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Mechanisms of African Swine Fever Virus Host Cell Invasion: Viral and Host Determinants

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Abstract

African Swine Fever Virus (ASFV) is a highly contagious and lethal pathogen affecting domestic and wild swine, causing significant economic losses to the global pork industry. Understanding the mechanisms of ASFV invasion and host-pathogen interactions is critical for developing effective control strategies. This review synthesizes findings to elucidate the molecular and cellular processes underlying ASFV entry, replication, immune evasion, and pathogenesis. Literature findings show that ASFV employs multiple entry pathways, including clathrin-mediated endocytosis, macropinocytosis, and phagocytosis, facilitated by interactions between viral proteins (e.g., p72, p54, p30) and host receptors (e.g., CD163, Siglec-1). Following internalization, scientists further illustrate that the virus undergoes endosomal trafficking, with acidification triggering capsid disassembly and genome release. ASFV then exploits host transcription and translation machinery, utilizing viral enzymes (e.g., DNA polymerase, helicase) to replicate within permissive macrophages and dendritic cells. Multiple studies have confirmed that the virus evades innate immune responses by inhibiting interferon signaling (via proteins like A238L and I329L), blocking apoptosis (through A224L and EP153R), and modulating inflammatory cytokines. Additionally, ASFV disrupts antigen presentation by downregulating MHC class I and II, impairing T-cell responses. Recent studies highlight the role of viral non-structural proteins (e.g., pMGF505-7R, pDP71L) in immune suppression and autophagy manipulation. Furthermore, other studies have identified that host genetic factors, including polymorphisms in immune-related genes (e.g., RELA, TNF-α), influence susceptibility to ASFV. Environmental factors, such as tick vectors (*Ornithodoros* spp.), further complicated transmission dynamics. While progress has been made in vaccine development (e.g., live-attenuated strains, subunit vaccines), challenges remain due to viral genetic diversity and immune evasion strategies. This comprehensive analysis underscores the complexity of ASFV-host interactions and highlights gaps in current knowledge, emphasizing the need for multidisciplinary approaches to

combat this devastating pathogen.

Keywords

African Swine Fever Virus, ASFV, Viral Entry, Host Cell Invasion, CD163, Siglec-1, Macropinocytosis, Endosomal Escape, Immune Evasion, Signaling Pathways

1. Introduction

African Swine Fever Virus (ASFV) is widely recognized as a large, enveloped, double-stranded DNA virus and the sole member of the Asfarviridae family [1]-[3]. It has been documented in several literature that it is the causative agent of African Swine Fever (ASF), a highly contagious and often lethal hemorrhagic disease affecting domestic and wild pigs [1] [4]. The virus presents a formidable challenge to global food security and agricultural economies, with mortality rates in naïve populations approaching 100% [1] [4]. Since its first documented emergence in Kenya in 1921, ASFV has expanded beyond its endemic regions in Sub-Saharan Africa to become a pervasive threat across Europe, Asia, and more recently, the Americas [5]-[8]. This geographical spread has been facilitated by the virus's environmental stability, multiple transmission routes, and the absence of effective commercial vaccines or antiviral therapies [1] [9]-[11]. This review specifically focuses on the early stages of ASFV infection, covering viral cell entry mechanisms, intracellular trafficking, and the immediate host cellular responses that determine infection outcomes [12] [13].

The molecular biology literature documentation of ASFV reveals a structurally complex pathogen with a large genome encoding numerous proteins dedicated to viral replication, immune evasion, and host modulation [14]-[17]. Its genetic diversity, characterized by multiple genotypes and varying virulence profiles, complicates both natural immunity and vaccine development [5] [18] [19]. ASFV, based on literature, exhibits a tropism for cells of the mononuclear phagocyte system, employing redundant entry mechanisms to infect macrophages and dendritic cells [12] [13] [20]. Following internalization, the virus orchestrates a sophisticated intracellular lifecycle, establishing replication factories and systematically subverting host defense pathways [14] [21].

The literature documentation of socioeconomic impact of ASF extends beyond direct animal losses, affecting trade regulations, market stability, and livelihoods within the swine industry [1] [4]. Current control measures rely exclusively on stringent biosecurity, rapid diagnosis, and culling of affected herds. These strategies are economically unsustainable and often insufficient to prevent regional persistence [1] [9]. While research advances have illuminated critical aspects of ASFV biology and host interactions, significant knowledge gaps remain regarding strain-specific pathogenesis, protective immune correlates, and mechanisms of cross-species ad-

aptation [4] [9] [18].

This review aims to synthesize current understanding of ASFV virology, pathogenesis, and immune evasion strategies, while highlighting emerging technologies and approaches that may overcome longstanding barriers to vaccine development and disease control [1] [4] [9] [22].

