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Review of immunological responses to porcine coronaviruses and implications on population based control strategies in epidemic and endemic infections

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Abstract

Five major porcine coronaviruses (COVs) have been identified which cause severe gastrointestinal (GI) and respiratory disease in pigs. They include transmissible gastroenteritis (TGEV), porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus, porcine respiratory coronavirus, and porcine hemagglutinating encephalomyelitis. These diseases, especially TGEV and PEDV, have caused epidemics in Europe, Asia, and the Americas over the past 50 years, causing significant economic losses to swine producers. As pigs are a major protein source worldwide there is great interest in understanding, controlling, and preventing these diseases. These diseases have no cure, and current vaccines are not fully protective. On-farm prevention and biosecurity are difficult to enforce and have not stopped the spread of these diseases between herds. Recent advances in the immunology of porcine COVs has revealed that the immune response to porcine COVs shares many similarities with the response to human COVs, leading to increased interest in pigs as models for human disease. Highlights of these advances include the key role of local antigen presenting cells in the gastrointestinal tract in stimulating a protective immune response. This understanding has lead to new proposed vaccines. Advances in the understanding of the ways the viruses evade and degrade the host immune system have also lead to novel proposed therapies. Many of these therapies are in the early development stages, as researchers attempt to create efficacious, cost-effective, and practical therapies for these diseases.

Key words: Immunology; Porcine; Corona viruses; Population; Control; Zoonotic; Epidemic

Core tip: Coronaviruses (COVs) cause severe disease in both pigs and humans. New immunological research in pigs has revealed many similarities between porcine and human responses to COVs. Understanding the immunological responses of pigs to COVs may prove

that they are a viable human model to study these diseases, as well as providing new and more efficacious control mechanisms for veterinarians and swine producers worldwide.

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INTRODUCTION

Coronaviruses (COVs) are pathogens that naturally infect both humans and pigs, as well as other mammals and birds[1]. These viruses mutate rapidly and spread easily, and present a complex prevention and control challenge[2]. Known and emerging COVs continue to cause dangerous and costly diseases in humans and pigs, and some have proven zoonotic potential. For example, the global outbreak of severe acute respiratory syndrome in 2003, which killed 774 people, was caused by a COV of animal origin[3]. Emerging COVs have also caused outbreaks with high mortality in pig populations. A recent example was the 2010 outbreak of porcine epidemic diarrhea virus (PEDV) in China, which killed over 1000000 piglets[4]. New research has also shown that pigs may be a viable human model to study COVs in both species[5]. Recent research has made many advances in the field of coronavirus immunology, which will be crucial in improving future prevention and control techniques of COVs in both humans and pigs.

BACKGROUND

Review of COVs

COVs are enveloped, single strand positive-sense RNA viruses that primarily target the upper respiratory and gastrointestinal (GI) tract of humans, pigs, and a variety of other mammals and birds[2,6,7]. They belong to the *Coronaviridae* family, subfamily *Coronavirinae*[8].

Once in the body, COVs bind to surface receptors on susceptible cells[2]. This allows the virus to enter the cell, and replicate in the cytoplasm[8]. New copies of the virus are then assembled in the rough endoplasmic reticulum[9]. From there, move through the Golgi apparatus, fuse with the cell membrane of the host cell, and are released[10].

Porcine COVs

Five COVs have been identified in pigs. They include: Transmissible gastroenteritis (TGEV), PEDV, porcine deltacoronavirus (PDCoV), porcine respiratory coronavirus (PRCV), and porcine hemagglutinating encephalomyelitis (PHEV). Of these, the most damaging to the worldwide pork production industry and therefore the most studied are TGEV and PEDV[2]. These two diseases will be the focus of this review.

TGEV was the original COV shown to cause clinical disease in pigs. It was first documented in the United States in 1946, and today has high seroprevalence in pig operations throughout the world[2]. Because of its longevity and negative impact worldwide, it has received the most attention and research. Epidemics are less common in the United States and Europe today since PRCV has become more ubiquitous which seems to provide immunological protection against TGE, but outbreaks in naïve herds still occur[11]. However, TGEV continues to cause major outbreaks in Asia to date, and a new strain has been documented in 2012 that shares a common ancestor with the United States strain[12]. This highlights the ability of COVs to mutate rapidly, and the challenges and risks of our increasingly global economy, which may bring new virulent strains back to the United States and Europe.

