William T. Golde
Charles K. Nfon
Felix N. Toka

Immune evasion during foot-and-mouth disease virus infection of swine

Author's address
William T. Golde[1], *Charles K. Nfon*[1], *Felix N. Toka*[1,2]
[1]Plum Island Animal Disease Center, ARS, USDA, Greenport, NY, USA.
[2]Department of Preclinical Science, Faculty of Veterinary Medicine, Warsaw University of Life Science, Warsaw, Poland.

Correspondence to:
William T. Golde
Plum Island Animal Disease Center
ARS, USDA
PO Box 848
Greenport, NY 11944-0848, USA
Tel.: +1 631 323 3249
Fax: +1 631 323 3006
e-mail: william.golde@ars.usda.gov

Acknowledgements
We acknowledge our colleagues and collaborators on the work conducted at Plum Island Animal Disease Center (PIADC). In particular, we thank Dr Elida Bautista, Boerhinger Ingleheim Animal Health, who initiated the DC work, Mr Geoffery Ferman, Department of Safety, PIADC, who initiated the lymphopenia studies when formally a member of the laboratory, and Dr Juan Pacheco, who developed most of the animal inoculation protocols and did the analysis of antibody response to FMDV in swine. In addition, we thank Drs D. Mark Estes and Janis Endsley, University of Texas Medical Branch at Galveston, for initiating the development of the NK cell assays in cattle, which we adapted to swine. This work was supported by CRIS #1940-32000-052-00D from the Agricultural Research Service, US Department of Agriculture (W. T. G).

Immunological Reviews 2008
Vol. 225: 85–95

Immunological Reviews
0105-2896

Summary: The interface between successful pathogens and their hosts is often a tenuous balance. In acute viral infections, this balance involves induction and inhibition of innate responses. Foot-and-mouth disease virus (FMDV) is considered one of the most contagious viruses known and is characterized by rapid induction of clinical disease in cloven hoofed animals exposed to infection. Viral shedding is extensive before the equally rapid resolution of acute disease. This positive strand RNA virus is an extremely successful pathogen, due in part to the ability to interrupt the innate immune response. Previous reviews have described the inhibition of cellular innate responses in the infected cell both *in vitro* and *in vivo*. Here, we present a review of virus inhibition of cells that are a source of antiviral function in swine. Particularly in the case of dendritic cells and natural killer cells, the virus has evolved mechanisms to interrupt the normal function of these important mediators of innate function, even though these cells are not infected by the virus. Understanding how this virus subverts the innate response will provide valuable information for the development of rapidly acting biotherapeutics to use in response to an outbreak of FMDV.

Keywords: *swine, infectious disease, innate immunity*

Introduction

Pathogenicity of infectious agents is determined by many factors bearing on transmission such as susceptibility and type of exposure of the host to the agent. In addition, transmission of disease is also dependent on virulence of the pathogen and whether other infectious disease processes are already ongoing in the exposed individual. The evolution of successful pathogens seems remarkable in the face of what we know about mammalian immune systems after decades of intense investigation. However, pathogens in general and viral pathogens in particular rapidly mutate and evolve to counteract the non-specific, innate responses of mammals and the highly specific adaptive immune response.

Upon infection, a virus is faced with the immune response of the host. Because viruses rely on the host for growth, survival, and transmission, they have evolved diverse methods to escape elimination by the host's immune system. Generally,

this is referred to as immune evasion, and the simple reason for a virus to evade the host's protective mechanisms is to acquire time for replication and transmission of progeny to other cells or hosts or to hide from surveillance by the immune system in a latent state. A large body of literature now exists on the main mechanisms engaged by viruses to evade the immune system. Immune evasion can be achieved by the interruption of various host responses including the innate response, cellular responses, humoral responses, and inhibition of immune effector molecules (1). The result of immune evasion is clinical disease or infection that is protracted over time.

Viral subversion of cellular immune responses is often associated with reducing the influence of the most efficient mechanism of virus clearance, $CD8^+$ cytotoxic T lymphocytes (CTLs). Eliminating the possibility of recognizing viral epitopes presented in context of major histocompatibility complex (MHC) class I molecules is common during infection by successful viral pathogens (2–5). To evade humoral immunity, viruses may undergo antigenic variation or encode proteins that induce polyclonal activation of B cells, leading to hyperproduction of immunoglobulins (Igs) not necessarily protective against the virus (6, 7). Other viruses encode highly glycosylated envelope proteins such that the glycosylation can mask the neutralizing epitopes, thus rendering the antibody response ineffective (8, 9). In some infections, viral proteins have been shown to inhibit complement-mediated killing of infected cells (10) or target effector molecules by encoding either homologous cytokines or cytokine receptors, having acquired such genes through modification or capturing of host cellular genes (11–13).