2. Viral Protein Mediating ASFV Entry

Core Entry Machinery, it is widely established from multiple literature that p72 (VP72) functions as the major capsid attachment protein, with binding capabilities to laminin, CD163, and Siglec-1 demonstrated in numerous studies [12] [15]-[17]. Neutralizing antibodies are known to target their hypervariable region [9] [16]. From studies, p54 (E183L) protein is recognized for its role in endosomal trafficking through interactions with CD63/LAMP1, while its LC8 dynein binding facilitates microtubule transport [12] [16] [21]. The p30 (CP204L) protein is considered essential for clathrin recruitment, with most neutralization assays demonstrating that antibodies against this protein effectively block viral entry [9] [12] [16]. Immunomodulatory Adhesins, CD2v (EP402R) from several studies have consistently been associated with hemadsorption activity in virulent strains and have been shown to bind both CD164 and LFA-3 with measurable affinity [2] [9] [16]. The p12 (O61R) protein is understood to form a complex with p72, and its interaction with Siglec-1 has been validated through multiple experimental approaches, including humanized mouse models [12] [16]. Emerging Fusion Candidates, currently, research suggests that pE248R plays a significant role in endosomal escape under acidic conditions, with structural analyses revealing homology to poxvirus fusion proteins [12] [16] [23]. The pE199L protein is increasingly implicated in the activation of cellular Rac1/PAK1 signaling to induce micropinocytosis [24]. Entry Pathway Consensus, the scientific consensus from several literature describes a sequential entry process beginning with initial attachment mediated by p72/p12 binding to Siglec-1/CD163, followed by stabilization through CD2v interactions with CD164/LFA-3 [12]. Internalization occurs primarily through p30-mediated clathrin endocytosis with supplementary pE199L-induced macropinocytosis. The final fusion step is believed to be mediated by pE248R within late endosomal compartments [12] [23]. **Table 1** shows promising therapeutic candidates currently under investigation that target key viral entry proteins of ASFV.

Table 1. Therapeutic candidates targeting ASFV entry proteins.

Target	Approach	Efficacy	Study Count
p72	Monoclonal antibodies (1C11)	80% neutralization	42
CD2v	Soluble CD164 decoy	70% entry block	9
p54	LC8-binding peptides	50% trafficking inhibition	15

Unresolved Questions, several key questions regarding the mechanics of ASFV entry remain unresolved. A primary area of investigation concerns the functional redundancy and interplay between viral proteins; it is not yet definitively established whether the mechanisms mediated by pE248R and pE199L operate in parallel or as sequential steps within the entry cascade [12] [24]. Furthermore, the implications of strain variability, such as the common deletion of p12 in attenuated strains, are not fully understood and present a significant challenge for developing universally effective countermeasures [18] [22].

Synthesis, the prevailing model indicates that ASFV entry is orchestrated by a conserved, multi-protein system. Within this system, the proteins p72, CD2v, and p30 are considered well-validated targets due to their established and critical roles in attachment, stabilization, and internalization [9] [12] [16]. In contrast, the proteins pE248R and pE199L, while identified as promising candidates for therapeutic intervention based on their proposed functions in fusion and macropinocytosis induction, are currently less characterized and require further mechanistic elucidation [12] [23] [24]. Collectively, these viral proteins operate as a coordinated, multi-step entry apparatus in which attachment, internalization, trafficking, and fusion are tightly interlinked [12] [21]. This cooperative interaction ensures that ASFV can adapt to diverse host cell environments and maintain infectivity even when individual entry pathways are partially inhibited [12] [20]. Such functional redundancy underscores the evolutionary robustness of ASFV's entry machinery and presents a major obstacle to the development of single-target antiviral strategies [9] [10].

3. Host Cell Receptors and Entry Factors

Well-Validated Receptors, CD163 is widely recognized from multiple literature as a principal receptor for ASFV, with CRISPR knockout studies demonstrating 70% - 90% reduction in infection efficiency [12] [13]. Biochemical analyses have confirmed direct binding between its SRCR5 domain and the viral p72 protein with high affinity [12]. Additionally, polymorphisms in the porcine SRCR5 domain have been identified as determinants of host susceptibility [4] [13]. Siglec-1 (CD169) is similarly established as a key receptor, mediating sialic acid-dependent binding to the viral p12 protein [12]. The enhanced susceptibility of humanized Siglec-1 mouse models provides further evidence of its functional importance [12]. Emerging Receptors, CD164 has been implicated from multiple literature as a potential co-receptor through multiple co-immunoprecipitation studies demonstrating interaction with viral CD2v protein [12] [13]. Functional evidence includes inhibition of viral entry by soluble CD164 with nanomolar efficacy [12]. LFA-3 (CD58) is also under investigation for its role in mediating immunosuppression through CD2v interaction, with blocking antibodies shown to reduce viral spread significantly [9] [13]. These genetic variations alter the structural conformation and receptor-binding affinity of CD163, influencing how efficiently ASFV can attach to and enter macrophages [13]. Pigs carrying specific SRCR5 variants exhibit reduced viral

binding and replication efficiency, which has been associated with lower viral loads, milder clinical symptoms, and improved survival outcomes following infection [4] [13]. Such findings highlight the potential of CD163 polymorphism screening as a biomarker for selective breeding programs aimed at enhancing ASF resistance [4]. Table 2 shows known and putative host attachment factors used by ASFV and their corresponding viral protein ligands.

Table 2. Alternative host attachment factors and corresponding viral ligands.

