Open Veterinary Journal, **(2023), Vol. 13(12): 1517-1535**
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Review Article
DOI: *10.5455/OVJ.2023.v13.i12.2*

Submitted: 08/09/2023 Accepted: 22/11/2023 Published: 31/12/2023

Host immune responses against African swine fever virus: Insights and challenges for vaccine development

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Abstract
The African swine fever virus (ASFV) poses a serious threat to global swine populations, underscoring the urgent need for effective preventive strategies. This comprehensive review investigates the intricate interplay between innate, cellular, and humoral immunity against ASFV, with a focus on their relevance to vaccine development. By delving into immunopathogenesis and immunological challenges, this review article aims to provide a holistic perspective on the complexities of ASFV infections and immune evasion. Key findings underscore the critical role of innate immune recognition in shaping subsequent adaptive immune defenses, potential protective antigens, and the multifaceted nature of ASFV-specific antibodies and cytotoxic T-cell responses. Despite advancements, the unique attributes of ASFV present hurdles in the development of a successful vaccine. In conclusion, this review examines the current state of ASFV immune responses and offers insights into future research directions, fostering the development of effective interventions against this devastating pathogen.
Keywords: African swine fever virus, Immune response, Swine industry, Vaccine development.

Introduction

One hundred years ago, the emergence of African swine fever (ASF) in Kenya devastated domestic pigs (*Sus scrofa domesticus*) (Costard *et al.*, 2013). ASF is attributed to ASF virus (ASFV), an enveloped icosahedral arbovirus with a double-stranded DNA structure that displays genomic similarities to poxviruses, housing approximately 160 genes sequenced in its genome. ASF's incubation period of ASF spans a variable timeframe contingent on factors such as virus virulence, host attributes, viral load, and route of infection (Guinat *et al.*, 2016). Clinically, ASF symptoms resemble those of other hemorrhagic illnesses and manifest in diverse forms, including peracute, acute, subacute, and chronic. In cases of peracute manifestation induced by potent ASFV strains, pig mortality can increase to 100% within a mere four (dpi), often without discernible lesions (Gallardo *et al.*, 2015).

In addition to domestic pigs, ASFV carriers include warthogs (*Phacochoerus africanus*), bush pigs (*Potamochoerus porcus*), and parasitizing ticks (*Ornithodoros moubata* spp.), potentially serving as reservoirs that sustain infections for over five years, contributing to the endemicity of ASF within regions (Penrith, 2020). Notably, ASFV persistence within ticks for extended periods, such as the documented four-year presence in unoccupied domestic pig premises in Madagascar, highlights the virus's resilience (Jori *et al.*, 2023). The presence of ASF since its first documentation in Kenya in 1921 has led to its dissemination across African and non-African countries (Fig. 1), affirming its endemic nature and persistent threat to the swine populations across approximately 32 African countries (Penrith, 2013).

ASFV primarily targets immune cells of myeloid lineage, such as dendritic cells, macrophages, and monocytes (Sánchez *et al.*, 2012). The virus enters host cells through either clathrin-mediated endocytosis or macropinocytosis (Duan *et al.*, 2022). Intracellularly, ASFV possesses distinct layers: a large DNA genome at the core, encased by a core shell, inner lipid envelope, and icosahedral capsid. As ASFV buds through its plasma membrane, it acquires an external envelope. Although both extracellular and intracellular virions are infectious, the precise role of the outer envelope is still uncertain (Wang *et al.*, 2019). The ASFV genome size

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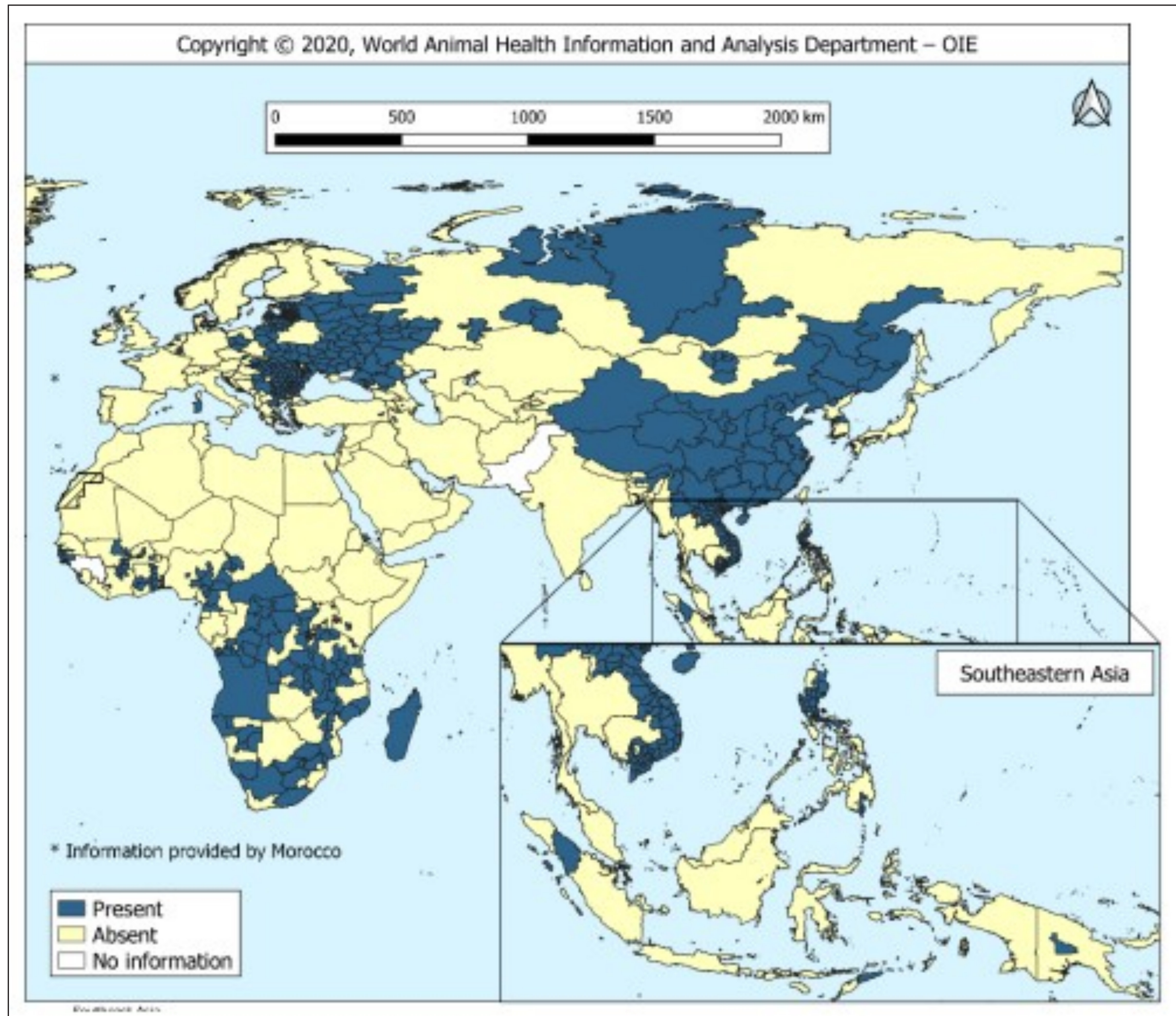

Fig. 1. Global distribution of ASFV (OIE, 2020).

varies significantly (170–194 kb) across geographical isolates and encompasses over 150 open reading frames (ORFs) (Dixon *et al.*, 2013). ASFV genes are categorized into immediate-early, early, intermediate, and late classes (Cackett *et al.*, 2020).

Vaccination serves as a potent strategy against viral infections; however, the development of an effective vaccine against ASFV remains elusive (Karger *et al.*, 2019; Orosco, 2023a). The challenge lies in the incomplete understanding of the host immune responses triggered by ASFV (Dixon *et al.*, 2019). Complex evasion tactics and genetic variability hinder the development of an effective ASF vaccine (Correia *et al.*, 2013; Orosco, 2023a). The ongoing epidemics in Asian nations and the resurgence of ASF in Europe (Table 1) underscores the necessity for an ASF vaccine. Fortunately, dedicated efforts are underway to explore anti-ASFV immune responses and identify potential vaccine antigens.