Rodent models of infectious disease depend upon similarities in the pathogenic progression of infection with the modeled species, i.e. human, companion pet, livestock, etc. There are many good rodent models of disease including severe acute respiratory syndrome coronavirus (SARS-CoV) in the aged mouse (over 12 months) (14) and some requiring adaptation of viral strains to mice such as influenza. However, other mouse 'models' have been reported extensively where disease pathology does not have any relationship to the pathology in the host of interest. A clear example in livestock is vesicular stomatitis virus (VSV), which does not cause vesicles at all in mice but rather causes encephalitis. Contrarily, this virus gains its name from the natural disease pathology in cattle, horses, and swine, which is characterized by vesicular lesions on the lips, tongue, snout, and coronary bands of the feet (15). Similarly, rodents are a poor model for foot-and-mouth disease virus (FMDV) infection, as infection of mice requires high doses of virus and there is no clinical vesicular disease. Further, important gene loci such as Ig and T-cell receptor diverged after separation of rodent and livestock species during evolution (16), thereby compromising translation of results in mice to the natural hosts. Here, we focus our discussion to one of the natural hosts of FMDV infection, swine.

The present review focuses on the evasion of early innate responses of swine to FMDV and the clearance of virus during infection of naive animals. The role of important innate cell populations has been a focus of recent research efforts, and many aspects of the response of these cell types have been studied. A comprehensive understanding of the innate response to viral infection still requires a great deal more investigation, as knowledge of the relationship of this virus and the infected cell is extensive but not yet comprehensive. By contrast, information on the immunopathology of FMDV in infected animals is sparse, though recently there has been more activity on this subject. Here, we endeavor to inform about current knowledge as well as identify areas lacking in information and understanding. Understanding FMDV immunopathology *in vivo* remains a very high priority, given an outbreak of FMDV in North America is anticipated to cause losses in the billions of dollars to the economies of Mexico, Canada, and the United States.

Immune evasion in porcine viral infections

Understanding the immune processes mediating immune evasion is made more relevant by studying diseases in their natural hosts. In swine, viral infections that can be studied to more fully understand immune evasion in a natural host include pseudorabies virus (PrV). This virus belongs to the alphaherpesviridae subfamily of *Herpersvirinae*, the same subfamily as the human herpes simplex virus (HSV) and bovine herpes viruses (BoHV), and has many features in common with these viruses. It infects the central nervous system (CNS) and establishes latency. PrV induces apoptosis in the host inflammatory cells, while it inhibits apoptosis of the trigeminal ganglion neurons where it resides during the latency period (17). While the genes involved in similar anti-apoptotic mechanisms in HSV-1 or -2 infection are known, in PrV the most likely candidates are the Us3 and LAT genes, which share RNA homology with anti-apoptotic molecules in muscle cells (18).

The same virus exhibits another mechanism that also may affect viral immune evasion. Infected cells display viral glycoproteins on the plasma membrane, but in the presence of virus-specific antibodies, redistribution of viral glycoproteins on the cell surface occurs, which leads to their aggregation, capping, and finally shedding. Further, when infected monocytes are incubated with glycoprotein-specific antibodies, viral

glycoproteins are endocytosed. In both cases, the result is no display of viral proteins on the cell surface membrane of infected cells, rendering such cells invisible to CTLs. This is particularly important for glycoproteins B and D (19, 20). Likely, PrV subverts the antibody response phase of infection until it establishes latency. However, it is not known whether such a mechanism is operational *in vivo*.

African swine fever virus belongs to the *Asfarviridae* family and uses a different mechanism to evade immune surveillance. Within 10 h of infection, this virus induces alteration of the secretory pathway by interfering with the trans-Golgi network (TGN). This interference leads to a loss of TGN46, a protein responsible for maintenance of the morphology of the TGN (21), and API, an adapter protein involved in sorting secretory bound proteins exiting the TGN. The obvious consequence of these viral mechanisms is to disrupt the translocation of MHC class I molecules to the cell membrane surface (22) so as to eliminate a crucial immunosurveillance component.