PEDV was the next virus to emerge as a global problem in pig production. PEDV initially caused outbreaks in Europe. It was identified as the cause of outbreaks of diarrhea in 1971. In 1984 PEDV was identified in Asia. The most recent outbreak in Asia was 2010-2012, when the appearance of new strains caused massive vaccine failures and high mortality among affected pigs[13]. From 2013-2014, two new strains of PEDV were identified in the United States. Despite control efforts, these strains spread rapidly through the United States, Canada, and Mexico, causing the death of several million piglets[14]. Additionally, an outbreak in Germany in 2014 revealed strains nearly identical to those found in the United States[15]. The fact that multiple strains are present worldwide that may be related again highlights the need for new, more effective control measures[2].

PDCoV is the most recent porcine COV to be identified, it was detected for the first time in the United States in 2014. Although the consequences of infection tend to be less severe than TEGV and PEDV, it is an emerging disease that requires further study[16].

PRCV is a variant of TGEV caused by small deletions in the TGEV genome, and generally causes an asymptomatic infection. Its emergence in Europe in the early 1980's corresponded with the disappearance of TGEV in Europe, as it acted as a natural modified-live vaccine to produce active immunity in infected sows, who then passively transferred immunity to their suckling piglets. Infections are usually subclinical, or a mild respiratory disease can be detected. It is endemic in most swine herds in affected countries, and pigs are not routinely tested for the disease. However, it still remains of interest because of potential protection against TGEV afforded by previous exposure to PRCV[2].

PHEV is a ubiquitous virus, and causes mostly subclinical infections. It can cause clinical disease upon entering a naïve herd that can affect both the GI and central nervous system[17]. However, morbidity and mortality are low for both forms, making this disease

less of a concern for pig producers and researchers at this time[2].

TGEV and PEDV pathogenesis and clinical signs

TGEV and PEDV enter *via* the oral cavity, survive the low pH of the stomach, and primarily infect villous epithelial cells of the intestinal brush border of the small intestine. Both viruses can also infect the alveolar macrophages of the respiratory tract resulting in pneumonic lesions[2].

TGEV uses its spike (S) glycoprotein to bind to the receptor aminopeptidase N expressed by epithelial cells of the small intestine. The virus then destroys the mature villous cells, leading to reduction and blunting of the villi, and reducing normal enzymatic activity. This leads to alterations in digestion, cellular transport, and hydrolysis of lactose from milk. Tight junctions between the intestinal epithelial cells are also weakened by both TGEV and PEDV infections[18]. The result is a malabsorptive syndrome caused by increased osmotic force in the unhealthy intestine as lactose and sodium accumulate, leading to diarrhea, metabolic acidosis, dehydration, and death in a naïve animal[2].

The impact of TGEV on a herd depends on the immune status of herd that is infected. The most devastating is the epizootic infection, which occurs when a herd with no prior immunity herd is exposed. It is common in these herds for neonatal mortality to reach 100% for as long as 4 wk secondary to profuse diarrhea and the resulting dehydration. In young growing pigs and adults inappetence, vomiting, and diarrhea are commonly seen with morbidity approaching 100% but very low to no mortality. At the herd level the infection is usually self-limiting, and ends within several weeks. However, recovered adults often become carriers[19]. Other herds which have an endemic or enzootic infection, or where adult females have been previously exposed and can provide passive immunity to the piglets, may experience outbreaks, but those outbreaks have much lower mortality rates, usually not exceeding 20%. PEDV is closely related to TGEV in both pathogenesis, behavior in a herd, and clinical signs[2]. It is necessary to use diagnostic tests to differentiate the diseases from each other on an infected farm[20].

Epidemiology of TGEV and PEDV

TGEV is shed in both the feces and the nasal secretions of infected animals during an epidemic infection. The virus has been found in the feces of young pigs for up to 2 wk, and in the nasal secretions for 10 to 11 d. The virus is then spread to other animals *via* fecal-oral contact or aerogenously over short distances, and can travel between pens and farms by means of mechanical vectors which include farm workers and equipment, dogs, cats, foxes, starlings, and flies[2].