This review aims to comprehensively examine the innate, cellular, and humoral immune responses against ASFV, relevant to the development of a safe and effective vaccine against the pathogen. The immunopathogenesis and immunological challenges in ASFV vaccine development are also discussed.

African swine fever virus (ASFV): An overview

ASFV is a large enveloped DNA virus and represents the only member of the *Asfarviridae* family and *Asfivirus* genus (Arias *et al.*, 2018). The genetic makeup of the virus encodes 150–165 proteins that are indispensable for the evasion of host immunity and virus replication (Dixon *et al.*, 2013). A comprehensive proteomic map of ASFV has been outlined previously (Alejo *et al.*, 2018; Mahedi *et al.*, 2023). Notably, both structural and

Table 1. The number of outbreaks and losses caused by ASF in different regions (2016–2022) (OIE, 2022).

Locations	Outbreaks		Cases		Losses*	Total Outbreaks
	Domestic pigs	Wild boar	Domestic pigs	Wild boar	Domestic pigs	
Africa	277	-	74,085	-	105,509	277
Americas	210	-	8,592	-	14,972	210
Asia	10,967	2,149	204,344	2,746	7,132,038	13,116
Europe	7,607	33,565	1,553,645	57,185	2,643,923	41,172
Oceania	4	-	500	-	397	4
Total	19,065	35,714	1,841,166	59,931	9,896,839	54,779

*Losses in the establishments affected by the outbreaks and it does not include the animals culled in areas around the outbreak for controlling the disease. Data reported since January 2020 covers only epizootic situations.

infection-related proteins play roles in regulating host immune evasion mechanisms. ASFV enters the host cells through cell surface receptors, often following an infection route through the tonsils proximate to the lymph nodes (Reis *et al.*, 2017).

Subsequent to viremia, ASFV translocates to tissue organs, with primary transmission occurring through direct contact with infected pigs, contaminated fomites, or swills (Guinat *et al.*, 2016; Orosco *et al.*, 2023b). *Ornithodoros* ticks acquire the virus while feeding on infected pigs; within them, the virus undergoes replication in the gut tissues and subsequently migrates to the salivary glands. These infected ticks act as vectors and can transfer the virus from one pig to another through bites. ASFV's entry of ASFV into pig cells occurs via endocytosis (Fig. 2), encompassing both clathrin-mediated and receptor-mediated pathways (Galindo and Alonso, 2017).

In new herds, ASF presents with extensive mortality, marked by elevated fever, reduced appetite, limited mobility, and congregating pigs. In severe cases, death can precede the emergence of other clinical indications for up to four days. The onset of clinical symptoms can be influenced by factors such as incubation period, viral genotype, exposure route, environmental circumstances, and animal breed (Jori and Bastos, 2009).

Less severe cases of ASF are characterized by petechial hemorrhage, mucoid diarrhea, and skin reddening near the ears, abdomen, and limbs (Pikalo *et al.*, 2019). Post-mortem examinations revealed hemorrhages within internal organs, including the liver, lungs, intestines, kidneys, heart, and lymph nodes. The spleen has an increased size and abnormally darkened appearance (Sánchez-Cordón *et al.*, 2018; Orosco *et al.*, 2023c). The contributions of free-living hosts, scavenging pigs, and pigs recovering from ASF to the epidemiology of the disease remain uncertain, although several investigations suggested their potential roles in virus transmission (Probst *et al.*, 2019).

Immunopathogenesis of ASFV

ASF is distinguished by profound leukopenia, particularly lymphopenia, and a widespread immunodeficiency (Patil *et al.*, 2020). Initial pig infection stems from the oral-nasal route or bites from infected ticks. The virus reproduces within regional lymph nodes or tonsils (Pikalo *et al.*, 2019) and subsequently disseminates via the blood and lymph to secondary replication sites within 2–3 days (Colgrove *et al.*, 1969; Orosco, 2024a) before migrating to other organs, enabling replication across diverse cell types (Heuschele, 1967).

Macrophages and monocytes are the primary targets of ASFV (Dixon *et al.*, 2019). Although ASFV is a DNA virus, its replication occurs in the cytoplasm, not within the nucleus (Coelho and Leitão, 2020). Infected monocyte-macrophages exhibit enlargement and nuclear chromatin margination and contain an intracytoplasmic juxtanuclear inclusion body. These bodies reveal viral factories by transmission electron microscopy. Virus replication leads to necrosis in host cells, and virions are released via budding (Salguero, 2020).

ASF-triggered monocyte-macrophage destruction has been linked to ASFV-induced apoptosis or necrosis (Afe *et al.*, 2023). The ASFV genome encompasses genes that influence programmed cell death, with both inhibitory (i.e., A179L, A224L, DP71L, and EP153R) and inductive roles (i.e., A199L and E183L) (Netherton *et al.*, 2019a). Certain genes can enhance the survival of infected cells whereas apoptosis is believed to be an improbable cause of cell death within the infected monocyte-macrophage subset (Dixon *et al.*, 2017).

ASF is distinguished by the extensive degradation of lymphoid organs, including the lymph nodes, spleen, tonsils, and thymus (Sánchez-Cordón *et al.*, 2021). In acute ASFV infection, notable proportions of T and B lymphocytes, along with macrophages, undergo cell death (Schäfer *et al.*, 2022). Replication of the virus within monocyte-macrophages triggers its activation, leading to increased secretion of proinflammatory cytokines during the early stages of the disease (Gómez-Villamandos *et al.*, 2013). The heightened expression of proinflammatory cytokines recognized as a "cytokine storm," underpins the considerable lymphocyte