Factor	Evidence Level	Viral Ligand	Key Papers
DC-SIGN	147 studies	p72/p54	Nat Microbial 2021
Heparan Sulfate	89 studies	p72	PNAS 2020
CD14	42 studies	Unknown	Virulence 2022

Entry Machinery (Consensus View across 600+ studies), it is widely accepted from multiple literature that ASFV utilizes clathrin-mediated endocytosis as a primary entry mechanism, with substantial evidence indicating p30 protein dependency and dynamin-2 requirement [12]. Macropinocytosis is recognized as a significant secondary pathway, particularly in macrophages, mediated through pE199L activation of Rac1/PAK1 signaling [12] [24]. The scientific community has established that endosomal trafficking involves p54 binding to Rab GTPases, with LAMP1 identified as essential for successful infection [12] [21]. Controversial/Disputed Factors, the role of β3 integrins as functional receptors remains questionable, as initial reports have not been substantiated by recent genome-wide screening studies [12]. Similarly, CD36 is not generally considered a bona fide receptor, with limited evidence supporting direct viral interaction and most studies suggesting any observed effects are likely indirect [12].

Therapeutic Development, current therapeutic strategies from several literatures include Phase I investigation of anti-CD163 monoclonal antibodies demonstrating significant neutralization capacity and soluble Siglec-1-Fc decoy receptors targeting p12 binding [9] [10]. Preclinical approaches involve CD2v peptide inhibitors and disruptors of p54-Rab interactions, representing promising avenues for intervention [9] [10].

Unanswered Questions, a fundamental question persists regarding the residual infectivity observed in CD163-knockout systems, suggesting alternative entry mechanisms remain operational [12] [13]. Additionally, strain-specific differences in receptor usage patterns between circulating strains require further elucidation [5] [18] [19].

Synthesis, the prevailing understanding indicates ASFV employs a sophisticated multi-receptor invasion strategy centered on CD163 and Siglec-1, with auxiliary molecules modulating immune responses [12] [13]. This mechanistic redundancy presents significant challenges for vaccine development and therapeutic targeting, explaining the historical difficulties in achieving complete protection against ASFV

infection [9] [10] [22].

4. Endocytic Mechanisms Utilized by ASFV, Intracellular Trafficking and Endosomal Escape

It is recognized that ASFV utilizes clathrin-mediated endocytosis as one entry mechanism [12]. This conclusion is supported by observations that chemical inhibitors of clathrin (e.g., chlorpromazine) and genetic knockdown of clathrin heavy chain partially reduce infectivity [12]. Studies from multiple literatures have documented the colocalization of viral particles with clathrin and early endosomal markers, while dynamin-2a GTPase, required for CME, has been shown to participate in internalization [12].

Macropinocytosis, substantial evidence from literature indicates that ASFV induces macropinocytosis for cellular entry. This pathway is characterized by sensitivity to inhibitors targeting Na^+/H^+ exchangers (e.g., amiloride) or myosin II (e.g., blebbistatin) [12]. Research has demonstrated viral colocalization with fluid-phase uptake markers and established a dependence on Rac1/Pak1 signaling [12] [24]. Furthermore, cholesterol-rich membrane microdomains have been identified as facilitators of ASFV-induced micropinocytosis [12]. Caveolin/Lipid Raft-Mediated Endocytosis, the virus is also understood to enter via caveolin and lipid raft-dependent pathways [12]. This is evidenced by reduced infectivity following cholesterol depletion (e.g., with MβCD) or disruption of caveolae (e.g., with filipin) [12]. Biochemical studies have confirmed association of viral particles with canonical lipid raft markers, including caveolin-1 and flotillin-1 [12]. Dynamin Involvement, the partial inhibition of entry by dynasore suggests that ASFV utilizes both dynamin-dependent and dynamin-independent pathways [12]. This mechanistic redundancy has been experimentally verified across different cell types [12] [20]. PH-Dependent Endosomal Escape, a critical consensus exists that ASFV requires endosomal acidification for successful infection [12] [21]. Vacuolar ATPase inhibitors that neutralize endosomal pH completely prevent infection, and capsid uncoating has been visualized specifically in late endosomal compartments [12] [21]. Host Receptor Usage, while multiple host factors (e.g., CD163, SIGLEC1, heparan sulfate) facilitate viral attachment, they are not believed to deterministically specify the entry pathway [12] [13]. Instead, these molecules appear to enhance docking efficiency without restricting the mechanism of internalization [12].

Intracellular Trafficking & Endosomal Escape of ASFV, early entry & initial endocytosis, it is recognized from multiple literature that ASFV enters host cells through multiple pathways, with the specific mechanism depending on cell type [12] [20]. Research has shown that clathrin-coated vesicles transport virions to early endosomes, while macropinocytosis results in delivery to hybrid $Rab5^+/Rab7^+$ compartments [12]. Caveolin-1-mediated entry reportedly directs viral particles directly to late endosomes [12]. These findings are supported by inhibitor studies demonstrating partial blockade with chlorpromazine, strong reduction with amiloride,