The infection may become endemic on the farm after an epidemic. Those pigs that survived the initial TGEV infection may continue to shed the virus, and further outbreaks have been documented up to 9 mo later. Also, in the absence of thorough cleaning and disinfection, the virus may persist in the environment, especially during colder months[21].

PEDV offers similar epidemiological challenges, and has been documented to be shed in the feces of recovered pigs for up to 42 d [13]. However, the numbers of studies currently available for PEDV epidemiology are far fewer than those available for TGEV, and offers another avenue for continued study that may yield important information for the control of both diseases.

THE PORCINE IMMUNE RESPONSE

When a pig is infected with TGEV or PEDV, both single stranded RNA (ssRNA) viruses, the virus primarily targets the epithelial cells of the small intestines[2]. As the virus invades the cells and begins its process of replication, the immune system attempts to mount a response. However, given the high mortality rate among pigs less than four weeks of age (neonates), it is known that the response is unable to protect very young animals.

Innate immune response

The major difference in the behavior of the villous epithelial cells in neonates compared to older animals is the rate of replacement of the mature cells. Neonatal pigs require up to 10 d to replace these cells, while older pigs only require 2 to 4 d[2]. Therefore, clinical signs caused by the destruction of the villous epithelium rapidly overwhelm younger pigs, while older pigs can repair the epithelium quickly enough to mount an appropriate immune response and recover.

The first immune cells to respond to TGEV, PEDV, or other viral infections are the professional antigen presenting cells (APCs) of the innate immune system[19]. These are a family of white blood cells including monocytes and macrophages, B lymphocytes, and, of most recent interest in TGEV and PEDV, dendritic cells (DCs)[22]. After antigen recognition, APCs migrate to lymphoid tissues and interact with T lymphocytes, which are responsible for cell-mediated immunity[5]. This interaction leads to the activation of B cells, which are responsible for humoral immunity (antibody production). Memory cells of both types are also produced in response to an antigen that are present and prepared to respond upon antigen reexposure[23].

The DCs are of great interest in viral infections such as TGEV because these cells are the only APCs capable of presenting antigen to naïve T lymphocytes. The other APCs are only able to stimulate existing memory T lymphocytes[22]. In pigs, these APCs, including DCs, are present in various organs and locations throughout the body, but of particular interest to TGEV, are found in the gut-associated lymphoid tissue (GALT) of the small intestines[24]. The GALT is the largest immune organ in the body, and is composed of peyer's patches, lymphoid follicles, and mesenteric lymph nodes[14].

The DCs and other APCs in the GALT recognize TGEV using pathogen associated molecular patterns

(PAMPs) on the virus particles that correspond to pattern recognition receptors (PRRs) on the APCs[25]. One of the primary families of PRRs involved in ssRNA virus detection are called toll-like receptors (TLRs)[25].

DCs in pigs, as in humans, are divided into two families, and have slightly different roles in mediating the initial immune response to a virus like TGE. These two families are: Plasmacytoid and myeloid. Myeloid dendritic cells (mDCs) are also called conventional myeloid dendritic cells (cDCs) and are most similar to monocytes. Plasmacytoid dendritic cells (pDCs) are most similar in appearance to plasma cells, but share characteristics of the mDCs. pDCs have a further subset of special interest called natural interferon producing cells (NIPCs). pDCs, including NIPCs, differ from mDCs in several ways, including that they can be triggered by viral glycoprotein structures without a live virus present or without a concurrent viral infection[26].

Once the TGEV PAMP matches to the DCs TLRs, a process called dimerization occurs. Initial signaling pathways are then initiated which lead to the MyD88 signaling cascade, ultimately resulting in the production of interferon-alpha (IFN-α) and other proinflammatory cytokines[25]. IFN-α is a type 1 interferon that can help to induce viral resistance as well as initiating its own signaling cascade to activate the adaptive immune response[27]. Specifically, it can mediate inhibition of viral replication, and activate local cell-mediated immunity[28].

Adaptive immune response

In TGEV, the adaptive response involves both cell-mediated and humoral immunity. The production of the antibody IgA has been identified as particularly important in protecting pigs against future infection[27]. The importance of stimulating this local IgA and cell-mediated response is a finding that has led to an increased interest in oral vaccines, which may be a more effective route of administration than traditional injectable vaccines[28].