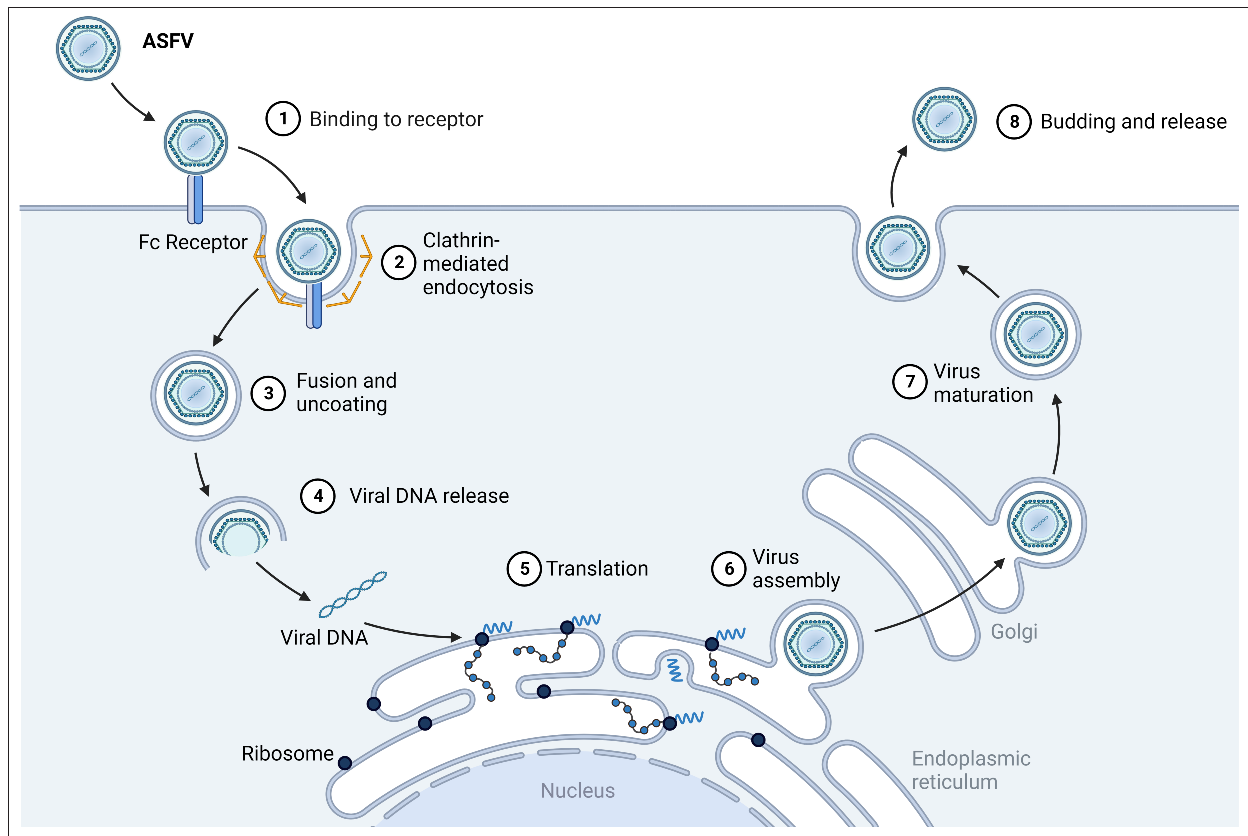

Fig. 2. Replication cycle of ASFV.

apoptosis in proximity to activated/infected monocyte-macrophages within tissues (Machuka *et al.*, 2022).

Innate immunity against ASFV

Innate immune sensing

The innate immunity constitutes the initial defense against infection (Riera Romo *et al.*, 2016). Pathogens carry distinctive conserved elements termed pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors (PRRs) in the cytoplasm or on cell surfaces (Roers *et al.*, 2016). This recognition triggers innate immunity, culminating in the induction of proinflammatory cytokines and type I interferons (IFNs) to coordinate innate immunity (Iwasaki and Medzhitov, 2015), as illustrated in Figure 3. Among these, IFNs, particularly type I IFNs, play a pivotal role in antiviral defense (Ivashkiv and Donlin, 2014). Specific IFN-stimulated genes, including IFN-induced transmembrane (IFITM) genes and myxovirus resistance (Mx), which contribute to anti-ASFV defense, have been described previously (Muñoz-Moreno *et al.*, 2016).

ASFV primarily multiplies within mononuclear-phagocytic cells, which is pivotal for instigating innate and adaptive host immunity (Sánchez *et al.*, 2012). Consequently, cytoplasmic PRRs, such as cyclic AMP-GMP synthase (cGAS), Toll-like receptors (TLRs), RIG-I-like receptors (RLRs) and/or stimulators of interferon genes (STING), have a crucial role in the detection of ASFV infections (Iwasaki and Medzhitov, 2015). ASFV also produces proteins capable of subduing TLR and cGAS recognition, thereby evading antiviral immunity (García-Belmonte *et al.*, 2019). In contrast to the virulent strain Armenia/07, attenuated ASFV NH/P68 promptly triggers the cGAS-STING-IRF3 cascade, leading to early STING phosphorylation and trafficking through cGAMP mediation. Following TBK1 and IRF3 activation, substantial IFN-β production occurs during NH/P68 infection, whereas virulent ASFV impedes IFN synthesis, a pivotal link between innate and adaptive immunity (García-Belmonte *et al.*, 2019). Detailed insights into IFNs' regulatory mechanisms in ASFV infection across *in vitro* and *in vivo* investigations have been reviewed previously (Dixon *et al.*, 2019).

ASFV's interference of ASFV with innate immunity begins by evading PRR recognition (Dixon *et al.*, 2019). As such, the I329L protein resembles TRIF in its C-terminal region and acts as a type I transmembrane protein, curtailing dsRNA-triggered IRF3 and NF-κB activation, which are the central components of innate immunity. This suggests that the viral modulation gene could potentially target TRIF, a key MyD88-independent adaptor. In addition, ASFV's A238L

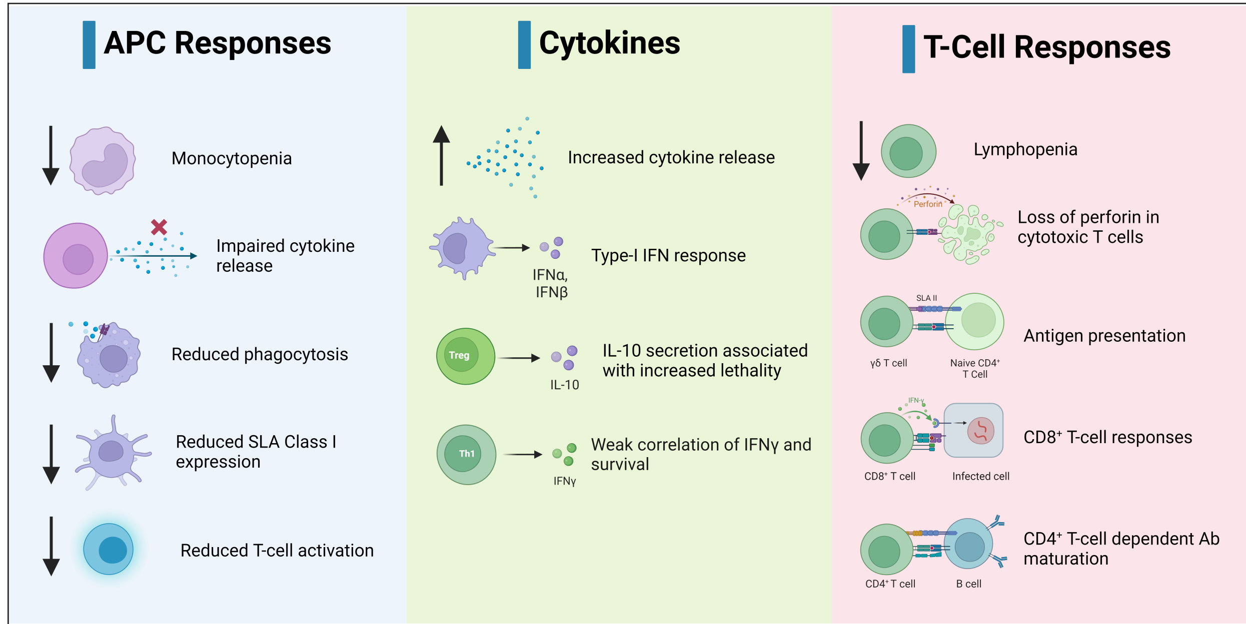

Fig. 3. Host immune responses against ASFV infection.

protein binds the p65 subunit of NF-κB, thereby inhibiting its functionality (Hong *et al.*, 2022).

Apoptosis and autophagy

Viruses often employ diverse tactics to modulate apoptosis and autophagy as natural antiviral responses (Leonardi *et al.*, 2021). ASFV inhibits apoptosis in infected macrophages to support viral replication and the dissemination of new virions (Dixon *et al.*, 2019). ASFV produces several proteins, A179L, A224L, and DP71L, which govern host autophagy and apoptosis. As such, A179L inhibits autophagy and apoptosis by binding to Beclin-1. The apoptosis inhibitor A224L suppresses caspase 3 activation and amplifies NF-κB, curtailing apoptosis via anti-apoptotic genes, such as Bcl-2 and IAP (Shi *et al.*, 2021). DP71L hampers CHOP induction *in vitro*, indicating that ASFV encodes analogous functional genes (Zhang *et al.*, 2010). Although the specifics of apoptosis inhibition remain unclear, ASFV's effects on autophagy are less explored, with limited knowledge beyond A179L inhibiting starvation-induced autophagosome formation and LC3B presence at ASFV replication sites upon overexpression (Banjara *et al.*, 2019). A comprehensive investigation of the factors affecting apoptosis in uninfected lymphocytes and macrophages during acute ASFV stages holds the potential for vaccine development (Gómez-Villamandos *et al.*, 2013; Orosco, 2024b).

