Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.016

SHORT COMMUNICATION

Integrins and Heparan Sulfate Play Crucial Role in Pathogenesis of Foot-and-Mouth Disease Virus

Muhammad Nauman Zahid[1*], Muhammad Akbar Shahid[2*], Hafiz Muhammad Imran[3], Muhammad Oneeb[3], Mushtaq Ahmed[4], Zia Ur Rehman[5], Nida Arooj[6] and Tahir Yaqub[3]

[1]Department of Biology, College of Science, University of Bahrain, Kingdom of Bahrain; [2]Department of Pathobiology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan; [3]University of Veterinary and Animal Sciences, Lahore, Pakistan; [4]Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan; [5]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan; [6]Provincial Disease Diagnostic Laboratory, Lahore, Pakistan
*Corresponding author: makbar@bzu.edu.pk; nzahid@uob.edu.bh

ARTICLE HISTORY (19-547)

Received: December 02, 2019
Revised: January 09, 2020
Accepted: January 15, 2020
Published online: February 08, 2020

Key words:
Anti-receptor antibodies
FMDV
Heparan sulfate
αvβ1
αvβ3
αvβ6

ABSTRACT

Foot-and-mouth disease virus (FMDV) is one of highly contagious pathogens of cloven-footed animals, attaches with host cell surface receptors, including the integrins and heparan sulfate, for entry into the epithelial cells. FMDV is endemic in Pakistan, but limited studies are available on the interaction of local FMDV serotypes with host-entry factors. Here, we used anti-receptor antibodies and studied the interaction of Pakistani serotypes of FMDV serotypes (A, O and Asia-1) with integrins and heparan sulfate. We observed that by blocking integrins αvβ1, αvβ3 and αvβ6 as well as heparan sulfate by specific monoclonal antibodies, FMDV infection of serotype A, O and Asia-1 was prevented. In conclusion, anti-receptor antibodies are critically important to inhibit FMDV infection.

To Cite This Article: Zahid MN, Shahid MA, Imran HM, Oneeb M, Ahmed M, Rehman ZU, Arooj N and Yaqub T, 2020. Integrins and heparan sulfate play crucial role in pathogenesis of foot-and-mouth disease virus. Pak Vet J, 40(3): 403-406. http://dx.doi.org/10.29261/pakvetj/2020.016

INTRODUCTION

Foot-and-mouth disease virus (FMDV), belonging to *Aphthovirus* genus and *Picornaviridae* family, is a single stranded RNA virus that causes highly contagious, devastating and acute disease of cloven-hoofed animals (Basagoudanavar *et al*., 2019). FMD is endemic in Asia, Africa, Middle East and South America. There are seven FMDV serotypes (A, O, C, Asia-I, SAT1, SAT2 and SAT3), with many subtypes with each serotype. FMDV is a non-enveloped, single-stranded, positive sense 8.4 kb RNA genome which encodes four structural proteins (VP1, VP2, VP3 and VP4) and eight non-structural proteins. VP1-VP3 makes the outer capsid shell, while VP4 forms the internal surface. VP1 contains an outer flexible loop called G-H-loop (major antigenic site) and an Arg-Gly-Asp (RGD) motif, which is important in host receptor binding (Chamberlain *et al*., 2015). The virus exhibits tropism for epithelial cells of the upper respiratory tract. FMDV initially attaches to the hosts' cell surface receptor(s) and subsequently enters the cell(s) by receptor-mediated endocytosis. Many receptors, including integrins and heparan sulfate proteoglycans (HSPGs), have been reported for FMDV. The virus enters into the cells after binding to the integrins via clathrin-mediated endocytosis or by binding to HSPGs via caveola-mediated endocytosis (Wang *et al*., 2015).

Foot-and-Mouth Disease (FMD) is endemic in Pakistan, with an estimated seroprevalence of 47.1% and causes heavy fiscal losses to livestock industry due to poor production performance, ban on the export of livestock and livestock products to the international market, high morbidity and mortality (Nawaz *et al*., 2019; Sajid *et al*., 2019). Limited studies are available on the interaction of host-cell entry factors with FMDV. Current study was conducted, for the first time in Pakistan, to determine the role of integrins and heparan sulfate receptors in FMDV infection using local FMDV serotypes prevalent in Pakistan.