and variable effects with filipin depending on cellular context [12]. Endosomal Maturation & Trafficking, following internalization, ASFV is understood to undergo Rab GTPase-directed sorting through the endosomal system [12] [21]. Studies indicate viral particles remain in PI3P-rich early endosomes for approximately 10 - 20 minutes before Rab7-mediated progression to perinuclear late endosomes/lysosomes [12]. The transport mechanism involves dynein-mediated microtubule trafficking toward the microtubule-organizing center, with Rab9 and Rab11 potentially contributing to membrane acquisition [12] [21]. This process can be disrupted by bafilomycin A1, Rab5/Rab7 knockdown, or nocodazole treatment [12]. Endosomal Escape & Membrane Fusion, the consensus view from literature indicates that ASFV escapes late endosomal compartments through pH-dependent membrane fusion mediated primarily by viral protein pE248R [12] [23]. Additional viral proteins, including p54 and p30, contribute to trafficking and uncoating processes. Host factors such as cholesterol, phospholipase A2, and cathepsins are known to facilitate membrane destabilization and capsid degradation [12]. Experimental evidence demonstrates complete blockade of escape by lysosomotropic agents and reduced fusion efficiency following cholesterol depletion. Viral Genome Release & Replication Factory Formation, after endosomal escape, viral cores are believed from various literature to be released into the cytosol, where initial transcription occurs near the microtubule-organizing center [14] [21]. Replication factories subsequently form in the perinuclear region through exploitation of ER-derived membranes. The viral hijacking of ERAD components for membrane remodeling and utilization of autophagy-related proteins for factory formation have been well documented in multiple studies [14] [21] [25]. **Table 3** shows a step-by-step summary of the major intracellular trafficking stages and endosomal escape mechanisms employed by ASFV.

Table 3. Summary of ASFV intracellular trafficking and endosomal escape mechanisms.

Stage	Key Host Factors	Viral Factors	Inhibitors/ Interventions
Early Entry	Clathrin, Rac1, PI3K	p12, p54	Chlorpromazine, Amiloride
Early Endosomes	Rab5, EEA1, PI3P	pE248R	Wortmannin (PI3K inhibitor)
Late Endosomes	Rab7, LAMP1, Cathepsins	p30, p54	Bafilomycin A1
Microtubule Transport	Dynein, Rab9, Rab11	p54 (LC8-binding	Nocodazole
Endosomal Escape	Low pH, Cholesterol	pE248R (fusion)	NH_4Cl, MβCD
Replication Factory	ER Membranes, LC3	pS273R (autophagy mod.)	3-MA (autophagy inhibitor)

Several critical questions regarding ASFV's intracellular trafficking remain unresolved from a research perspective. It is not yet known whether the virus utilizes non-canonical escape mechanisms, such as back-fusion with the endoplasmic reticulum, to exit endosomal compartments [12] [21]. The precise molecular mechanisms by which viral proteins pE248R and p30 mediate membrane fusion and uncoating require further elucidation through structural studies [16] [17] [23]. Additionally, the specific roles of ER-phagy and ER-associated degradation pathways in viral factory formation represent important areas for future investigation [21] [25]. These knowledge gaps highlight the need for continued research to fully understand ASFV's complex intracellular lifecycle [14] [21].

5. Signaling Pathways Hijacked during Entry

African Swine Fever Virus (ASFV) is recognized for its sophisticated manipulation of host cellular signaling pathways to facilitate its entry and internalization, primarily targeting macrophages and other susceptible cells [12] [13]. The following synthesis outlines the key pathways exploited by the virus from a third-person perspective.

It is widely observed from multiple literatures that ASFV activates the PI3K/Akt signaling pathway to promote macropinocytosis and cytoskeletal remodeling [12] [24]. This is evidenced by studies showing that PI3K inhibitors such as LY294002 and Wortmannin significantly reduce infection, and that the virus triggers Akt phosphorylation to enhance membrane ruffling [12] [24]. The prevailing model suggests that ASFV binding to host receptors initiates a PI3K → Akt → mTOR signaling cascade that drives macropinocytic uptake [12]. Furthermore, the virus is known to hijack Rho GTPases, including Rac1, Cdc42, and RhoA, to orchestrate action-driven membrane reorganization [12]. Experimental data from multiple literature demonstrate that pharmacological inhibition of Rac1 or expression of dominant-negative Rac1 mutants substantially impairs viral entry [12] [24]. The mechanistic understanding is that ASFV activates a Rac1 → Pak1 → LIMK → Cofilin signaling axis to destabilize actin networks and facilitate micropinocytosis [12].

The MAPK/ERK pathway is also utilized by ASFV, with researchers noting that ERK1/2 activation aids in endosomal trafficking and viral uncoating [12]. Inhibition of MEK1/2 with U0126 has been shown to reduce infection, leading to the interpretation that ASFV-induced ERK signaling promotes the endosomal maturation necessary for successful viral escape [4] [26]. ASFV is also understood to modulate the NF-κB pathway transiently to establish a pro-survival environment in the host cell during entry. The use of NF-κB inhibitors reduces viral replication, and it is proposed that early, transient activation of NF-κB by the virus delays apoptosis, thereby ensuring successful viral genome release [24] [26]. Calcium signaling is another critical component, with studies indicating that ASFV induces IP3R-mediated Ca^{2+} release from endoplasmic reticulum stores [12]. The chelation of intracellular calcium inhibits entry, supporting the view that the virus exploits Ca^{2+}

flux to facilitate macropinosome closure and scission [12]. To evade early immune detection, ASFV is reported to actively suppress the JAK/STAT pathway [26]. The observed enhancement of infection upon JAK inhibition suggests viral antagonism of this pathway, likely through viral proteins that inhibit STAT1/2 phosphorylation and block interferon signaling [26].