A somewhat novel mechanism of immune evasion is exhibited by porcine circovirus Type 2 (PCV2). It causes an ailment termed post-weaning multisystemic wasting syndrome in piglets aged 5–12 weeks and has tropism for cells of the monocytic lineage. PCV2 escapes into dendritic cells (DCs) or macrophages and shows no evidence of replication and neither does the virus kill the cells nor alter any of the activation markers on the cells harboring the virus. There is no cytokine profile modification (23). Once the virus is released from the cells, it is infectious to other cells. Given that DCs circulate to perform their function, it is possible that the virus is kept and spread in the host through DCs and macrophages without detection by the immune effector cells such as natural killer (NK) cells and CTLs. Moreover, PCV2 causes depletion of NK cells, γδT cells, and B cells (24). However, what is not clear is whether there is any level of viral antigen presentation during the period that DCs carry the virus.

Porcine respiratory and reproductive syndrome virus (PRRSV), an enveloped, positive, single-stranded RNA virus that is a member of the *Arteriviridae* family in the order *Nidovirales* (25, 26), causes a persistent infection of respiratory and reproductive tracts of pigs. The virus targets the DCs and down-regulates expression of MHC class I and II and costimulatory molecules such as CD80/86 (27). Although this virus is capable of inducing the translocation of activated nuclear factor-κB (NF-κB) to the nucleus followed by transcription of some genes such as matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) (28) and interleukin-10 (IL-10) (29), it fails to activate transcription of interferon (IFN) regulatory factor 3 (IRF3), an important IFNβ transcription factor (30). It does so by inhibiting the activation of IPS-1 within the RIG-I signaling pathway leading to diminished production of IFNβ. The lack of this type 1 IFN leads to reduced DC–NK cell cross-talk. Together with the upregulation of IL-10 impairing function of T lymphocytes, the virus is allowed to persist.

PRRSV also appears to attack the antibody responses as well. An elegant report by Butler *et al*. (7) shows that this virus manipulates the immune system by inducing polyclonal B-cell activation. In an isolator piglet model, PRRSV causes immune dysregulation characterized by heavy lymphoid hyperplasia and hypergammaglobulinemia. The generalized polyclonal B-cell activation may produce autoantibodies to double-stranded (ds) DNA, Golgi glycoproteins, and other autoantigens, presumably leading to subversion of normal B-cell repertoire development. The likely consequence of this is a delay in the development of effective PRRSV-specific adaptive immunity. Mulupuri *et al*. (31) report the delayed appearance of antibodies against crucial PRRSV antigens such GP5, supporting this hypothesis.

Immunopathology during FMDV infection

FMDV induces vesicular lesions on the feet, mouth, tongue, and teets of susceptible species such as cattle and swine. FMDV is often referred to as the most contagious virus known and can spread very rapidly through naive herds. A positive strand RNA virus, FMDV rapidly mutates in the course of an outbreak of infection, creating quasi species within the broader serotype (32). This virus also induces a highly acute infection, with clinical disease developing rapidly after exposure and a high level of viremia early in infection. Fever and viremia last only 1 or 2 days, and lesions normally resolve rapidly, in 7–10 days. In a small percentage of cases, a carrier state can develop lasting more than a few months, and this is often associated with exposure of vaccinated cattle to virulent virus (33, 34).

The understanding of the host pathogen relationship between susceptible species and this virus is extensive on the cellular level. Receptors for the virus have been identified and are all in the αv integrin family. In addition, *in vitro*, this virus can use heparin sulfate as a receptor (35). The *in vivo* role of this latter interaction is not well understood (reviewed in 36).

Viral proteins are encoded in a single open reading frame, and the polypeptide is translated and subsequently processed by viral encoded proteases. The leader protease self-cleaves and, among other activities, cleaves the elongation factor 4 (elf-4) of the cellular transcription complex. This arrests cap-dependent mRNA and cellular protein synthesis, and because the virus has an internal ribosome entry sight, the protein synthesis complexes are bound by the viral RNA genome.

Infected cells therefore have mostly viral protein synthesis and little cellular proteins translated (37, 38). This is likely to have a significant effect on inducing immune responses, as infected cells have been shown to lose class I MHC expression. With no new protein synthesis, these effects likely lead to no viral protein peptides getting loaded into newly synthesized MHC molecules via the endogenous pathway, and therefore, no induction of a $CD8^+$ T-cell response can occur. Data for $CD8^+$ T-cell responses in animals infected with FMDV is minimal, and no infected cell killing by CTLs has ever been reported.