MATERIALS AND METHODS

Virus and cells: Baby Hamster kidney (BHK) cell line was used in this study as BHK cells express integrins and heparan sulfate on their cell surface to facilitate FMDV binding and entry. BHL cells were procured from the cell

culture collection of Quality Operation Laboratory (QOL), University of Veterinary and Animal Sciences (UVAS), Lahore-Pakistan. BHK cells were grown in Dulbeco Modified Eagle Medium (DMEM) (Invitrogen, UK) supplemented with 10% fetal bovine serum (FBS), penicillin 100 U/mL, streptomycin 100 µg/mL, Amphotericin B 2.5µg/mL, HEPES 10mM, and glutamine 2mM. The local isolates of FMDV serotypes A, O and Asia-1 were obtained from culture bank of QOL, UVAS, Lahore-Pakistan. Biological titration of the locally isolated FMDV serotypes was performed as tissue culture infective dose$_{50}$ ($TCID_{50}$) as described by Reed and Muench.

Anti-receptor antibodies: Antibodies against heparan sulfate, integrin αvβ3 and αvβ6 (Millipore, USA) and anti-αvβ1antibody (Chemicon International, USA) were diluted (1:10) in DMEM and used at final concentration of 10µg /mL. Anti-CD81 (Chemicon International, USA) was used as a negative control antibody at a similar concentration.

Experimental design: Three different experiments were performed to study the role of heparin sulfate and integrins, using one FMDV serotype infections in each experiment. BHK cells were grown at density of 2.5×10^4 cells/well in 48-well plates in DMEM by incubating at 37°C by incubating under 5%CO_2 for 24 hours. BHK cells were pre-incubated 1ith 100 µL of anti-αvβ3, anti-αvβ1, anti-αvβ6, and anti-heparan sulfate antibodies (10 µg/mL), in separate wells, for 1 hour at 37°C under 5% CO_2, then FMDV serotype (either A, O, or Asia-1) was added to the cells at multiplicity of infection (MOI) of 10 $TCID_{50}$/cell and incubated for another 4 hours at 37°C. Then, media containing unbound virus and antibodies was removed and replaced with fresh medium. To serve as positive control in each experiment, BHK cells were infected, without pre-treatment with anti-receptor antibodies, with one FMDV serotype (either A, O, or Asia-1) at MOI of 10 $TCID_{50}$/cell for 4 hours under 5% CO_2 at 37°C. For negative control, BHK cells were incubated with anti-CD81 for 1 hour at 37°C, followed by infection with FMDV (either A, O, Asia-1) at MOI of 10 $TCID_{50}$/cell. Cytopathic effects (CPEs) were observed after 24 hours under an inverted microscope. In each experiment, triplicate wells of BHK cells were used for each condition.

RESULTS AND DISCUSSION

Normal BHK cells were observed as shown in Figs. 1A, 2A, and 3A. CPEs When BHK cells were incubated with FMDV serotype A, O and Asia-1, CPEs were observed, showing complete destruction of the monolayers (Figs. 1B, 2B, and 3B). As BHK cell line is an adherent cell line, dead cells were floating while viable cells were strictly attached as observed under an inverted microscope. Anti-CD81, used as a negative control, did not inhibit any FMDV serotype from infecting the BHK cells (Figs. 1C, 2C and 3C). It was evident that this receptor has no role in FMDV infection. Interestingly, when BHK cells were pre-incubated with anti-αvβ3 (Figures 1D, 2D and 3D), anti-αvβ1 (Figures 1E, 2E and 3E), anti-αvβ6 (Figs. 1F, 2F and 3F) and anti-heparin-sulfate (Figs. 1G, 2G and 3G) antibodies, and then infected with either FMDV serotype A (Figs. 1D-1G), O (Figs. 2D-2G) or Asia-I (Figs. 3D-3G), no CPEs were observed after 24 hours of infection. It is also obvious from percentages of live and dead cells (Table 1-3). These results suggested that integrin αvβ3, αvβ1, αvβ6, and heparan sulfate play significant role in establishing FMDV infection.