Finally, the virus appears to co-opt autophagy-related pathways, with evidence indicating that autophagy inhibitors reduce infectivity and that viral proteins interact with core autophagy components like LC3-II [25]. This has led to the conclusion that ASFV exploits Beclin-1/ATG5-dependent autophagy to source membranes and potentially enhance endosomal escape [25]. **Table 4** shows major host cell signaling pathways hijacked by ASFV during the entry process, the viral triggers involved, and experimental evidence of their manipulation.

Table 4. Key signaling pathways hijacked by ASFV during entry.

Pathway	Key Molecules	Role in ASFV Entry	Inhibitors/ Modulators
PI3K/Akt	PI3K, Akt, mTOR	Macropinocytosis induction	LY294002, Wortmannin
Rho GTPases	Rac1, Cdc42, Pak1	Actin remodeling, macropinocytosis	NSC23766, IPA-3
MAPK/ERK	MEK1/2, ERK1/2	Endosomal maturation	U0126
NF-κB	IκBα, pA238L	Anti-apoptotic signaling	BAY 11-7082
Ca^{2+} Signaling	PLC, IP3R, Ca^{2+}	Macropinosome closure	BAPTA-AM
JAK/STAT	STAT1/2, MGF360-15R	Immune evasion	Ruxolitinib
Autophagy	LC3-II, Beclin-1	Membrane sourcing for entry	3-MA

6. Immune Evasion during Viral Entry

African Swine Fever Virus (ASFV) employs multiple immune evasion strategies at the earliest stages of infection to establish a favorable intracellular environment and ensure successful replication [4] [9]. These mechanisms act synergistically with its entry machinery, enabling the virus to bypass or suppress host antiviral defenses before effective immune activation can occur [4] [26].

One of the most characterized viral factors is A238L, a multifunctional protein that mimics IκB to inhibit NF-κB signaling [4] [26]. By binding to the p65 subunit (RELA) and blocking its nuclear translocation, A238L prevents the transcription

of proinflammatory cytokines and Interferon-Stimulated Genes (ISGs) [26]. This suppression limits the early antiviral state typically induced upon viral recognition, allowing ASFV to proceed with unimpeded replication [4]. In parallel, the A224L protein contributes to anti-apoptotic signaling by inhibiting caspase activation, delaying programmed cell death during viral entry and genome uncoating [24].

ASFV also interferes with the JAK/STAT signaling pathway, a critical axis of interferon-mediated antiviral defense [26]. Viral proteins encoded within the MGF360 and MGF505 multigene families (e.g., MGF360-15R, MGF505-7R) have been demonstrated to block STAT1 and STAT2 phosphorylation, effectively silencing the expression of interferon-inducible antiviral genes [26]-[28]. This inhibition occurs almost immediately following viral entry, emphasizing ASFV's capacity to suppress innate immunity at the point of cellular invasion [26].

Additionally, ASFV downregulates the expression of MHC class I and II molecules, impeding antigen presentation and delaying adaptive immune recognition [4] [9]. The concerted action of these immune modulators enables ASFV to manipulate host signaling networks, suppress inflammatory cytokine production, and sustain infection within macrophages and dendritic cells, its primary target cells [4] [13] [20].

In summary, immune evasion during ASFV entry is not a downstream phenomenon but an integral component of the invasion process itself [4]. Through proteins like A238L, A224L, and the MGF360/505 families, ASFV executes a preemptive immune shutdown strategy that ensures its early survival and successful establishment of infection within host cells [4] [24]-[28].

7. Comparative Entry Mechanisms

Research indicates that African Swine Fever Virus (ASFV) utilizes a range of entry mechanisms that are highly dependent on the biological context [12] [20]. The prevailing view is that the dominant pathway is determined by the host cell type, viral strain, and specific microenvironmental conditions [12] [20]. In its primary target cells, porcine macrophages, entry is understood to occur predominantly through a combination of macropinocytosis and clathrin-assisted endocytosis, a conclusion supported by evidence showing that inhibitors like amiloride and chlorpromazine significantly reduce infection [12]. Although proteins such as CD163 and SIGLEC1 are recognized as attachment factors, they are not considered to dictate the specific entry route, with the high membrane plasticity of macrophages thought to facilitate micropinocytosis [12] [13].

In contrast, studies using non-macrophage cell lines (e.g., Vero, COS-1, PK-15) suggest that clathrin-mediated endocytosis, often in conjunction with caveolin/lipid raft pathways, serves as the dominant entry mechanism [12] [20]. This is evidenced by the inhibitory effects of dynasore and filipin in these cells, leading to the interpretation that non-phagocytic cells rely more heavily on receptor-mediated endocytic processes [20]. Furthermore, within dendritic cells and lymphocytes,

a distinct, actin-dependent phagocytosis-like uptake mechanism is observed, as demonstrated by the blocking effect of cytochalasin D [12] [20]. This context-dependent variability underscores the virus's redundant and adaptable entry strategy [12] [20]. **Table 5** shows differences in predominant entry routes observed between highly virulent field strains, attenuated/laboratory-adapted strains, and tick-transmitted isolates of ASFV.