Cytokines and chemokines

Cytokines critically regulate innate immune and inflammatory responses, including IFNs, chemotactic factors, interleukins (ILs), and tumor necrosis factors (TNFs) (Schwartz *et al.*, 2016). Macrophages, which is ASFV's primary target, engage in pivotal interactions (Fig. 3). *In vitro* studies indicate that the attenuated NHV strain of ASFV triggers higher IL-12p40, TNF-α, and IFN-α levels than the virulent strain (Schäfer *et al.*, 2022). Proinflammatory cytokines increase in ASFV-infected pigs, peaking at 7 dpi, underlining their role in ASFV pathogenesis (Zakaryan *et al.*, 2015). In acute ASFV infections, these cytokines promote lymphocyte apoptosis (Salguero, 2020). Detailed analysis of ASFV interactions with macrophage and monocytes revealed that field isolates such as 22653/14 replicate more efficiently in monocytes and moMΦ, while inducing lower IL-1α, IL-1β, and IL-18release than avirulent BA71V (Franzoni *et al.*, 2017). A subsequent investigation on porcine monocyte-derived macrophages demonstrated attenuated strain NH/P68-infected moM1 secreting higher levels of IL-18, IL-1β, and IL-1a than the virulent strain 22653/14, suggesting compromised immune response development due to ASFV 22653/14 covert macrophage replication (Franzoni *et al.*, 2020). Transcriptome analysis revealed that TNF family cytokines, along with upregulated IL-17F and IFNs and downregulated IL-10, significantly contribute to ASF pathogenesis and tissue inflammation (Zhu *et al.*, 2019). Infection of porcine monocyte-derived DCs (moDCs) with ASFV strains leads to efficient replication, functional modulation, and reduced cytokine responses, potentially hindering protective immune activation (Franzoni *et al.*, 2018).

Cellular immunity against ASFV

Numerous studies have underscored the crucial role of T-cell responses in combatting ASFV infections. While extensive research exists on human and murine T cell

responses, understanding of porcine T cell immunity, especially against non-zoonotic diseases such as ASF, remains limited. In this section, existing insights and gaps in understanding porcine T cell immunity to ASFV infections and vaccination are discussed.

Lymphocyte activation

Evidence indicates that attenuated ASFV infection in pigs results in survival and subsequent protection against virulent strain challenges of the same genotype (Acton and Reis e Sousa, 2016). Attenuated ASFV infection triggers ASFV-specific CTLs and memory T cells (Fig. 3), as shown by lymphocyte proliferation assays, highlighting the protective immune responses (Lokhandwala *et al.*, 2016). Anti-CD4 and anti-CD8 mAbs hinder ASFV-specific T-cell proliferation differently, depending on the viral state, underscoring the complexity of immune interactions (Mair *et al.*, 2014). Certain ASFV antigens that offer protection without antibodies emphasize the significance of T-cells in eliminating infected cells (Acton and Reis e Sousa, 2016). Beyond T cells, NK cells and $\gamma\delta$ T cells are also activated upon ASFV infection; however, their roles in protection and potential cross-protection through T-helper cells require further verification (Takamatsu *et al.*, 2013).

αβ T Cells

All mammalian T cells share hallmark features, such as CD3 expression and TCR, enabling antigen recognition and cellular activation. The TCR comprises α and β chains or γ and δ chains in $\alpha\beta$ and $\gamma\delta$ T cells, respectively. In pigs, the prevalent $CD3^+$ T cell subset comprises $\alpha\beta$ T cells, encompassing $CD4^+$ T helper (Th) cells, $CD8\alpha^+$ CTLs, and a distinctive $CD4^+/CD8\alpha^+$ double-positive (DP) T cell population, serving as effector and memory cells (Schäfer *et al.*, 2022). This section explores the characteristics and roles of these subsets in combating ASFV infections.

$CD8\alpha^+$ αβ T cells and cytotoxic responses

$CD4^-/CD8\alpha^+$ $\alpha\beta$ T cells, commonly identified as CTLs, recognize antigens presented by SLA class I molecules, thereby responding to intracellular antigens (Fig. 3). Their function involves eliminating the infected cells to hinder the propagation of intracellular pathogens, including viruses. The initial indications of T-cell involvement surfaced in the early 1980s, as leukocytes from pigs infected with attenuated ASFV strains displayed specific cytotoxicity against infected cells (Pabst, 2020). Notably, animals infected with low-virulence ASFV strains exhibit persistent cytotoxic responses and the establishment of T cell memory up to two years post-infection, in contrast to virulent ASFV-infected animals (Schäfer *et al.*, 2022). Extensive investigations characterized CTL responses in pigs that survived low-virulence ASFV infection (Galindo and Alonso, 2017). Utilizing ASFV-immune pig PBMCs, they demonstrated specific lysis through CTL assays, employing NHV-infected macrophages as targets. This process showed specific CTL activity, with reduced lysis observed when SLA-mismatched PBMCs were used or when SLA I was blocked with a specific antibody. Moreover, the depletion of $CD8\alpha^+$ cells significantly reduced specific lysis in $CD8\alpha$-depleted samples, establishing the role of $CD8\alpha^+$ CTLs in conferring protective immunity (Galindo and Alonso, 2017). These ex *vivo* analyses marked the initial description of protective immune responses mediated by $CD8\alpha^+$ CTLs.

A study highlighting the essential role of $CD8\alpha^+$ T cells in immune protection was conducted (Sánchez-Cordón *et al.*, 2018). They utilized low-virulence OURT88/3-infected animals, which cleared the infection and subsequently depleted surviving animals of $CD8\alpha^+$ lymphocytes using specific antibodies. Upon challenge with the highly virulent OURT88/1, depleted animals succumbed to infection, whereas nondepleted animals survived with mild clinical signs. Although various porcine lymphocyte subsets express $CD8\alpha$, the exact contributors to immunity remain unclear. Attempts to specifically deplete CTLs using a different antibody (clone PPT22) resulted in partial depletion in one out of ten animals, and this particular animal succumbed to the challenge. Although this study focused on memory responses, it provided definitive *in vivo* evidence of the crucial role of $CD8\alpha$ + T cells in immune memory against ASFV (Sánchez-Cordón *et al.*, 2018).

Recent investigations of cytotoxic responses involved domestic pigs and wild boars during distinct ASFV infections at up to 10 dpi. Moderate virulent Estonia2014 infection induced $CD8\alpha^+$ CTL frequency increases 5–10 dpi in infected domestic pigs, paralleled by similar responses in wild boars. In addition, wild boars displayed T-bet-dependent differentiation at 10 dpi, indicating antiviral Th1 response initiation (Oura *et al.*, 2005). Highly virulent Armenia2007 infection prompted DP T cell dominance in both subspecies, with $CD8\alpha^+$ CTLs showing no variation until 7 dpi (Schäfer *et al.*, 2021).

An intriguing and consistently reproducible discovery emerged from multiple independent trials: a marked reduction in the expression of the key lytic effector protein perforin within various cytotoxic effector populations at 4–5 dpi during both moderately and highly virulent ASFV infections. This phenomenon was particularly pronounced in domestic pigs infected with the Armenia2007 strain, and to a lesser degree after the Estonia2014 infection. While wild boars also exhibited reduced perforin expression, the effect was generally milder than that in domestic pigs (Hühr *et al.*, 2020).

