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Review Article

Antiviral Immunity Evoked Post Foot-and-Mouth Disease Virus (FMDV) Infection and Vaccination

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ABSTRACT

Foot-and-mouth disease (FMD) is an important transboundary disease of domestic and wild cloven hoofed animals. Both, innate and adaptive immunity play an important role in combating FMDV infection. Interferons, CD4+ helper cells and CD8+ cells are the key factors in developing anti-FMDV immunity inside host's body. In this review a detail of FMDV pathogenesis and anti-FMDV immunity has been discussed in detail.

INTRODUCTION

Foot-and-Mouth Disease (FMD) is a highly contagious and economically important disease of domestic and wild cloven hoofed animals. The disease is caused by foot-and-mouth disease virus (FMDV) which belongs to genus Aphthovirus of the family Picornaviridae [1].

The mechanism of spread of FMDV is primarily in the form of aerosolised droplets, saliva or through indirect contact by personnel or contaminated surfaces [2]. However, infection can also happen *via* skin or mucous membranes, but these are inefficient means of entry, unless abrasions or cuts are present [3,4]. Cattle sheep and goats can exhale up to 5.2 log 10 TCID50 virus/day and pigs up to 8.6 log 10 TCID50 virus/day which shows that infected pigs are bigger source of infection than infected ruminants [2,5]. In comparison to pigs, ruminants excrete less number of viruses in breath but are more susceptible than pigs to infection through respiratory route. Ruminants can be infected experimentally by airborne exposure of 10 TCID50 whereas pigs get infected by 103 TCID50 viruses [5,6]. However, the infective experimental oral doses for pigs are 104-105 TCID50 viruses and for ruminants 105-106TCID50 viruses [7] which shows that they are relatively insensitive to experimental infection by oral route. The incubation period for FMDV is from 2 to 14 days.

FMDV enters cells through binding to surface receptors of host cells. The major receptors for FMDV are integrins and heparan sulphate (HS) proteoglycans [8]. The integrin binding is mediated by a highly conserved arginine-glycine aspartic acid (RGD) motif located in GH-loop of VP1. The principal receptor used by field strains of FMDV to initiate infection is αvβ6 integrin which is due to its epithelial cell restricted expression [9-12]. The other 3 integrins recognized as receptors for field strains of FMDV are αvβ 1, αvβ 3 and αvβ 8 [13-15]. The role of these 3 integrins in pathogenesis is not clear and αvβ3 has been found as a poor receptor for FMDV [16]. However, cell culture adapted viruses often use heparansulphate (HS) as receptors and can initiate infection *via* an integrin-independent process [8,17-19].

After binding to the receptor, virus is endocytosed by clathrin dependent endocytosis into endosomes and then acidic pH of endosomes dissociates the capsid to release viral RNA [20]. The hydrophobic regions of capsid fuse with the endosomal lipid bilayer leading to a pore formation and then subsequently release of viral RNA into cytoplasm [21]. The viral RNA is later on translated into viral structural and non-structural proteins [22]. After FMDV infection, primary replication occurs in the mucosa-associated lymphoid tissue (MALT) of the nasopharynx and thereafter in the pulmonary alveolar septa. As viraemia approaches in 24-48 hours [23], the replication increases in the lungs and decreases in the nasopharynx [9,10,23]. Then lesions appear in the mouth and feet of susceptible animals. The viraemic phase is defined by vesiculation and erosion of epithelia of mouth, feet, teats, prepuce and ruminal pillars [24,25]. It has been shown that

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cattle are non-infectious until 0.5 days after appearance of clinical signs and the infectious period is usually shorter (1.7 days) [26]. After clearance of the virus from lesion sites, some ruminants may develop chronic asymptomatic infection called the carrier state [5,27].

Animals from which live FMDV can be recovered after 28 days of infection are referred as carriers [28,29]. Carriers have been recorded in cattle [30], African buffalo [31], sheep [32], and goats but not in pigs [33]. Carriers have been recognized for all serotypes of FMDV and in both experimentally and naturally infected animals [33]. The maximum duration observed for the carrier state in African buffalo, cattle, sheep and goats is 5 years, 3.5 years, 9 months and 4 months, respectively [3,34]. 45%-50% of FMD-infected ruminants may turn into virus carriers. Although pigs can clear infection and do not turn into carriers [3], however, in some studies live virus was been isolated till 14 days of infection from tissues like tonsil, spleen, thymus and lymphnodes [35]. FMDV RNA was detected in the nasopharynx [36], dorsal palate [36] and tonsils [37] of FMDV infected pigs up to 33 days, 48 days and 36 days post infection, respectively.

Apart from oropharynx, FMDV may persist in other organs like mammary gland, testicles, pituitary, pancreas and thyroid [38-42]. Tonsil is the prominent site of persistence of FMDV in sheep [43,44].