Leader protease (Lpro) is the first protein to be translated from the viral genome, and its effect on type 1 IFN responses has received much attention. Like many viruses, FMDV is very sensitive to these cytokines (39–41). The bulk of knowledge in this area has been obtained by using a serotype A virus derived from an infectious clone (A12) that has the leader protease removed (LLA12). Deletion of Lpro in the LLA12 virus results in a highly attenuated virus that fails to replicate in otherwise susceptible primary cell lines (39). In addition, infection of animals with the LLA12 virus rarely causes viremia, and lesions are restricted to the inoculation sites, resulting in the rapid clearance of the virus before induction of neutralizing antibodies (42, 43). However both the wildtype virus and the LLA12 replicate to almost the same degree in BHK-21 cells or in suckling mice (44) due to defects in type 1 IFN response machinery in these systems (45, 46). Furthermore, supernatants from LLA12-infected porcine or bovine fibroblasts had a stronger type 1 IFN-mediated antiviral activity relative to those from wildtype infection (40). These data indicate that Lpro contributes to the virulence of FMDV and plays a significant role in suppressing the innate immune response to this virus, permitting replication and transmission between susceptible species.

Further research has demonstrated that in addition to blocking type 1 IFN translation, Lpro also inhibits transcription of IFNβ and the IFN-stimulated genes 2′5′ oligoadenylate synthetase, dsRNA protein kinase, and MX1 (41). NF-κB is a ubiquitous protein that regulates genes (including IFNβ) responsible for innate and adaptive immunity. Inactive NF-κB is found in the cytoplasm coupled to an inhibitory protein, IκB. Viral infections and other stressors induce a signaling cascade via pathogen-associated molecular pattern receptors leading to the activation of NF-κB (47). Activated NF-κB translocates to the nucleus, where it mediates gene transcription. Infection of BHK-21 cells with FMDV wildtype or LLA12 results in translocation of NF-κB into the nucleus (48). However, at later stages of the infection, there is degradation of the nuclear NF-κB in wildtype infected cells but not in the LLA12 infected cells. The degradation of NF-κB correlates with translocation of FMDV Lpro into the nucleus. Furthermore, NF-κB degradation is observed when Lpro is introduced into the cells in the absence of other FMDV proteins (48). Thus, degradation of NF-κB by FMDV Lpro might be another mechanism by which FMDV mediates the downregulation of inflammatory gene transcription and hence inhibition of innate immunity.

The 3C protease (3Cpro) of picornaviruses is responsible for the cleavage of the viral polyproteins. FMDV 3Cpro has also been shown to not only cleave elF-4G but also elF-4A. However, 3Cpro accumulates at a slower rate than the Lpro, but the accumulation eventually exceeds the level required for processing viral polyprotein, and the excess can begin to cleave other substrates (49). Based on the cleavage of elF-4G, it can be concluded that 3Cpro potentially exerts a similar immunosuppressive effect on infected cells as does the Lpro, albeit at late stages of infection.

FMDV Lpro, 3Cpro, and possibly other FMDV proteins (50) suppress transcription and/or translation of inflammatory cytokines in infected cells. Lysed cells harboring these proteins probably serve to supply inhibitory signals for cells such as DCs, macrophages, NK cells, and T cells, which are otherwise refractory for FMDV infection.

Induction of lymphopenia during FMDV infection

Some viral infections of swine cause immunopathology by inducing a decline in circulating lymphocytes. Classical swine fever virus, for example, induces a severe lymphopenia of 7–10 days duration (51–53). PRRSV causes a transient leukopenia (54, 55) and pathology in lymphoid tissues of infected swine (56). Similarly, multiple strains of FMDV have been shown to cause lymphopenia in swine involving multiple subsets of peripheral lymphocytes (57, 58, Nfon *et al.*, manuscript submitted). This lymphopenia is characterized by a decline in the absolute numbers as well as percentage of lymphocytes in peripheral blood and closely correlates with peaks of viremia. The more virulent strains for pig (O1 Campos, O Taiwan 97, A24 Cruzeiro, C3 Resende, and Cs8C1) induce more profound lymphopenia compared with the less virulent strains (O-SK2000 and A12), inducing mild and delayed lymphopenia. Active infection of lymphocytes is not responsible for the lymphopenia, as virus could not be recovered from peripheral blood mononuclear cells (PBMCs) of infected pigs despite high viremia. In addition, PBMCs were not infected *in vitro* (59, 60). However, a plaque purified, tissue culture strain of serotype C, Cs8C1, inoculated into young pigs has been reported to infect lymphocytes (58). There is no

significant increase in apoptosis of peripheral and tissue lymphocytes, and the recovery from lymphopenia is usually rapid.