However, the cause of this observation remains unclear. In addition to perforin, CTLs employ other cytotoxic molecules, including the TNF-related apoptosis-inducing ligand (TRAIL) or Fas ligand (FasL or CD95L/CD178), to trigger death receptor-mediated apoptosis instead of direct cell lysis (Schäfer *et al.*, 2021). It is plausible that ASFV-infected CTLs switch to apoptosis induction via FasL or TRAIL, which

can lead to a complete loss of perforin expression (Prager and Watzl, 2019). This adaptation might be advantageous, as certain ASFV strains encode a viral homolog (A179L) of the anti-apoptotic Bcl-2 protein (Meiraz *et al.*, 2009), which most effectively counteracts perforin-mediated killing (Sutton *et al.*, 1997). Reduced perforin in domestic pigs could signify a shift toward death receptor-mediated killing, whereas the response in wild boar might have been caused by the virus. Liver-based antiviral perforin responses have been linked to substantial tissue damage (Welz *et al.*, 2018), offering a potential explanation for the observed Kupffer cell degeneration in wild boars and broader differences in disease outcomes (Sehl *et al.*, 2020). Another hypothesis involves perforin consumption, possibly due to T cell exhaustion or hyperinflammatory responses. The immediate secretion of synthesized perforin, which is not detectable by the mAb employed (Hersperger *et al.*, 2008), suggests proinflammatory and polyclonal activation. Intense inflammatory cues and high antigen burdens during acute ASFV infections can lead to continuous T-cell activation, culminating in T-cell exhaustion (Erickson *et al.*, 2015). However, the exact mechanism requires further investigation.

CD4⁺ and CD4⁺/CD8α⁺ αβ T cells

Porcine $CD4^+$ T cells encompass $CD4^+/CD8\alpha^-$ $\alpha\beta$ Th cells, which play roles in antibody production, immune response orchestration, and affinity maturation (Fig. 3). Unlike humans and murine T cells, activated or memory $CD4^+$ T cells express CD8α (Pabst, 2020). This phenomenon has been observed across species, including monkeys, chickens, and dogs (Overgaard *et al.*, 2015). This chapter explores $CD4^+/CD8\alpha^+$ DP T cells that contribute to immune processes. Despite the focus on CTL responses, emerging studies have shed light on $CD4^+$ Th cell responses during ASFV infection. Early investigations have demonstrated $CD4^+$ T cell proliferation in response to live or UV-inactivated ASFV, highlighting their participation (Bosch-Camós *et al.*, 2022). Blocking experiments indicated $CD4^+$ Th cell involvement, although discerning $CD4^+/CD8\alpha^-$ from DP T cells is challenging (Bosch-Camós *et al.*, 2022). Functional relevance was observed when attenuated BA71 ASFV-immunized PBMCs showed reduced antibody production upon $CD4^+$ Th cell depletion, thus emphasizing their significance (Lopera-Madrid *et al.*, 2017).

It is noteworthy that the depletion experiments conducted by Oura *et al.* likely affected DP T cells formed during primary ASFV infection, making it challenging to distinguish the repercussions of depleting $CD4^-/CD8\alpha^+$ or DP T cells (Oura *et al.*, 2005). This, along with other studies demonstrating DP T-cell development after ASFV infection, underscores their role in secondary responses to ASFV. While investigations into DP T cell function during immunity generally focus on memory responses due to their gradual development over infection, comparative studies involving highly virulent Armenia2007 and moderately virulent Estonia2014 ASFV infections examined primary T cell responses (Schäfer *et al.*, 2021). Domestic pigs infected with Armenia2007 exhibited a substantial increase in DP T cells at 5–7 dpi. Notably, these cells did not proliferate, as indicated by the absence of Ki-67 expression. The inverse correlation with observed $CD4^+$ T cell levels suggested the differentiation of DP T cells from $CD4^+/CD8\alpha^-$ Th cells. Conversely, in wild boars, DP T cells underwent extensive proliferation without a significant increase during the study. These cells could have spread to unexamined tissues or undergone apoptosis during antiviral responses (Schäfer *et al.*, 2021).

Estonia2018, a moderately virulent strain, triggered increased DP T cell frequencies in the livers, lungs, and spleens of domestic pigs and the livers and lungs of wild boar. Notably, DP T cell proliferation is exclusive to the spleen in infected domestic pigs (Sauter-Louis *et al.*, 2021). Interestingly, perforin loss observed in CTLs was not mirrored in the DP T cells. As proliferating DP T cells were solely identified in the spleen, it was inferred that during moderately virulent ASFV infection, DP T cells likely assume orchestrating, rather than cytotoxic, roles (Sauter-Louis *et al.*, 2021). This contrasts with prior evidence of perforin increase in DP T cells after infection with the low-virulence OURT88/3 strain, although this study employed different infection strains and was conducted with only two pigs. Collectively, these investigations underscore the substantial contribution of DP T cells, which serve as both memory cells guarding against recurrent infections and evolving effector cells during primary ASFV infections.

Regulatory T-cell responses

Regulatory T cells (Tregs) share the $CD3^+/CD4^+/CD25^+/FoxP3^+$ phenotype with other mammals and merit dedicated discussion because of their distinctive immunoregulatory role (Żabińska *et al.*, 2016). Tregs are a subset of $CD4^+$ $\alpha\beta$ T cells that secrete IL-10, an immunomodulatory cytokine with dual effects, suppressing antigen presentation and proinflammatory Th1 cytokines (Fig. 3), while enhancing antibody production and B cell responses (Catalán *et al.*, 2021). The comprehension of Treg responses in ASFV-infected pigs remains incomplete, as infected animals typically succumb or require euthanasia before Treg responses manifest. However, insights from ASFV and other viruses suggest that Tregs play a pivotal role in modulating porcine antiviral response. For instance, porcine Tregs were linked to viral persistence during porcine reproductive and respiratory syndrome virus (PRRSV) infection via IL-10 secretion, which dampens Th1-mediated antiviral responses (Silva-Campa *et al.*, 2012). Notably, several ASFV vaccination studies observed elevated IL-10 levels in nonresponsive pigs that eventually succumbed to challenge infections, accompanied by increased IFNγ levels, which is a

potential sign of skewed immune responses in moribund animals (Sánchez-Cordón *et al.*, 2020).

Numerous infection investigations have highlighted the detrimental link between IL-10 secretion and survival outcomes. For instance, Cabezón *et al.* conducted a study involving wild boars exposed to classical swine fever virus (CSFV) immediately after birth, followed by infection with the moderately virulent E75 ASFV strain. All CSFV-infected animals succumbed to infection within 6–7 dpi, whereas CSFV-negative animals secreted detectable IL-10 levels. Interestingly, some of these IL-10-producing wild boar survived, indicating a complex relationship between survival, infection, and IL-10 (Cabezón *et al.*, 2017). A separate investigation demonstrated that vaccinated wild boars showed no increase in IL-10 levels after contact with shedding ASFV-infected pigs. In contrast, unvaccinated controls display elevated IL-10 levels and later succumb to ASFV infection (Barroso-Arévalo *et al.*, 2021). Similarly, Reis *et al.* noted increased IL-10 levels in animals fatally challenged with the virulent OURT88/1 ASF strain, following immunization with an I329L deletion mutant derived from the attenuated OURT88/3 strain. These observations collectively suggest that elevated IL-10 levels might indicate a system unable to recover rather than a coordinated immune response (Reis *et al.*, 2020). Consequently, previous investigations in wild boars and domestic pigs have identified increased and early Treg frequencies in wild boars during moderately and highly virulent ASFV infections, aligning with higher lethality and disease severity. However, cytokine measurements were not performed in those studies (Schäfer *et al.*, 2019).

Conversely, Post *et al.* (2017) presented a contrasting perspective, associating elevated IL-10 levels with survival during moderately virulent ASFV infections. However, the relatively modest IL-10 concentrations and notable age and quantity distinctions between survivors and nonsurvivors raise questions about the robustness of these findings, suggesting that age-related disparities might play a role.