FMDV structural proteins have been located till 38 days post infection in the germinal centres of lymphoid tissue of cattle [45] and these FMDV particles located in the lymphoid tissue could be a possible source of infectious material encountered during probang sampling of infected cattle [45].

There is no experimental evidence of virus transmission from carrier cattle or sheep to uninfected animals [46]. The only evidence available for virus transmission from a carrier to a susceptible animal is from African buffalo to cattle during the outbreaks in Zimbabwe in 1989 and 1991 [47]. Furthermore, it has been reported that FMD transmission can occur sexually from infected buffalo harbouring virus in oesophageal-pharyngeal (OP) fluids to uninfected cattle [48].

IMMUNE RESPONSE

Immune defense against FMDV has been related to circulating antibody titres and humoral antibody responses are the most important factor which protects animal against FMD [49-51]. The antibodies are produced by B lymphocytes by their interaction with Th lymphocytes. Hence, both humoral and cell mediated immunity play important role in providing protection. In addition, the innate immune response also protect animals during early FMDV infection [52].

INNATE IMMUNE RESPONSE

The main components of the innate immune system are macrophages and dendritic cells. Macrophages play an important role in early phases of FMDV infection. Macrophages infected with FMDV have been observed to play role in acute infection [53] acting as infectious carriers and disseminating virus to other parts of body. Infection in these virus infected macrophages can be cleared within 10-14 hours *in vitro*. Macrophages are considered to play role in immune responses through opsonisation and subsequently destruction of virus.

Natural killer (NK) cells obtained from FMDV restimulated bovine PBMC (peripheral blood mononuclear cells) of vaccinated cattle, have been shown to be cytotoxic against FMDV-infected target cells [54]. In addition, γ δ T-cells have been shown to proliferate and produce cytokines in response to FMDV antigen [55].

Dendritic cells (DCs) are the major antigen presenting cells in mammals and plasmacytoid DCs (pDCs) produce significant amounts of IFN-α early after infection [56]. Studies in humans and mice have demonstrated that DCs have roles in linking innate and adaptive immune responses [57]. Various subsets of porcine dendritic cells including plasmacytoid dendritic cells (PDC), monocyte-derived dendritic cells and bone marrow-derived dendritic cells have shown to be susceptible to infection [58].

FMDV is highly sensitive to type 1 IFNs *in vitro* [59] and FMDV Leader proteinase (Lpro) and 3C block the expression of type 1 IFN *in vitro* [60-62]. This blocking of interferon may help the virus to replicate and disseminate effectively [63]. It has been demonstrated that FMDV replication in cell culture is inhibited by IFN-α and delivery of IFN-α protects swine from subsequent FMDV challenge [59,64]. IFN-α and/or IFN-β mRNA production after experimental infection has been reported in skin [65], nasal associated lymphoid tissue [66], mononuclear cells of lung [67], and epithelial cells of bovine tongue, coronary band and dorsal soft palate [66]. Recently, Segundo et al., reported that Ad5-boIFN-λ 3 induced systemic antiviral activity and boIFN-λ3 has potential as a biotherapeutic candidate to inhibit FMDV or other viruses in cattle.

ADAPTIVE IMMUNE RESPONSE

Adaptive (acquired) immunity includes both humoral and cell mediated immunity (CMI). It involves antigen presenting cells (APCs) such as macrophages and dendritic cells, the activation and proliferation of antigen specific B cells and T cells and the production of antibody molecules, cytotoxic T lymphocytes (CTLs) and cytokines.

Humoral immunity

There is a strong correlation between the circulating humoral antibody titre against FMDV and protection against the virus [51,68]. The first neutralising antibody is IgM, appearing after 3-4 days of infection/vaccination, peaking after 10-14 days and thereafter declining [69,70]. IgG appears 4-7 days post infection/vaccination, and becomes the major neutralising antibody after 2 weeks [71,72]. The titre of IgG1 is reported to be higher than IgG2 [34]. IgM followed by IgA and IgG are the major antibody subclasses found in the upper respiratory tract [34]. IgM and IgA mediated neutralising activity has been observed in the pharyngeal fluid after 7 days of virus exposure [73]. However the presence of IgA antibody in early stage of infection was considered to be the leakage of tissue fluid and serum whereas presence of IgA antibody in the pharyngeal fluid after 20 days of infection was reported to be synthesized at mucosal surface rather than serum transudation. Salt et al. [72] considered the possibility of mucosal IgA detection in the oropharyngeal fluids of persistently FMDV infected animals and later on developed an IgA assay to detect the persistently infected (carrier) cattle and demonstrated that IgA is the indicator of oropharyngeal replication of FMDV.