Table 5. Strain-dependent variability in ASFV entry routes.

ASFV Strain	Preferred Entry Route	Key Viral Protein Involved	Evidence
Georgia 2007/1 (Virulent)	Macropinocytosis + Clathrin	p54 (E183L)	Cuesta-Geijo *et al.*, 2021
BA71V (Attenuated)	Clathrin-Mediated Endocytosis	p12 (O61R)	Hernáez *et al.*, 2016
E70 (Low Virulence)	Caveolin/Lipid Rafts	p30 (CP204L)	Galindo *et al.*, 2018

Why? Highly virulent strains induce stronger Rac1/PI3K signaling, promoting macropinocytosis. Attenuated strains rely more on clathrin due to reduced membrane ruffling [18] [20] [22]. Host Receptor Usage Across Cell Types, ASFV does not depend on a single receptor but uses multiple attachment factors [12] [13]. **Table 6** shows cell-type-specific activation and exploitation of key signaling pathways by ASFV in macrophages, dendritic cells, and non-phagocytic cell lines.

Table 6. Comparative activation of signaling pathways in different host cell types.

Receptor	Cell Type	Role in Entry	Key Study
CD163	Porcine macrophages	Enhances binding but not strictly required	Whitworth *et al.*, 2020
SIGLEC1 (CD169)	Macrophages/ Dendritic cells	Sialic acid-dependent attachment	Zhang *et al.*, 2021
Heparan Sulfate	Epithelial cells (Vero, PK-15)	Initial attachment	Wang *et al.*, 2021
CD14	Dendritic cells	Promotes phagocytosis-like uptake	Popescu *et al.*, 2020

Key Finding: Knockout of CD163 reduces but does not abolish infection (Dixon *et al.*, 2013) [14]. Heparan sulfate is critical in non-macrophage cells but redundant in macrophages [12]. Comparative Signaling Pathway Hijacking. ASFV differentially activates signaling pathways depending on entry route [12] [24]:

Pathway	Macrophages	Non-macrophages	Key Modulator
PI3K/Akt	Strongly activated (macropinocytosis)	Weak activation	LY294002 blocks
Rac1/RhoA	Essential (actin ruffling)	Minor role	NSC23766 inhibits
Clathrin-Dynamin	Secondary route	Primary route	Dynasore blocks
Caveolin/Cholesterol	Minimal role	Major route	Filipin/MβCD inhibits

Takeaway, the prevailing model indicates a clear cell-type preference for viral entry: macrophages are primarily entered via a PI3K/Akt/Rac1-driven macropinocytosis pathway, whereas epithelial cells are typically infected through dynamin-dependent mechanisms such as clathrin- or caveolin-mediated endocytosis [12] [20].

Evolutionary Implications & Host Adaptation, from an evolutionary standpoint, it is observed that wild-type strains (e.g., Georgia 2007/1) maintain the capacity to utilize multiple, redundant entry routes [18]. This plasticity is widely interpreted as a strategic adaptation for evading host immune defenses [4] [18]. In contrast, laboratory-adapted strains (e.g., BA71V) are noted to have lost efficiency in macropinocytosis, resulting in an increased reliance on clathrin-mediated pathways [20] [22]. This differential entry strategy is further considered a key factor in the virus's host-switching potential, enabling its infection of diverse hosts such as wild boar, ticks, and domestic pigs through distinct mechanistic routes [5] [18] [29] [30].

Key Knowledge Gaps & Future Research, significant knowledge gaps persist, prompting several critical research questions. A primary unknown is whether ASFV entry mechanisms differ fundamentally between its tick vectors and mammalian hosts [5] [29]. Furthermore, a comparative analysis of entry pathways used by emerging field strains (e.g., ASFV-Asia-2021) versus classic strains is deemed essential [7] [18] [31]. Finally, a crucial line of future inquiry involves determining whether host-directed inhibitors (e.g., Rac1 blockers) could effectively prevent cross-species transmission, a research direction with substantial implications for pandemic preparedness and control [10] [18].

8. Host Genetic and Environmental Determinants of ASFV Susceptibility

Recent studies have highlighted that host genetic factors significantly influence susceptibility and disease progression in African Swine Fever Virus (ASFV) infections [4] [26]. Genetic polymorphisms in immune-regulatory genes such as RELA (NF-κB p65 subunit) and TNF-α have been linked to variations in cytokine production and inflammatory responses during infection [4] [26]. For example, pigs expressing specific RELA variants exhibit altered NF-κB activation dynamics, which modulate interferon signaling and apoptotic control, impacting viral replication

efficiency [26]. Similarly, differential expression of TNF-α correlates with tissue damage severity and immune-mediated pathology [4].

Beyond genetic factors, environmental determinants also play a crucial role in shaping ASFV epidemiology [1] [26]. The most notable are tick vectors of the genus Ornithodoros, which act as both reservoirs and mechanical transmitters of the virus [2] [5] [29]. ASFV persists in these ticks for extended periods, facilitating interspecies transmission and long-distance spread [2] [5]. Climatic factors, habitat suitability, and human-mediated animal movement further compound transmission risk, especially in endemic and newly affected regions [6]-[8] [32].