The stimulation of a protective adaptive response in pregnant pigs is crucial in allowing for the transfer of effective lactogenic immunity to newborn piglets[29]. Lactogenic immunity refers to the passive transfer of antibodies to newborn pigs through the mother's milk. This is necessary for disease protection in piglets because in pigs, unlike in humans, maternal antibodies are not passed to the neonate *in utero*. They must be consumed in the first milk produced by the mother that is very rich in antibodies called colostrum, and continue to be consumed in subsequent nursing for the first several weeks of life as their immune systems develop[14].

IgA has been identified as the key antibody produced that must be present in milk to protect piglets from severe disease. Once it is stimulated in the intestines, IgA travels to both the lamina propria of the intestines and the mammary glands[30]. The mammary gland then produce specific IgA that is transferred to the milk. Protective levels for piglets in milk are only produced by mothers exposed to a virulent form of the virus *via* the oral route. Attenuated oral vaccines given to pregnant mothers offer only partial protection to neonates[14].

To achieve the best level of protection in the neonate, piglets must be continually nursing or being fed milk with high levels of anti-TGEV or PEDV IgA. However, even with continuous consumption of IgA the lactogenic immunity is incomplete. Limiting the amount of virus in the environment is also critical for the protection of neonates[30].

CURRENT CONTROL STRATEGIES

Given what is known about the pathogenesis, immune responses, and transmission of TGEV and PEDV, current population based control strategies focus on prevention of disease, as there is no curative treatment available. These strategies include herd management, strict biosecurity on the farm, and vaccination protocols[30].

In epidemic infections, affected piglets are treated with fluids in an effort to maintain hydration and improve survival rates. In those facilities with adequate space, mothers who are due to give birth are isolated in order to protect the newborn piglets. In those where space is not available, the only effective option to stimulate lactogenic IgA production or offer future protection from disease in naïve animals is to feed the feces of infected piglets to expectant mothers or other herd members[30].

However, this is both time and labor intensive, and offers its own challenges and risks. Because only general guidelines have been agreed upon in terms of amount of feces that should be used to inoculate mothers, in some cases the doses have been more virulent than expected and caused disease. In other cases they have been less virulent, and were not protective. Also, there is no guarantee that the animals are only being infected with TGEV or PEDV - other diseases can be transmitted throughout the herd in the feces. Furthermore, intentionally infecting animals leads to more challenges with biosecurity to avoid transmission to neighboring farms[30].

Those farms not currently experiencing an outbreak or where the diseases are endemic are not able to use this method to stimulate immunity in their herds. They must turn to available injectable vaccines, which do not offer full protection against the disease, and do not stimulate sufficient lactogenic immunity to protect newborn piglets. Available oral attenuated vaccines also lack effectiveness in preventing disease in naïve animals[30]. However, the vaccines have been shown to be effective as boosters in previously exposed pigs, and are able to stimulate appropriate levels of IgA to offer lactogenic immunity to piglets[14].

FUTURE CONTROL STRATEGIES

Therefore, future control strategies for TGEV and PEDV

are largely focused on the creation of more effective vaccines to control these diseases and prevent further spread. Proposals for improved modified live and attenuated vaccines are currently under investigation.

Other areas of research include improved treatment options for those animals that have already been infected to lessen clinical signs and decrease mortality. Research in these areas include methods to directly stimulate the innate immune response to overcome the dysregulatory effects of these viruses, as well as preventing the extensive cell death that also leads to the debilitating and often fatal clinical signs.

Proposed vaccines

Given the recent increase in understanding of the gut-mammary gland-IgA axis as described above, creating a safe, effective, and cost-effective live oral vaccine to induce lactogenic immunity as well as providing immunity to naïve herds is one of the main goals of TGEV and PEDV vaccine research. Several modified live (attenuated) vaccines have been proposed. Some strategies for creating these vaccines include deleting proteins and genes in order to decrease virulence. However, modified live vaccines still carry the risk of reverting to virulence and causing disease[31].