The production of antibody after FMDV infection has been demonstrated to be T cell independent in both mice [74] and

cattle [74,75]. The induction of IgG after FMDV immunization has been shown to be T-cell-dependent in a murine experimental model [71,76]. However, T cells have been shown to play a role in induction of antibody responses in ruminants which is demonstrated by FMDV specific CD4 T-cell proliferation after infection/vaccination [77-79]. The NSPs of FMDV have many T-cell epitopes [77,79] which might elicit prolonged immune response in infected animals [80]. The presence of non-replicating FMDV antigen in the light zone of the germinal centre of mandibular lymph node may elicit long-term antibody response in infected animals [45]. Pega et al. [81] demonstrated a rapid local antibody response on aerosol exposure to FMDV, where all the infected animals developed antibody secreting cells after 4 days of infection in all the lymphoid organs along the respiratory tract. Recently the role of CD4+ cells in induction of neutralizing antibodies against FMDV infection and isotype switching has been demonstrated by Carr et al. [82]. They found that after depletion of CD4+ cells in vaccinated animals there was significant decrease in the titre of neutralizing antibodies and delay in the isotype switching from IgM to IgG suggesting the possible role of CD4+ in induction of humoral immunity against FMDV.

Cell-mediated immunity

The cellular immune response is evoked by CD8+ cytotoxic T-cells and CD4+ helper T-cells [83]. The Th1 secrete interferon gamma (IFNγ), IL-2 and tumour necrosis factor beta (TNFβ) which stimulates phagocytosis to remove the intracellular microbes [84]. It has been reported in mice that Th1 cells promote an IgG2 antibody response [84]. The Th2 cell identifies the antigen presented by B lymphocytes and secrete IL-4, IL-5, IL-6, IL-10 and IL-13 which stimulates production of IgG1 antibody [85].

Although it is a well-known fact that protection against FMDV infection is provided by virus neutralizing antibodies, however, specific antibody response does not always provide clinical protection against FMDV infection [49]. Intriguingly, in many cases animals are found protected even in the absence of humoral response against FMDV [86]. Hence it has been suggested that a cell mediated immune response is required for protection against FMDV infection [87]. Both CD8+ and CD4+ antiviral responses have been observed after FMDV infection, however, the role of these responses in providing protection is unclear [88,89].

The T cell response to FMDV is cross reactive between FMDV serotypes hence it forms an important tool for the vaccine design [90-93]. It has been shown that the CD4+ cell mediated T cell response is required for providing protection against FMDV infection by the production of antiviral antibodies [88,89,94]. Hence, IFN-ϒ has been used for measurement of antigen-specific T cell activation [95]. The proliferation and production of IFN-ϒ from lymphocytes derived from FMDV infected animals have been demonstrated after restimulating with vaccine antigen [79,82,96]. Moreover, a memory CD4+ T-cell population has been detected in vaccinated cattle [97,98]. Recently a positive correlation has been found between IFN-ϒ and vaccine induced protection [68]. It was found that CD4+ T cells are the major proliferating cells and produce interferon gamma in stimulated cells of vaccinated and subsequently infected animals [68].

The FMDV specific MHC-I restricted CD8+ T cells response has been detected in cattle using a sensitive IFN-restimulation ELISpot assay [99]. Recently a highly conserved putative cross reactive cytotoxic CD8+ T-cell epitope was detected in the VP1 protein of FMDV [100]. The cell-mediated immune response against FMDV has been demonstrated by induction of IFN-γ [94]. Also, induction of FMDV-specific CD8+ cytotoxic T lymphocytes killing of MHC matched target cells in an antigen specific manner has been demonstrated [101]. Recently, Zhang et al. [102] reported that IL-2 and GM-CSF can be used as adjuvants with VP1 to enhance both humoral and cell mediated immune responses. Proliferation of CD8+ cells has been demonstrated starting from 10-14 days post vaccination up to 3-4 weeks following FMDV infection [87]. However, the role of CD8+ immune responses could be limited due to the rapid decrease in MHC 1 expression that occurs with FMDV infection. This has been demonstrated *in vitro* with MHC-1 expression down to about 53% that of normal at 6 hours post infection [103].

During FMDV infection, impairment of T-cell function has been observed. During acute phase of infection transient lymphopenia has been observed in swine [104] with T cell function returning to normal after 4-7 days of infection [105]. It has been demonstrated that incorporation of T cell epitope along with B cell epitope in a peptide based vaccine followed by vaccination and subsequent challenge leads to significant reduction in virus excretion and clinical score [106]. Moraes et al. [107] found that inclusion of 2B peptide in adenovirus vectored based vaccine increased CD8+ and CD4+ response which correlates with protection.

CONCLUSION

Thus, both innate and adaptive immunity play an important role in providing anti-viral immunity against FMDV. The humoral anti-FMDV response is T cell dependent and in some of the instances in the absence of humoral antibody response the cell mediated immune response has been found to be active.

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