We have consistently observed a transient serum IFNα response in FMDV-infected swine. Early after infection (48 h), all swine had detectable IFNα in serum regardless of virus serotype and strain used to infect. Peak levels of serum IFNα ranged from 200 to 1500 pg/ml on day 3 post-infection and dropped sharply to background levels by days 4–10 (Nfon *et al.*, manuscript submitted). IFNα protects swine from FMDV infection when administered 24–48 h before challenge (61, 62). IFNα may contribute to early clearance of viremia by preventing further propagation of virus in susceptible cells (42). Despite this activity, FMDV replicates rapidly and attains peak viremia within 48–72 h of infection. This observation suggests a potential for swine to mount an innate response to FMDV that is insufficient to stop the early establishment of infection but plays a significant role in viral clearance. For instance, IFNα secretion may activate cells mediating an innate response and/or induce an antiviral state in susceptible tissue, although not in time to completely block infection. Either of these possibilities is consistent with published data analyzing exogenous delivery of IFNα (61).

In pigs infected with CSFV, lymphopenia correlates with the levels of IFNα in serum (53). Similarly, our observation of peak serum IFNα in porcine FMDV infection coincided with lowest lymphocyte numbers in blood. Thus, the serum IFNα response could partly account for the FMDV-associated lymphopenia in pigs. The connection between serum IFNα and lymphopenia has been clearly demonstrated in mice. Mice injected with poly I:C exhibit a lymphopenia linked to their serum IFNα levels (63). Studies in gene-targeted mice show that infection with VSV is associated with lymphopenia, which is dependent on signaling through the type 1 IFN receptor (IFNαR) (64). Kamphuis *et al.* (64), using adoptive transfer studies in mice with a B- or T-cell-specific IFNαR deletion, showed that IFNα/β exerted a direct effect on lymphocytes sufficient to induce lymphopenia.

At peak viremia and minimum lymphocyte numbers, the functional capacity of residual T cells is significantly reduced. These cells show little or no proliferation in response to stimulation with the mitogenic lectin concanavalin A (ConA) (57, 58). In addition, they fail to secrete IFNγ in response to ConA, and these functional deficiencies in T cells persist for up to 7 days post-infection, despite the rapid recovery in lymphocyte numbers by day 4. These rapid FMDV effects on T cells likely create a transient immunosuppressive state, further enabling the propagation and shedding of virus.

DCs and FMDV

DCs are professional antigen-presenting cells, yet many DC subsets play a major role in innate responses to pathogens. These cells therefore are critical in regulating the early, non-specific response to viral infection and transitioning to the induction of highly specific immune responses via activation of helper T cells if viral antigens are still present. DCs previously described in swine peripheral blood include myeloid/monocyte-derived DCs (MoDCs) (65–67) and plasmacytoid DCs (pDCs) (68). pDCs are specialized IFNα-secreting cells and produce large amounts of this cytokine in response to many viral infections. However, pDCs only respond to FMDV in the presence of immune serum (69), suggesting that these cells might be less effective early in FMDV infections. MoDCs secrete type 1 IFNs in response to FMDV; however, stimulation of MoDCs with synthetic dsRNA, polyI:C (60), induces a stronger IFN response than FMDV.

We have previously reported the characterization of DCs isolated from porcine skin (70). Recently, we have identified the majority of these cells as Langerhans cells (LCs) populating the epidermis (71). Uniquely, porcine LCs constitutively express IFNα and secrete this cytokine after encountering FMDV *in vitro* (57, 60). This finding may indicate a prominent role of LCs in FMDV infection.

IFNα secretion by MoDCs propagated from peripheral blood of animals infected with FMDV is transiently depressed during acute infection, specifically at 48 h post-infection (60). In that study, we only analyzed cells harvested on days 2, 7, and 14 following infection as generation of the MoDC population for analysis required 7 days of *in vitro* propagation. MoDC secretion of IFNα recovered by day 7 to levels similar to preinfection samples. Subsequently, we analyzed PBMCs for pDC function by assessing the response to an innate immune stimulator. Daily analysis of PBMCs from FMDV-infected pigs revealed there is a transient decline in the number of IFNα-producing cells when stimulated with CpG 2216, a Toll-like receptor 9 (TLR-9) agonist. In addition, the IFNα in supernatants of these cultures is significantly lower on days 2–4 post-infection (Nfon *et al.*, manuscript submitted).

LCs harvested from FMDV-infected pigs secrete little or no IFNα in response to *ex vivo* stimulation. The suppression of IFNα secretion of LCs following infection is more protracted, beginning on day 2 post-infection and lasting beyond 35 days. This timing apparently coincides with the turnover period for LCs, suggesting those LCs that have encountered viruses are permanently anergized for IFNα secretion (60). The ability to make IFNα is a function of repopulation of the skin with naive LCs.