Prevalence of FMDV is one of the major constraints in exporting livestock and livestock products from developing countries including Pakistan (Sajid *et al*., 2019). Therefore, development of new approaches, e.g., using artificial microRNAs (amiRNAs) to inhibit FMDV replication, are necessary to control and eliminate FMDV (Basagoudanavar *et al*., 2019). The findings of current study, using anti-receptor antibodies against Pakistani FMDV serotypes, are analogous to previous studies on integrin αvβ3 and αvβ1. It has been shown that integrin αvβ3 binds to all serotypes of FMDV (Berinstein *et al*., 1995). This finding is important in understanding that blocking only one receptor is sufficient to inhibit infection. The specificity of αvβ1, αvβ3, and αvβ6 for FMDV infection has been studied for three strains of FMDV serotype A and two strains of FMDV serotype O (Jackson *et al*., 2004). However, in current study, we have

Table 1: Inhibition of FMDV serotype A by anti-receptor antibodies

Conditions	Live cells (%)	Dead cells (%)
BHK cells + DMEM	100	0
BHK cells + FMDV serotype A*	0.66	99.33
BHK cells pre-incubated with anti-αvβ3 + FMDV serotype A	98.66	1.33
BHK cells pre-incubated with anti-αvβ1 + FMDV serotype A	97.66	2.33
BHK cells pre-incubated with anti-αvβ6 + FMDV serotype A	98.66	1.33
BHK cells pre-incubated with anti-heparan sulfate + FMDV serotype A	97.33	2.66

*Mean.

Table 2: Inhibition of FMDV serotype O by anti-receptor antibodies.

Conditions	Live cells (%)	Dead cells (%)
BHK cells + DMEM	100	0
BHK cells + FMDV serotype O*	0.33	99.66
BHK cells pre-incubated with anti-αvβ3 + FMDV serotype O	97.66	2.33
BHK cells pre-incubated with anti-αvβ1 + FMDV serotype O	98.33	1.66
BHK cells pre-incubated with anti-αvβ6 + FMDV serotype O	99.66	0.33
BHK cells pre-incubated with anti-heparan sulfate + FMDV serotype O	97.66	2.33

*Mean

Table 3: Inhibition of FMDV serotype Asia-1 by anti-receptor antibodies

Conditions	Live cells (%)	Dead cells (%)
BHK cells + DMEM	100	0
BHK cells + FMDV serotype Asia-1 *	0.66	99.33
BHK cells pre-incubated with anti-αvβ3 + FMDV serotype Asia-1	98.66	1.33
BHK cells pre-incubated with anti-αvβ1 + FMDV serotype Asia-1	97.33	2.66
BHK cells pre-incubated with anti-αvβ6 + FMDV serotype Asia-1	99	1
BHK cells pre-incubated with anti-heparan sulfate + FMDV serotype Asia-1	98.66	1.33

*Mean.

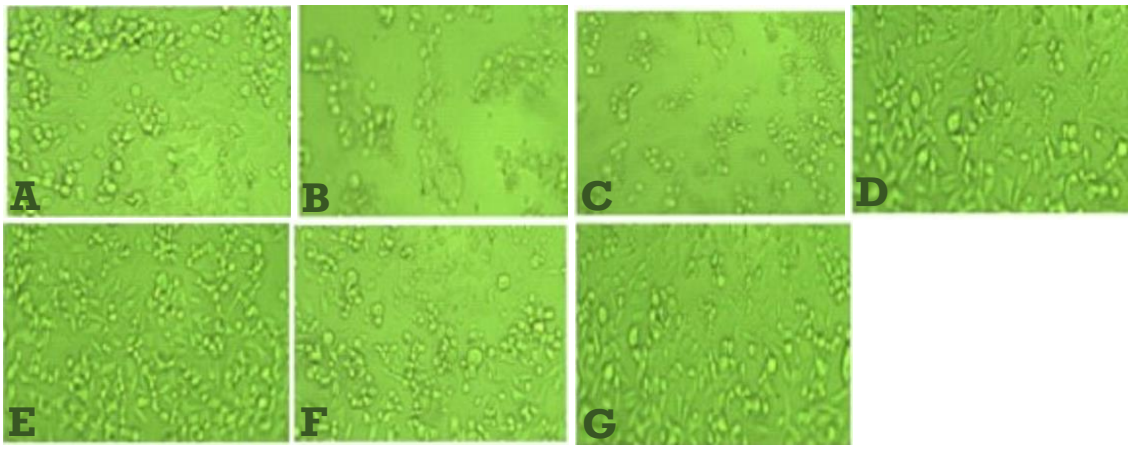

Fig. 1: Inhibition of FMDV serotype A by anti-receptor antibodies. (A) BHK cells with DMEM. (B) BHK cells with FMDV serotype A. (C) BHK cells pre-incubated with anti-CD81, followed by FMDV serotype A infection. BHK cells pre-incubated with anti-αvβ3 (D), anti-αvβ1 (E), anti-αvβ6 (F) and anti-heparan sulfate (G), followed by FMDV serotype A infection (Magnification ×200).

