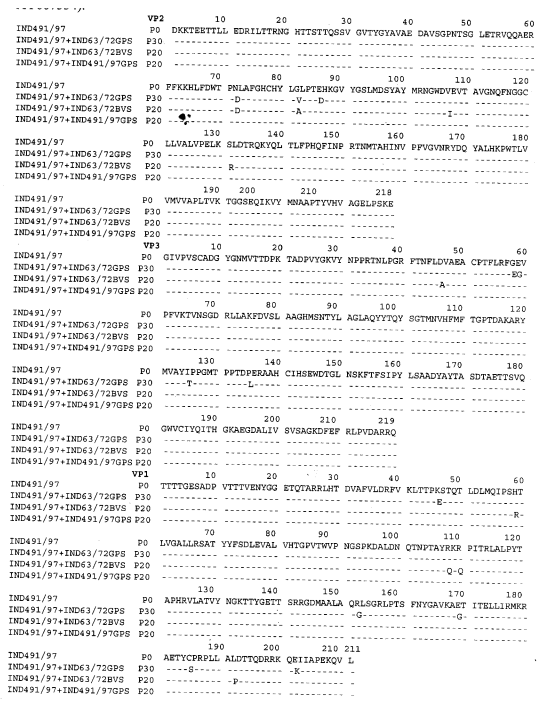

Fig. 1: Amino acid substitution in the Nr viruses of the vaccine strain IND 491/97 selected with antibodies raised both in bovine and guineapig. P0 is the consensus sequence (Gen/Bank Ac. No. AY687334)

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 [N_{72}→D, G_{82}→V, E_{86}→D], VP3

[G_{58}→E, E_{59}→G, I_{125}→T, P_{135}→L] and VP1 [K_{46}→E, R_{152}→G, E_{169}→G, C_{185}→S, E_{202}→K] and the variants generated with IND 63/72 BVS showed 9 changes and they are at residues, VP2 [N_{72}→D, G_{82}→A, V_{107}→I, S_{131}→R], VP3 [D_{56}→A] and VP1 [H_{59}→R, R_{108}→Q, R_{110}→Q, L_{192}→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 been 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 guineapig. 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.

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