Inhibition of type 1 IFN response of DCs is a feature of other viral infections (72–78). During dengue virus infection, pDCs, though not productively infected, decline in number and secrete less type 1 IFN (77). Similar effects on pDCs are observed in primary human immunodeficiency virus infections (76). Furthermore, dermal DCs in warts caused by human papilloma virus fail to respond to imiquimod, a TLR-7 ligand (72), and vaccinia virus suppresses the ability of a LC line to secrete proinflammatory cytokines in response to stimulation with lipopolysaccharide or poly I:C (75). Chronic hepatitis C virus affects both pDCs and MoDCs, causing these cells to secrete less cytokine in response to TLR ligands (73).

The mechanisms by which FMDV suppresses DC IFN responses are not clearly understood. No live virus was isolated from monocytes (including DCs and macrophages) of FMDV-infected swine, despite the presence of high titers of virus in blood. Furthermore, immune serum is required for an abortive infection of monocytes *in vitro* (79). Similarly, we found no evidence of productive infection of LCs *in vitro* and from LCs isolated from infected swine despite the high susceptibility of surrounding keratinocytes (59, 60). Viral products and replication intermediates released by lysed, infected cells and taken up by DCs may account for this immunosuppression. However, viral RNA can be detected by polymerase chain reaction (PCR) of RNA samples isolated from *in vitro* exposed LCs after 1 h adsorption, indicating that FMDV can bind to and be internalized by LCs (59). Indeed, by immunohistochemistry, Gregg *et al.* (80) detected low levels of FMDV antigen within LCs. Because FMDV is a positive sense RNA virus, viral protein synthesis can be initiated upon uptake and before virus inactivation. Thus, low levels of viral proteins, particularly the rapidly translated, self-cleaving Lpro, may occur within DCs even in the absence of replication.

Subversion of innate function of NK cells

Cellular responses are critical to clearance of the invading virus and are necessary for rapid response should reinfection occur. NK cells do not require expansion like the cells mediating antigen-specific adaptive responses, T cells and B cells, so there is no delay in NK-mediated responses. NK cells are able to recognize cells that are infected by a virus, usually via cell stress signals, and are capable of eliciting spontaneous cytotoxicity. These functions are regulated by NK cell-activating cytokines such as IL-2, IL-12, IL-15, IL-18, and IFNs (81). Apart from cytokines, NK cells express activating receptors, such as NKp30, NKp44, and NKp46, in humans. Binding of these receptors signals activation of the NK cell. Alternatively, inhibiting receptors such as killer-cell immunoglobulin-like receptor and CD94-NKG2A (82) transmit inhibitory signals.

NK cells may be targets for viral subversion of immunity. Most studies on how NK cells are affected by infection have been performed on viruses that are persistent or chronic in nature, although acute infections may also lead to dysfunction of NK cells (reviewed in 83). In fact, during porcine infection with FMDV, this may be the case. Pig peripheral blood NK cells are rather quiescent in nature and only increase their cytotoxicity after stimulation with NK cell-activating cytokines, such as IL-15 (84, Toka *et al.*, manuscript submitted). Peripheral blood NK cells from FMDV serotype O-infected pigs do not increase their cytotoxicity, as opposed to the responses of these cells in infections such as MCVM in the mouse (85, 86). Instead, the swine NK cell response declines beginning from the second or third day after FMDV infection. Such a state of dysfunction usually lasts for 2–3 days, after which reactivity returns to background levels. Higher viral titers in the serum of infected animals appear to coincide with the reduction in NK cell cytotoxicity. Curiously, when the effector protein expression such as perforin was compared in infected cells and non-infected cells, there was only a marginal difference (Toka *et al.*, manuscript submitted). This observation may indicate that while the granzyme/perforin killing mechanism may be intact, other pathways are possibly modulated by FMDV resulting in reduced antiviral activity of NK cells. However, intracellular expression of IFNγ was inhibited, which may largely contribute the inactive status of NK cells during FMDV infection. Inhibition of cytokines or chemokines is one of the strategies used by viruses to subvert the immune system. These processes take place in non-infected cells, requiring that the mechanism of this inhibition likely involves products, cellular or viral, from infected cells.

Some viruses encode proteins that interact with NK cells, and during this interaction, NK cells are inhibited in their primary function. Such is the case when CD81 directly binds E2 of hepatitis C virus (87). If such interaction occurs early in infection, the costimulatory effect of NK cells on DCs will be hampered, and as a result, DC-dependent activation of NK cell lytic activity. Commonly, NK cells recognize infected cells by virally induced lack of MHC expression; however, some viral proteins may upregulate expression of MHC class I molecules in infected cells, inhibiting NK function. Furthermore, viruses can interrupt NK receptor-mediated recognition of cells infected with virus by blocking cell-activating cytokines such as IFNα, IL-12, IL-15, and IL-18.