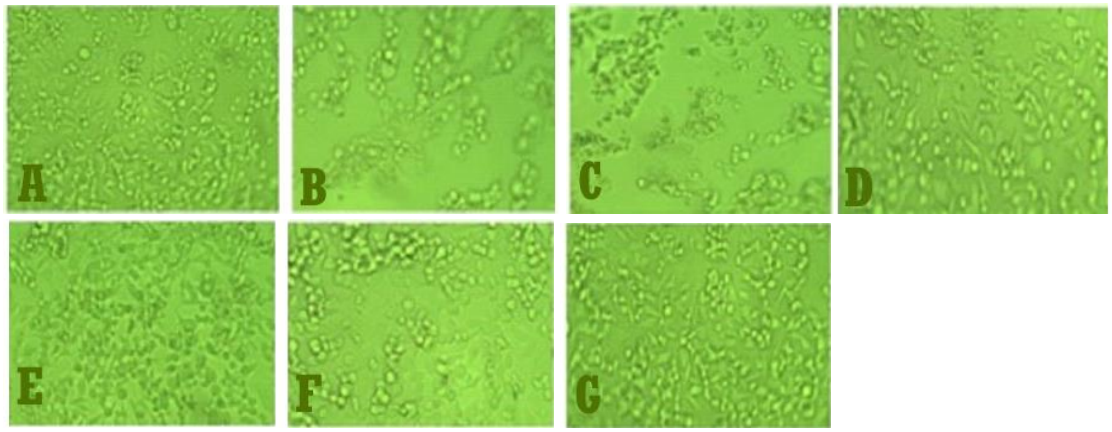

Fig. 2: Inhibition of FMDV serotype O by anti-receptor antibodies**.** (A) BHK cells with DMEM. (B) BHK cells with FMDV serotype O. (C) BHK cells pre-incubated with anti-CD81, followed by FMDV serotype O. BHK cells pre-incubated with anti-αvβ3 (D), anti-αvβ1 (E), anti-αvβ6 (F) and anti-heparan sulfate (G), followed by FMDV serotype O infection (Magnification ×200).

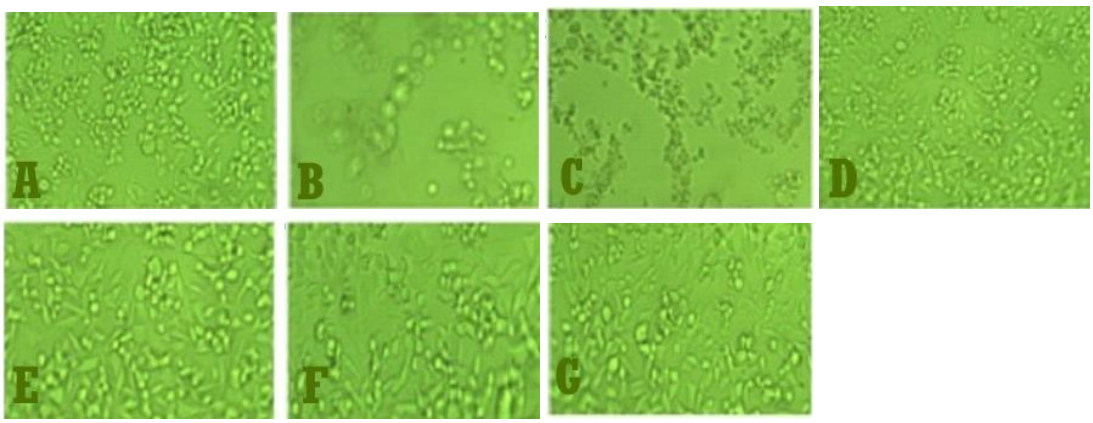

Fig. 3: Inhibition of FMDV serotype Asia-1 by anti-receptor antibodies**.** (A) BHK cells with DMEM. (B) BHK cells with FMDV serotype Asia-1 only. (C) BHK cells pre-incubated with anti-CD81, followed by FMDV serotype Asia-1 infection. BHK cells pre-incubated with anti-αvβ3 (D), anti-αvβ1(E), anti-αvβ6 (F) and anti-heparan sulfate (G), followed by FMDV serotype O infection (Magnification ×200).