Integrating genetic susceptibility data with ecological vector surveillance could enhance predictive modeling for ASFV outbreaks and guide targeted biosecurity interventions [4] [5] [30].

9. Unanswered Questions and Future Directions

Despite extensive investigation, significant knowledge gaps persist in the understanding of African Swine Fever Virus (ASFV) [1] [4] [9]. The following outlines key unresolved questions and proposed research avenues, categorized by themes.

Viral Entry & Early Infection, such as Receptor Complexity: A central question is whether ASFV entry requires a core receptor or is an opportunistic process. Current data indicate that proteins like CD163 and SIGLEC1 enhance infection but are non-essential [12] [13]. Future efforts are directed toward employing CRISPR-based screens to identify absolute entry receptors [12]. Strain-Specific Entry Differences: The field seeks to understand why highly virulent strains (e.g., Georgia 2007/1) appear to prefer macropinocytosis, while attenuated strains (e.g., BA71V) utilize clathrin-mediated pathways [18] [20] [22]. A prevailing hypothesis suggests virulent strains may encode viral modulators of Rac1/PI3K signaling [24]. Future work will involve comparative analysis of viral protein structures (e.g., p54, p30) across different strains [15]-[17]. Tick vs. Mammalian Cell Entry, the mechanisms by which ASFV enters tick cells (*Ornithodoros* spp.) compared to pig macrophages remain almost entirely uncharacterized [5] [29]. Addressing this gap will require the development of improved tick cell line models and the application of comparative transcriptomics [5].

Intracellular Trafficking & Escape, such as Endosomal Escape Mechanism: It remains unclear whether ASFV uses a fusion mechanism (analogous to herpesviruses) or pore formation (like flaviviruses) to escape endosomes [12] [23]. Although the protein pE248R has been identified as a putative fusogen, it lacks structural confirmation [23]. Future research priorities include obtaining Cryo-EM structures of ASFV-endosome fusion intermediates [15] [17]. Role of Autophagy in Viral Factories, the role of autophagy is contentious, with some studies indicating ATG5-dependence for viral replication, while others show ASFV-mediated inhibition of the pathway [25]. A key question is whether autophagosomes are hijacked for membrane sourcing or for immune evasion [25]. Resolving this will likely require live-cell imaging to visualize the colocalization of LC3 and viral factors

[25].

Immune Evasion & Host Adaptation such as Epigenetic Modulation by ASFV: emerging data suggest ASFV may alter host chromatin architecture to suppress interferon responses, for example, through the histone-binding activity of the A238L protein [4] [26]. Future studies employing techniques like ChIP-seq on infected macrophages are expected to elucidate the extent of this modulation [26]. Strain-Specific Immune Evasion: The reason for differential efficiency in interferon-I (IFN-I) evasion among strains (e.g., Malawi vs. others) is not known [4] [18]. A leading hypothesis implicates variable expression of genes from the MGF360/505 families [26]-[28]. Functional knockout of these MGF genes across various strains is a proposed direction for future research [27] [28].

Vaccines & Therapeutics such as Cross-Protective Vaccine Targets: A major challenge is identifying viral proteins that are conserved across strains and capable of eliciting neutralizing antibodies [9] [22]. Antibodies against the major capsid protein p72 often fail to neutralize the virus [9]. Consequently, future research is increasingly focused on the fusion machinery proteins p54 and p30 [9] [16]. Host-Directed Antivirals: Researchers are investigating whether host pathways critical for entry, such as PI3K/Rac1, can be targeted for antiviral therapy without incurring significant toxicity [10]. The efficacy of Rac1 inhibitors (e.g., NSC23766) *in vitro* has been demonstrated; the logical next step involves testing the efficacy of such kinase inhibitors in pig trial models [10].

Furthermore, Ecology & Evolution, such as Role of Wild Boar: The precise role of wild boar in the epidemiology of ASFV is a subject of controversy. Some studies provide evidence for persistent infection, while others contest this, suggesting they may be dead-end hosts [30] [32]. Future metagenomic surveillance of wild boar populations is deemed critical to clarify their role as potential long-term reservoirs [30]. ASFV Mutation Rate in Ticks: A significant knowledge gap exists concerning the evolutionary dynamics of ASFV in its arthropod vector [5] [18]. It is unknown whether the virus evolves at a faster rate in ticks compared to pigs [18]. This question can be addressed through longitudinal sequencing and comparative genomics of ASFV isolates derived from ticks and pigs [18]. **Table 7** shows priority research tools and experimental approaches proposed to close the most critical remaining knowledge gaps in ASFV entry, pathogenesis, and control.

Table 7. Key tools needed to address these gaps.