Although no evidence is yet available, the inhibition of activating receptors on NK cells may be involved in FMDV

infection of swine. Indeed, *in vitro* experiments with NK cells isolated from pigs infected with FMDV do not exhibit significant levels of cytotoxicity. Measurement of NK cell expression of activating receptors shows a rather unaltered profile. Of the three NK cell receptors (NCRs), only NCR3 is upregulated, at least in the first 3 days after infection, followed by GZMB, KLRC1, and KLRA1. The remaining genes, such as NCR1, KLRF1, GZMA, KLRB1, KLRK1, SH2DIB, and PRF1 are not upregulated, suggesting that there may be selective inhibition of expression of these genes during infection with FMDV (Toka *et al.*, manuscript submitted).

In other virus systems, inhibition of activating receptor function has been attributed to downregulation of NCR ligands, mainly on virus-infected cells. For instance, in human cytomegalovirus-infected cells, there is downregulation of leukocyte function-associated antigen-3, which renders these cells refractory to killing by NK cells (88). However, modulation of NK cell function by infection of NK cells has also been reported for vaccinia virus (89). Apparently, infection modulates the signaling in NK cells such that the cells become more sensitive to inhibitory signals likely derived from target cells.

As reviewed above, FMDV does not infect lymphocytes *in vivo* (57). Therefore, inhibition in terms of activating receptor manipulation by the virus may be a bystander effect exerted by viral protein products or simply binding of the virus particles to porcine NK cells. It remains to be determined what viral proteins might possibly be involved. Conversely, this inhibition may take the form of antagonism of the cytokines that regulate inflammatory and immune responses, often targets of subversion by viruses.

A few examples are Epstein–Barr virus, which encodes IL-10, and vIL-10 negatively regulates IL-12 by various leukocytes and subsequently IFNγ production by NK cells. Adenoviruses have evolved to express proteins that neutralize TNFα, and poxviruses encode soluble cytokine receptors able to block the most important regulators of immune and inflammatory responses such as IFNγ, IFNαβ, TNF, and IL-1 (90). Although there is no evidence of FMDV encoding cytokine and cytokine receptor homologues, it is clear that NK cells derived from FMDV-infected pigs are unable to secrete IFNγ (Toka *et al.*, manuscript submitted). The mechanism for inhibition is currently difficult to discern. But likely it may be through a virus protein cross-linking to NK cell inhibiting receptor, or the situation may be similar to that shown for measles virus, where virus binding to CD46 on monocytes leads to immunosuppression of cell-mediated responses (91).

Inhibition of adaptive immunity

Although IFNα secretion by DCs is blocked during acute FMDV infection of swine (60), the molecules required for adequate antigen-presenting function of DCs, MHC class II and CD80/86, are unaffected. Similarly, the ability of DCs to take up particulate matter and process protein antigens is undiminished by FMDV (59, 60). This finding suggests that these DCs retain the ability to process and present FMDV antigen, thus initiating the strong antibody response extensively reported in FMDV infection of swine (36). FMDV has evolved to establish acute infections characterized by rapid replication and spread between susceptible animals. Persistent infections are rare in swine. Suppression of innate function of DCs by FMDV creates a window for viral replication and shedding before the onset of an adaptive immune response.

The anti-FMDV antibody response supports the hypothesis that antigen presentation function remains intact. Various analyses of the quality of the antibody response in swine indicate that there is a broad induction of $CD4^+$ helper T cells that produce B-cell-activating cytokines. Anti-FMDV antibody of the IgM isotype is detected as early as 4–7 days, peaking by 10 days and waning by 14–21 days after infection. Using polyclonal anti-swine IgG antiserum, the IgG response is detectable around 14 days after infection and lasts for months. There are few reagents available to distinguish the six IgG isotypes of swine Ig, and the genetics of this locus indicate allelic differences will also be problematic to dissect using antibodies specific for these isotypes, if they were available.