γδ T cells

In contrast to the conventional TCR-MHC interaction that activates *αβ* T cells, *γδ* TCR is believed to function akin to PRRs (Chien *et al.*, 2014), with viral proteins identified as ligands of *γδ* TCRs (Halary *et al.*, 2005). Unlike humans or mice, pigs possess abundant circulating *γδ* T cells, which are grouped into terminally differentiated effector ($CD2^+/CD8α^+$), activated ($CD2^+/CD8α^−$), and naïve ($CD2^−/CD8α^−$) subsets based on CD2 and CD8α expression (Sedlak *et al.*, 2014). Given their functional flexibility and substantial numbers, the role of *γδ* T cells in ASFV infection has been explored. A recent investigation involving domestic pigs revealed that increased *γδ* T cell frequencies correlated with enhanced survival after low- or high-dose infection with moderately virulent Netherlands'86 ASFV (Post *et al.* 2017). Previous studies have indicated that porcine *γδ* T cells can present antigens to other T cells (Takamatsu *et al.*, 2002) and restore ASFV-specific proliferation without professional APCs (Takamatsu *et al.*, 2006). Thus, it is possible that the observed protection stems from heightened antigen presentation by *γδ* T cells, compensating for compromised presentation by APCs in early ASFV infection. However, the small survivor cohorts and the most pronounced impact in correlation with age suggest that survival is primarily influenced by immune maturity and age rather than specific cell frequencies (Post *et al.*, 2017).

The protective role of *γδ* T cell responses faces challenges from studies on ASFV infections in wild boars. These studies consistently reveal a *γδ* T cell-dominated response with more severe clinical manifestations and higher fatality rates following experimental infection, in contrast to domestic pigs (Zani *et al.*, 2018). Highly virulent ASFV Armenia2007 infection prompts heightened *γδ* T cell levels or $CD8α^+$ effector cell differentiation in the spleen, gastrohepatic lymph nodes, and blood of wild boar, distinct from domestic pigs (Hühr *et al.*, 2020).

Disparities between the two species were more pronounced during trials with the moderately virulent ASFV Estonia2018 strain. Notably, domestic pigs exhibit minimal *γδ* T cell responses, while wild boars display significant *γδ* T cell bias and elevated $CD8α^+$ *γδ* T cell frequencies at 4–7 dpi (Schäfer *et al.*, 2021). This trend is most pronounced in infected wild boar livers, correlating with severe Kupffer cell degeneration at 7–10 dpi (Sehl *et al.*, 2020). Increased $T\text{-}bet^+$ T cells are also exclusive to infected wild boar (Schäfer *et al.*, 2021). These findings, in contrast to prior studies, suggest a potentially pathogenic *γδ* T cell-driven immune response, potentially contributing to the higher fatality rate upon Estonia2018 infection (Sehl *et al.*, 2020). The divergent outcomes of ASFV infection in domestic pigs and wild boars may also stem from intrinsic differences in their immune responses, an area that has not yet been extensively explored.

Nonconventional T cells

Other T cell subsets exhibit distinct characteristics that differentiate them from the conventional *αβ* and *γδ* T cell populations. An extensively studied example is the invariant natural killer T (iNKT) cells. These cells share typical T-cell traits, including thymic development, CD3 and TCR expression, and antigen-specific responses upon activation. However, they also possess unique attributes that separate them from each other. iNKT cells show an antigen-experienced phenotype post-thymic egress, and their distinctive invariant TCR, with limited variability, recognizes a narrow range of antigens, typically glyco- and phospholipids or α-galactosylceramide (αGC) in research contexts, bound to MHC-related CD1d. Furthermore, cytokines, including type I IFN, IL-18, and IL-12, can trigger iNKT cell responses (Kohlgruber *et al.*, 2016). Despite some exploration of porcine iNKT cells (Thierry *et al.*,

2012), their role in ASFV infection remains largely unexplored.

Early studies have suggested the involvement of NKT cell-like lymphocytes in ASFV responses, as these cells expanded following *in vitro* culture of naïve PBMCs with SLA-matched ASFV-infected cells (Denyer *et al.*, 2006). Subsequent investigations using a murine CD1d tetramer loaded with the αGC analog PBS57 confirmed the presence of iNKT cells in ASFV infection. *In vivo* studies demonstrated a significant increase in iNKT cell frequency in the gastrohepatic lymph nodes, liver, peripheral blood, and lungs of domestic pigs infected with the highly virulent Armenia2007 strain (Schäfer *et al.*, 2019). Notably, although *in vivo* responses were evident, no activation was observed when porcine PBMCs were exposed to Armenia2007 *in vitro* because of the potential immune escape mechanisms. Conversely, during moderately virulent Estonia2014 infection, an upsurge in $ICOS^+$ iNKT cells in the blood and spleen was identified (Schäfer *et al.*, 2021), a marker associated with highly activated iNKT cells in pigs and mice. Collectively, these findings suggest the participation of porcine iNKT cells in antiviral responses, potentially linked to their robust IFNγ production capacity. However, further investigation is required to fully comprehend the scope and influence of iNKT cell responses (Kohlgruber *et al.*, 2016).

In addition to iNKT cells, mucosal-associated invariant T (MAIT) cells represent another subset of nonconventional T cells. These cells have been thoroughly studied in various species, including mice and humans, and have been implicated in antiviral responses against multiple viruses (Garner *et al.*, 2018). However, in pigs, only one study has reported the presence of cells expressing transcripts of the invariant MAIT cell TCR chain TRAV1-TRAJ33 (Xiao *et al.*, 2019). However, comprehensive cellular identification and analysis of their roles in response to infectious diseases in pigs remain unclear.

Humoral immunity against ASFV

Passive administration of convalescent swine sera has demonstrated the potential for protection against virulent ASFV challenge, providing significant insight into the development of an efficacious ASFV vaccine (Wang *et al.*, 2019; Orosco, 2024c). The generation of specific antibodies targeting p72 and p54 proteins during infection has been shown to neutralize approximately 10% more virus infectivity than antibodies derived from pigs immunized with recombinant proteins. These antibodies, specifically anti-p72 and anti-p54, exert inhibitory effects on virus binding to cells, whereas anti-p30 antibodies impede virus entry. Notably, antibodies raised against the dynein-binding domain of p54 have exhibited substantial neutralization of virus infectivity *in vitro* (Escribano *et al.*, 2013). On the other hand, antibodies interfering with viral spread can disrupt various entry mechanisms, including macropinocytosis and clathrin-mediated endocytosis (Sánchez-Vizcaíno *et al.*, 2012).

While vaccination with antibodies against p30 and p54 proteins displayed some disease course reduction upon challenge with the E75 isolate, complete immune protection against various virulent ASFV isolates remains elusive. Antibody responses targeting structural (A151R, A104R, K78R, B602L, and EP153R) and function-undefined proteins (CP312R, C44L, E184L, K205R, and K145R) were reported in recovered domestic swine (Lokhandwala *et al.*, 2019). Immunization with recombinant adenovirus cocktails confirmed the immunogenicity of CD2v, B602L, K205R, CP530R, and A104R but failed to provide protection against virulent ASFV strains, suggesting the potential for antibody-dependent enhancement (ADE) of disease (Lokhandwala *et al.*, 2017).

In summary, ASFV infection triggers both innate and adaptive immune reactions (Fig. 3). However, uncontrolled innate responses and compromised adaptive immunity can result in local and systemic tissue damage. While no definitive neutralizing antibodies have been identified for ASFV protection, the presence of anti-ASFV antibodies and CTLs appears to be crucial for defense. A comprehensive understanding of host immune responses and distinct cellular subsets is necessary to elucidate the immune responses at play during ASFV infection. The insight gained from discerning the specific CTL response and SLA-I sequencing, as well as the variations in protection seen with subunit vaccines, has implications for future vaccine designs.

Immunological challenges in ASFV vaccine development

The challenges in developing an ASF vaccine arise primarily from the intricate nature of the virus and gaps in our understanding of immune protection mechanisms and key antigens (Turlewicz-Podbielska *et al.*, 2021). This section discusses the various attributes of ASFV and extrapolates their potential implications for vaccine design, drawing insights from immunological research on other viruses.