studied the specificity of these integrins for FMDV serotype Asia-1 as well along with FMDV serotype O and A. Differential binding efficiencies of αvβ1and αvβ6, on the basis of amino acid residues, have also been studied, and shown that αvβ6 has more diverse expression on epithelial cells, especially at early stages of virus replication (Jackson *et al*., 2004). It has already been reported that αvβ6 plays a role in transporting the virus to early endosomes, and that αvβ6 is mainly expressed on epithelial cells that are site of FMDV replication (Monaghan *et al*., 2005). Fry and colleagues have shown that FMDV binding to heparan sulfate could increase integrin-mediated viral entry (Fry *et al*., 1999). Another study has described that attachment of FMDV to heparan sulfate can result in caveolae-mediated endocytosis of FMDV (O'Donnell *et al*., 2008). Remarkably, heparin sulfate also plays a critical role in binding Hepatitis C virus as well. As receptors are crucial for viral tropism

and pathogenesis, we have established the role of integrins and heparan sulfate receptors in the pathogenesis of Pakistan-origin FMDV serotypes.

Conclusions: FMDV interacts with different host cell factors at different phases of pathogenesis. This appears to be first report on crucial role of integrins and heparins in Pakistani FMDV pathogenesis.

Acknowledgments: This project was funded by Higher Education Commission (HEC) of Pakistan (PD-IPFP/HRD/HEC/2013/3025).

Authors contribution: MNZ, MAS, HMI, ZUR conceived and designed the experiments. MNZ, MAS, MA, and HMI performed and reviewed the experiments. MNZ, MAS, HMI, MO, NA, TY reviewed the results and wrote the manuscript.

REFERENCES

Basagoudanavar SH, Ranjitha HB, Hosamani M, *et al*., 2019. Efficient inhibition of foot-and-mouth disease virus replication in vitro by artificial microRNA targeting 3D polymerase. Acta Virol 63:475-9.

Berinstein A, Roivainen M, Hovi T, *et al*., 1995. Antibodies to the vitronectin receptor (integrin alpha V beta 3) inhibit binding and infection of foot-and-mouth disease virus to cultured cells. J Virol 69:2664-6.

Chamberlain K, Fowler VL, Barnett PV, *et al*., 2015. Identification of a novel cell culture adaptation site on the capsid of foot-and-mouth disease virus. J Gen Virol 96:2684-92.

Fry EE, Lea SM, Jackson T, *et al*., 1999. The structure and function of a foot-and-mouth disease virus-oligosaccharide receptor complex. EMBO J 18:543-54.

Jackson T, Clark S, Berryman S, *et al*., 2004. Integrin alphavbeta8 functions as a receptor for foot-and-mouth disease virus: role of the beta-chain cytodomain in integrin-mediated infection. J Virol 78:4533-40.

Monaghan P, Gold S, Simpson J, *et al*., 2005. The alpha(v)beta6 integrin receptor for Foot-and-mouth disease virus is expressed constitutively on the epithelial cells targeted in cattle. J Gen Virol 86:2769-80.

Nawaz Z, Siddique AB, Zahoor MA, *et al*., 2019. Detection of Foot and Mouth Disease virus shedding in milk of apparently healthy buffaloes and cattle of Punjab, Pakistan. Buffalo Bull 38:255-61.

O'Donnell V, Larocco M and Baxt B, 2008. Heparan sulfate-binding foot-and-mouth disease virus enters cells via caveola-mediated endocytosis. J Virol 82:9075-85.

Sajid S, Rehman SU, Sehrish N, *et al*., 2019. Emergence, existence and distribution of foot and mouth disease in Pakistan in comparison with the global perspective. GSC Biol Pharmac Sci 07:102-10.

Wang G, Wang Y, Shang Y, *et al*., 2015. How foot-and-mouth disease virus receptor mediates foot-and-mouth disease virus infection. Virol J 12:9.