In other species, the induction of particular isotypes of antibody is associated with the T-helper type 1 (Th1)/Th2 paradigm. These subsets of $CD4^+$, class II MHC-restricted T cells respond to activation by antigen-presenting cells and antigen by producing unique patterns of cytokine secretion. Th1 is characterized in all species by production of IFNγ, and Th2 cells are a prominent source of IL4, IL-5, and IL-13 following stimulation. In swine, analysis of the association of specific Ig responses with Th1 or Th2 T cells is not possible, given only two antibodies are presently available, anti-IgG1 and anti-IgG2. However, we have previously reported a reduced T-cell proliferation and lack of IFNγ secretion in response to mitogen during acute FMDV infection (57). Interestingly, when the antibody response to FMDV was analyzed using these limited reagents, we detected a putative IgG1 antibody reactivity with FMDV but no IgG2 antiviral reactivity. Porcine IgG2 antibody reactivity with a standard protein antigen, chicken ovalbumin, was readily detected (W. Golde,

unpublished data). This highly preliminary data may indicate an imbalance in the Th1/Th2 response during FMDV infection.

Infected cell expression of MHC proteins loaded with virally derived peptides is the hallmark of cellular immunity mediated by CTLs. This phenomenon allows detection and elimination of cells that are infected with virus. Class I MHC-restricted $CD8^+$ T-cell responses are diminished to absent in FMD-infected animals. Viruses have evolved several mechanisms to interrupt this critical infected cell labeling system.

The most common and well studied viral subversion strategies of MHC class I include inhibition of peptide translocation to endoplasmic reticulum, interference with cytosolic proteolysis resulting in inhibition of processing of antigenic peptides to be loaded into class I heterodimers, or retention and destruction of MHC class I. These effects hinder the presentation of viral antigens leading to non-recognition of infected cells by CTLs (78, 92–94). When assembly of the MHC molecules loaded with specific peptides is complete, they are delivered to the cell membrane surface, but viral proteins including the US3 protein of HCMV (95) and 2B and 3A proteins of poliovirus (96) can delay surface expression of these complexes. Even when the MHC-peptide complexes finally arrive at the cell membrane surface, viral proteins, such as B3 proteins of coxsackievirus (97) and Kaposi's sarcoma-associated herpes virus gene product K3 (98), can disguise these molecules.

Infection of porcine fibroblast cell lines with FMDV led to reduction of surface MHC class I molecules expression of approximately 50% by 10 h post-infection. On the contrary, swine alveolar macrophages incubated with FMDV increased the expression of both MHC class I and II molecules within the same time frame. Following acid treatment of the fibroblast cell lines infected with FMDV, there was no rapid reappearance of MHC class I molecules, indicating that there was inhibition of assembly of new molecules (99). The mechanism of reduction of MHC class I expression on infected fibroblasts could be that described by Moffat *et al*. (50). Using TsO45 mutant of VSV G protein to track movement of proteins from the endoplasmic reticulum to the cell surface membrane, they showed that co-transfection of cells with FMDV 2BC and TsO45-GYFP led to inhibition of delivery of the G-protein to cell surface. Therefore, it is possible that FMDV uses this strategy to inhibit the secretory pathway leading to retention of proteins such MHC class I molecules and cytokines that are necessary to induce an effective immune response against FMDV in swine.

The analysis of T-cell responses to FMDV antigens in cattle and swine is very limited. Attempts to identify peptide specificities have been difficult to quantify. A more sophisticated understanding of immune evasion of T-cell responses will require more basic analysis of T-cell responses to this virus.

Concluding remarks

The data reviewed here describe a very delicate balance between immunosuppression by FMDV and immune response by the host, which more often favors the virus in the acute phase of infection. Eventually, innate and ultimately adaptive responses favor the host, not only in the clearance of virus and recovery from clinical disease but also in protection from reinfection. Given that protection; is a serum antibody specific, the very definition of serotypes, such protection is mediated by antibody. There appears to be little contribution of the cellular response mediated by $CD8^+$ T cells acting as CTLs. $CD8^+$ T cells producing IFNγ is a more common observation but still poorly understood.

Previous reviews have elegantly summarized the virus life cycle in infected cells, cellular receptors for the virus, clinical progression of disease, and analysis of outbreaks of note. Here, we endeavored to focus on the innate response to this highly acute infection. In general, it would seem that such robust antiviral responses should protect at least swine from infection. However, we summarize here the subversion of many cells that mediate innate responses, especially DCs and NK cells. This subversion and that of other cells yet to be evaluated appear to be just sufficient to create the opportunity for the virus to replicate and spread to the next individual. The highly contagious nature of this picornavirus, therefore, is another critical factor in the success of this pathogen.

The innate response to this virus is a ripe target for creating new innovative approaches to develop rapidly acting biotherapeutics. Continued and more detailed analysis of the interaction of this virus with susceptible hosts will provide the information necessary to combat outbreaks and limit the devastation they can cause.

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