Infectious and abundant extracellular and intracellular virions

Similar to poxviruses, ASFV displays infectivity in both the intracellular and extracellular forms (McFadden, 2005). Infectious intracellular ASFV virions of various strains have been observed within the host cells at different time points, accompanied by extracellular virions (Canter *et al.*, 2022). In contrast to poxviruses, in which extracellular virions constitute a small fraction of the total, ASFV extracellular virions constitute a significant portion of the virus population (Payne, 1986). While poxvirus studies have identified factors affecting extracellular virion release, such as A33 and A34 proteins, the mechanisms of ASFV have been less explored (Su *et al.*, 2010). ASFV extracellular virions encompass both enveloped virions released directly

from the cell membrane and cell-associated enveloped virions (CEVs) that bud with actin tail propulsion (Mucker *et al.*, 2020). Intracellular ASFVs include immature and mature virions (Rodríguez *et al.*, 2004). Antibodies against intracellular virion surface proteins, such as p54, p72, and p30/32 have demonstrated partial protection and neutralization against ASFV (Barderas *et al.*, 2001). Given the diverse infectious forms, both intracellular and extracellular ASFV virions contribute to pathogenesis, posing challenges for vaccine development. This complexity may explain the limited success of vaccine trials targeting intracellular virion antigens (Neilan *et al.*, 2004).

Extremely glycosylated CD2v and C-type lectin

ASFV CD2v and C-type lectin proteins exhibit significant glycosylation, which is a common feature of viral envelope proteins synthesized in the Golgi complex and ER (Watanabe *et al.*, 2019). CD2v is recognized for its hemadsorption activity in infected cells and its presence in mature virions (Rodríguez *et al.* 1993). The exact location of ASFV's C-type lectin of ASFV is uncertain, as it was not detected in mass spectrometric analyses of extracellular virions, potentially due to its low abundance (Alejo *et al.*, 2018). Although vaccinia virus C-type lectins are outer envelope components, ASFVs might be below detection levels, such as C-type lectins in CEVs (Mucker *et al.*, 2020). Both CD2v and C-type lectins are markedly glycosylated, with CD2v possessing 14 potential N-linked glycosylation sites in its ectodomain and C-type lectin with eight such sites (Goatley and Dixon, 2011). Their glycosylation density, approximately one site per 13 amino acids, surpasses that of other ASFV structural proteins and poxvirus outer envelope proteins (Goatley and Dixon, 2011). Glycans constitute a considerable proportion of CD2v and C-type lectins with molecular weights of approximately 55% and 65%, respectively (Goatley and Dixon, 2011). Deletion of the CD2v and C-type lectin genes in attenuated ASFVs has shown reduced or abolished protection in pigs (Petrovan *et al.*, 2022). However, these highly glycosylated proteins present challenges for vaccine development owing to glycan heterogeneity, weak carbohydrate-protein binding affinities, and immune tolerance to self-like glycans (Crispin and Doores, 2015). This "glycan shield" negatively affects immune responses and peptide loading onto MHC molecules (Li *et al.*, 2021). Inducing neutralizing antibodies against highly glycosylated proteins, such as HIV Env spike or ASFV CD2v and C-type lectin, presents substantial challenges (Li *et al.*, 2021).

Very low estimated surface densities of most envelope proteins

Using mass spectrometry, a study found CD2v in two of three purified ASFV extracellular virion samples, whereas C-type lectin was not detected. CD2v's abundance among ASFV structural proteins was notably low, in contrast to all five outer envelope proteins of the vaccinia virus (Alejo *et al.*, 2018). The density of antigens on surfaces significantly affects antigenicity and immunogenicity. HIV, with its sparse spike surface density, evades the immune response (Amitai *et al.*, 2018). CD2v's estimated surface density is low (0.03 protein per 100 nm², whereas neutralizing antibody-inducing inner membrane proteins have higher densities, such as p12 (0.94), p30/p32 (0.13), and p54 (0.33) (Alejo *et al.*, 2018). Although these estimations might be influenced by factors such as tryptic sites, solubility, and modifications, the low surface densities of p30, CD2v, p54, and possibly C-type lectins could dampen antibody avidity and immunogenicity (Goatley and Dixon, 2011). CD2v might have the majority of its expression within infected cells, impacting its surface density, and its cleavage into glycosylated and nonglycosylated fragments further contributes (Goatley and Dixon, 2011). Hyperimmune sera recognize recombinant CD2v, but not convalescent sera from recovered pigs, hinting at a low CD2v presence on virus particles (Ruiz-Gonzalvo *et al.*, 1996). The interaction of C-type lectin with MHC-I proteins and the inhibition of MHC-I exocytosis could limit its expression on the cytoplasmic membrane (Hurtado *et al.*, 2011).

ASFV infection enhance by naïve sera

Naïve sera can amplify ASFV infection compared to the culture medium, indicating that certain serum components enhance infectivity (Canter *et al.*, 2022). Enveloped viruses, including dengue, Ebola, HIV, and poxviruses, exploit phosphatidylserine (PtdSer)-binding serum proteins as bridging molecules for TAM receptors on macrophages, promoting clathrin-mediated endocytosis (Chua *et al.*, 2019). PtdSer, normally exposed during apoptosis, becomes an "eat me" signal for macrophages to engulf dying cells (Lemke, 2019). Viral infection activates caspase-3, exposing PtdSer on infected cell surfaces, a phenomenon termed apoptotic mimicry (Amara and Mercer, 2015).

Exposing PtdSer to the plasma membrane of infected cells is crucial for ASFV budding, paralleling other enveloped viruses (Canter *et al.*, 2022). Monocyte-derived macrophages are notably more susceptible to ASFV infection compared to monocytes, likely because of their heightened phagocytic activity (Franzoni *et al.*, 2017). PtdSer on the ASFV envelope possibly contributes to macrophage preference and explains the sustained infectivity of CD2v or C-type lectin knockout ASFV (Canter *et al.*, 2022). The role of PtdSer in ASFV infection, if confirmed, could pose a significant challenge for ASF vaccine development.

Unknown but likely numerous virus receptors

ASFV's preference of ASFV for macrophage infection implies restricted expression of macrophage-specific receptors as the key entry point (Gaudreault and Richt, 2019). Clathrin-mediated endocytosis and macropinocytosis are both used in pig macrophage infections (Hernáez *et al.*, 2016). Elevated SWC9 (possibly CD80) expression renders monocytic cells

susceptible to ASFV infection (McCullough *et al.*, 1999). While the CD163 antibody inhibits ASFV infection and binding (Sánchez-Torres *et al.*, 2003), CD163 alone is not sufficient for infection (Lithgow *et al.*, 2014), as CD163-knockout pigs still succumb to infection (Popescu *et al.*, 2017). Therefore, other receptors are likely to be involved (Sánchez *et al.*, 2017). CD2v binding to CD58 and C-type lectin glycosylation suggest its involvement. N-linked glycans in viral spike proteins are crucial for infectivity (Li *et al.*, 2020), and CD2v and C-type lectin glycans may interact with host glycan-binding proteins. Macrophages possess these binding proteins (Park *et al.*, 2021), although neither CD2v nor C-type lectins are vital for ASFV replication (Galindo *et al.*, 2000), implying the presence of additional receptors.

PtdSer receptors, observed in the apoptotic mimicry of enveloped viruses, may also facilitate ASFV infection (Chua *et al.*, 2019). Host membrane adhesive proteins, such as ITG$\alpha 4\beta 7$ and CD54, have similarly increased infectivity in HIV (Burnie and Guzzo, 2019). Host membrane proteins, such as ITG$\alpha V\beta 1$, ITG$\alpha 3\beta 1$, and CD9, are found in ASFV extracellular virions, suggesting that they might enhance ASFV infectivity through receptor binding. The abundant presence of PtdSer receptors and GBPs in macrophages likely explains ASFV macrophage-targeting. A possible immune-mediated enhancement in vaccine challenge studies has been noted, yet Fc receptors have not been implicated (Alcami and Viñuela, 1991). The involvement of poorly immunogenic PtdSer and host proteins could be a key hurdle in ASF vaccine development.