One protein deletion investigation involves non-structural protein 1 (nsp1), which has been shown in mammalian studies to be the first mature viral protein to be expressed in the cytoplasm of the host cell. It may be responsible for the degradation of the host cell mRNA leading to apoptosis, as well as interfering with the IFN-α release that is one of the key components to effective innate immune response in early infection. A study in mice infected with a modified coronavirus where nsp1 had been deleted showed that the virus replicated as efficiently as the unmodified virus, but with greatly reduce virulence. It was able to stimulate efficient memory T cell responses that were protective[32].

Another study using swine testicular (ST) cells as a model targeted a gene called *ORF 7*, and showed that when it is targeted and inactivated, viral replication and expression of genes in ST cells are inhibited[33]. These types of investigations may lead to the development of more effective and safer modified live vaccines for swine producers. Because of the inherent risks involved in administering modified live vaccines, subunit vaccines are another area of interest in preventing TGEV and PEDV.

One study demonstrated that recombinant plasmids containing the "S" proteins of TGEV and PEDV could be created, and stimulated a robust proliferation of T lymphocytes and specific antibodies for the viruses[20]. Producing inexpensive vaccines made from transgenic plants expressing the S glycoprotein from TGEV have also been proposed[34].

Proposed treatments for infected animals

Some interest has also been generated in stimulating the innate immune response after an infection has occurred. How COVs like TGEV downregulate or dysregulate the immune system is not completely understood, but new evidence strongly suggests this viral ability[33].

One proposed treatment to counteract this dysregulation is to directly stimulate NIPCs. Research has shown that porcine NIPCs can recognize a PAMP called an unmethylated CPG motif, which is a short RNA sequence. Exposure to this PAMP leads to production of IFN-α and other inflammatory cytokines. This NIPC response has also been documented in humans. These unmethylated CPG motifs may be useful as adjuvants in new vaccines for both human and porcine COVs[35].

It is now understood that TGEV causes cell death (apoptosis) by irreversibly activating proteases found in the cytoplasm called caspases. These caspases lead to fragmentation of the host cell nucleus, resulting in the death of the cell. Studies have shown that apoptosis caused by TGEV can be significantly reduced using a caspase inhibitor called Z-VAD.fmk. This does not impede virus production, but does have a protective effect on infected cells[36]. Treatment with lithium chloride on cells infected with TGEV and PEDV also showed that early and late cell apoptosis was inhibited[37].

Protecting host cells during disease to reduce clinical signs and improve survival rates is another area of research that may offer another an alternative to supportive care or herd inoculation during an outbreak.

CONCLUSION

Members of the *Coronaviridae* family are important viruses today for both humans and animals. Emerging coronavirus diseases threaten health and productivity. More in-depth understanding of these viruses is the key to future control and safety.

In pigs, the study of TGEV and PEDV provide a window into the complexities of viral disease pathogenesis, control, and prevention. Study of these viruses in pigs may also have direct benefits on human coronavirus control, as porcine responses to these viruses have been increasingly found to be very similar immunologically to human responses.

Important advances have been made in the understanding of the immunological response of pigs to TGEV and PEDV over the last decade. Highlights include the role of NIPCs in early IFN-α production, the immunogenicity and function of the structural and non-structural proteins of the virus, the caspase-dependent pathways resulting in apoptosis, and the role and creation of lactogenic immunity in pig populations.

These advances have led to detailed and promising research for population based control strategies in epidemic and endemic infections. Increased understanding of the best methods to induce lactogenic immunity may play a role in slowing future epidemics on individual farms. The use of caspase inhibitors to

reduce the incidence of cell apoptosis may also increase survival rates for infected pigs during an outbreak.

New vaccines may be able to induce more complete immunity with fewer side effects for naïve herds, making vaccination a more reliable and cost-effective method of disease prevention, and enabling farmers to eradicate endemic infections with TGEV and PEDV over time. These efforts will need funding to facilitate new vaccine development, testing, and deployment.

Because these diseases pose such a threat to food security and both animal and human health, a worldwide effort to eradicate these diseases is likely an ultimate goal. Cooperation between human doctors and veterinarians will be essential in this endeavor. Increased understanding of the immunological responses to these diseases and future treatments will be at the forefront of new control strategies going forward.

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