Antibodies cannot completely neutralize ASFV

Virus neutralization by antibodies is a key defense mechanism; however, ASFV-specific antibodies exhibit only partial neutralization effects (Gómez-Puertas and Escribano, 1997). Even at high antibody concentrations, 5%–20% of virions remain non-neutralized (Pérez-Núñez *et al.*, 2019). Antibodies against p54 and p72 hinder attachment, while ASFV p30 antibodies impede internalization (Gómez-Puertas *et al.*, 1996). Inoculation with CD2v-expressing baculovirus yielded neutralizing activity, suggesting that p72, p54, p30, and CD2v are protective antigens (Ruiz-Gonzalvo *et al.*, 1996). However, antibodies to p12, despite inhibiting infection, lack neutralization potential (Carrascosa *et al.*, 1995). The neutralizing activity of C-type lectins p22 and p17 is unknown.

Extracellular virions of the vaccinia virus resist neutralization more than intracellular virions (Benhnia *et al.*, 2013), and both extracellular and intracellular ASFV virions evade complete neutralization (Canter *et al.*, 2022). The mechanism underlying incomplete neutralization remains unclear. Preincubation with non-neutralizing sera inhibits ASFV-neutralizing serum effects (Gómez-Puertas and Escribano, 1997), aligning with the enhancing impact of naïve serum. Strikingly, PtdIns removal from ASFV decreased neutralization, whereas adding PtdIns enhanced it (Gómez-Puertas *et al.*, 1997). As both PtdSer and PtdIns are anionic phospholipids, their distribution imbalance could affect the membrane curvature, which is essential for ASFV budding. Excessive PtdIns may hinder PtdSer-receptor binding, blocking apoptotic mimicry. This scenario could render ASFV more susceptible to antibody neutralization. Low antigen density, high glycosylation, host membrane proteins, and apoptotic mimicry on the envelope collectively contribute to incomplete neutralization of ASFV.

Viral proteins control apoptosis and/or inhibit MHC-I expression

ASFV infection induces apoptosis in host cells (Galindo *et al.*, 2012). Various ASFV proteins, including CD2v, MGF360, MGF505, and E183L, exhibit proapoptotic activity (Gao *et al.*, 2021). Conversely, ASFV also produces anti-apoptotic proteins such as A224L, A179L, DP71L, and EP153R (Wang *et al.*, 2020). Moreover, pEP153R reduces the expression of MHC-I molecules in infected cell membranes (Hurtado *et al.*, 2011). ASFV proteins blocking MHC-I expression and apoptosis may compromise cytotoxicity mediated by antigen-specific T-cells, NK cells, or antibodies. This interference could weaken the effectiveness of the vaccine-induced immune response.

Understudied protective immune mechanisms

Both cellular and humoral-mediated immunity have been investigated for their contributions to protective immunity. The significance of antibodies in safeguarding against ASFV infection has been comprehensively reviewed (Escribano *et al.*, 2013). Similar to the protective effect of passively transferred antibodies in mice against the vaccinia virus (Lustig *et al.*, 2009), the protective role of antibodies has been demonstrated in pigs through the passive transfer of serum or colostrum antibodies from convalescent animals (Onisk *et al.*, 1994). Although ASFV cannot be completely neutralized by hyperimmune sera from convalescent or protected pigs in *in vitro* assays, antibodies can induce cytotoxicity in ASFV-infected cells through antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CDC) *in vitro*. Although not reported, antibody-dependent cellular phagocytosis (ADCP) might also contribute to ASFV clearance. Despite these antibody activities, subunit vaccines containing potentially protective antigens from both intracellular and extracellular virions, such as p30, p54, p72, and CD2v, failed to provide full protection in pigs, despite the presence of antigen-specific antibodies with some neutralizing activity (Sunwoo *et al.*, 2019).

Cytotoxicity mediated by cytotoxic lymphocytes was reported using 51Cr release assays *in vitro* (Alonso *et al.*, 1997). Immunity against ASFV has been associated with increased NK cell activity (Leitão *et al.*,

2001) and cytotoxicity of $CD8^+$ T cells (Takamatsu *et al.*, 2013). Depletion of $CD8^+$ lymphocytes abolished the protection against virulent ASFV strain (Oura *et al.*, 2005). Pigs immunized with plasmid DNA containing ASFV antigen genes to selectively activate T cells displayed partial protection and elevated $CD8^+$ cell counts in the blood after day 3 of infection compared with control pigs (Argilaguet *et al.*, 2012). Cross-protection conferred by attenuated ASFV correlates with cell-mediated immunity parameters measured through T-cell epitope assays, lymphocyte proliferation, and IFN-γ ELISPOT (Bosch-Camós *et al.*, 2021). T effector cells induced by MHC epitopes of ASFV T cell epitope assays CD2v, x p30, p72, and pp62have been identified in infected, vaccinated, and/or protected pigs (Sun *et al.*, 2021). Although pigs vaccinated with a combination of multiple Ad5-vectored ASFV genes exhibited immune recall responses in IFN-γ ELISPOT assays, they did not protect against severe diseases (Lokhandwala *et al.*, 2017). Notably, ASFV T cell epitopes were identified through systematic screening using IFN-γ ELISPOT assays, and Ad5 vectors carrying genes containing dominant T cell epitopes reduced viremia but did not prevent severe disease in vaccinated pigs (Netherton *et al.*, 2019b). Thus, achieving robust pig protection through cell-mediated immunity remains challenging. It is important to note that a demonstration of cell-mediated cytotoxicity that prevents or reduces the release of infectious ASFV virions from infected cells has yet to be established.

Conclusion

Unraveling the intricate interplay between innate, cellular, and humoral immune responses against ASFV has provided crucial insights into the elusive quest for an effective vaccine against this pathogen. The immunopathogenesis of ASFV infection highlights the delicate balance between host immunity and viral evasion. While the innate response primes the immune landscape, adaptive immunity orchestrates defense mechanisms through both cell- and antibody-mediated pathways.

Despite the significant progress, several research gaps remain. The intricate glycosylation pattern of ASFV envelope proteins, exemplified by CD2v and C-type lectins, poses a challenge for vaccine design due to potential immune evasion. Further studies investigating the impact of antigen density, glycan shielding, and apoptotic mimicry on immune recognition are essential. The dual role of apoptosis in viral propagation and immune evasion necessitates comprehensive investigations of ASFV protein interactions and their influence on cytotoxicity.

While the significance of neutralizing antibodies has been established, the incomplete neutralization of ASFV virions presents another difficult problem. Understanding the mechanisms underlying this phenomenon, including the potential role of host membrane proteins and phospholipids, is a pivotal research frontier. In addition, deciphering the contribution of antigen-specific T cells to ASFV clearance and protection remains a critical knowledge gap.

Addressing these research gaps is key to advancing ASFV vaccine development. Innovative strategies can exploit the potential of nanoparticle-based immunogens, allowing controlled antigen presentation to enhance immune responses. The emerging field of synthetic biology may enable the rational design of antigens with improved immunogenicity. Moreover, leveraging cutting-edge techniques such as single-cell sequencing and systems immunology will reveal intricate immune dynamics during ASFV infection, facilitating the identification of correlates of protection. Therefore, the journey toward an effective ASFV vaccine is characterized by multifaceted challenges and opportunities. The intricate arms race between viral immune evasion strategies and host immune responses underscores the need for innovative approaches that harness the full potential of innate, cellular, and humoral immunities. By addressing current gaps and capitalizing on emerging technologies, we are poised to unlock new avenues in ASFV vaccine development, thereby safeguarding the swine industry against this devastating pathogen.

Acknowledgments

The author would like to thank the DOST S&T Fellows Program, the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) for funding this research project, and the Industrial Technology Development Institute (DOST-ITDI) for hosting this research project.

Conflict of interest

The author declares that there are no conflicts of interest.

Funding

This study was funded by the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development of the Department of Science and Technology (DOST-PCAARRD).

Data availability

All data are available in the published manuscript.

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