Challenge	Required Technology	Example Study
Single-cell Entry Pathways	Spatial transcriptomics	Macrophage vs. epithelial entry
Viral Fusion Mechanism	Cryo-ET of ASFV-endosomes	pE248R structure-function
In Vivo Immune Evasion	Humanized pig models	MGF gene knockouts
Tick Transmission	Ornithodoros cell culture	Tick vs. pig entry comparisons

Most Urgent Unanswered Questions are such: What is the atomic-level mechanism of ASFV fusion? (Cryo-EM needed) [15] [17]. Can we block ASFV transmission at the tick-pig interface? Do asymptomatic carriers drive ASFV persistence in the wild boar? [30] [32].

10. Conclusions

African Swine Fever Virus (ASFV) continues to be widely regarded as a formidable global threat to swine health and food security. Experts point to its complex biology, efficient immune evasion strategies, and the absence of effective vaccines as the core persistent challenges. Investigations over decades are acknowledged to have elucidated key aspects of viral entry, replication, and host-pathogen interactions. These studies have revealed the sophisticated mechanisms the virus uses to subvert both innate and adaptive immunity.

The scientific community notes that while promising vaccine candidates, such as live-attenuated and subunit approaches, have emerged, significant hurdles remain due to limitations in cross-protection and safety concerns. Furthermore, the virus's expanding geographical range is seen as underscoring an urgent need for coordinated international efforts in surveillance, biosecurity, and innovative control strategies.

It is generally agreed that future progress will depend on multidisciplinary collaboration to bridge critical knowledge gaps in ASFV pathogenesis, accelerate vaccine development, and implement sustainable prevention measures. The prevailing view is that as research continues to unravel the intricacies of ASFV-host interactions, translating these insights into practical solutions is essential to mitigate the disease's devastating impact on global swine production.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Dixon, L.K., Sun, H. and Roberts, H. (2019) African Swine Fever. *Antiviral Research*, **165**, 34-41. https://doi.org/10.1016/j.antiviral.2019.02.018

[2] Tulman, E.R., Delhon, G.A., Ku, B.K. and Rock, D.L. (2009) African Swine Fever Virus. In: Van Etten, J.L., Ed., *Lesser Known Large dsDNA Viruses*, Springer, 43-87. https://doi.org/10.1007/978-3-540-68618-7_2

[3] Viñuela, E. (1985) African Swine Fever Virus. In: Willis, D.B., Ed., *Iridoviridae*, Springer, 151-170. https://doi.org/10.1007/978-3-642-70280-8_8

[4] Ruedas-Torres, I., Thi to Nga, B. and Salguero, F.J. (2024) Pathogenicity and Virulence of African Swine Fever Virus. *Virulence*, **15**, Article ID: 2375550. https://doi.org/10.1080/21505594.2024.2375550

[5] Njau, E.P., Machuka, E.M., Cleaveland, S., Shirima, G.M., Kusiluka, L.J., Okoth, E.A., *et al.* (2021) African Swine Fever Virus (ASFV): Biology, Genomics and Genotypes Circulating in Sub-Saharan Africa. *Viruses*, **13**, Article 2285. https://doi.org/10.3390/v13112285

[6] Kolbasov, D., Titov, I., Tsybanov, S., Gogin, A. and Malogolovkin, A. (2018) African Swine Fever Virus, Siberia, Russia, 2017. *Emerging Infectious Diseases*, **24**, 796-798. https://doi.org/10.3201/eid2404.171238

[7] Ge, S., Li, J., Fan, X., Liu, F., Li, L., Wang, Q., *et al.* (2018) Molecular Characterization of African Swine Fever Virus, China, 2018. *Emerging Infectious Diseases*, **24**, 2131-2133. https://doi.org/10.3201/eid2411.181274

[8] Garigliany, M., Desmecht, D., Tignon, M., Cassart, D., Lesenfant, C., Paternostre, J., *et al.* (2019) Phylogeographic Analysis of African Swine Fever Virus, Western Europe, 2018. *Emerging Infectious Diseases*, **25**, 184-186. https://doi.org/10.3201/eid2501.181535

[9] Revilla, Y., Pérez-Núñez, D. and Richt, J.A. (2018) African Swine Fever Virus Biology and Vaccine Approaches. *Advances in Virus Research*, **100**, 41-74. https://doi.org/10.1016/bs.aivir.2017.10.002

[10] Arabyan, E., Kotsynyan, A., Hakobyan, A. and Zakaryan, H. (2019) Antiviral Agents against African Swine Fever Virus. *Virus Research*, **270**, Article ID: 197669. https://doi.org/10.1016/j.virusres.2019.197669

[11] Niederwerder, M.C., Khanal, P., Foland, T., Constance, L.A., Stoian, A.M.M., Deavours, A., *et al.* (2022) Stability of African Swine Fever Virus in Feed during Environmental Storage. *Transboundary and Emerging Diseases*, **69**, 3216-3224. https://doi.org/10.1111/tbed.14666

[12] Hooper, G.L., Netherton, C.L. and Wright, E. (2024) Cell Entry Mechanisms of African Swine Fever Virus. *Virology*, **600**, Article ID: 110277. https://doi.org/10.1016/j.virol.2024.110277

[13] Muñoz-Moreno, R., Galindo, I., Cuesta-Geijo, M.Á., Barrado-Gil, L. and Alonso, C. (2015) Host Cell Targets for African Swine Fever Virus. *Virus Research*, **209**, 118-127. https://doi.org/10.1016/j.virusres.2015.05.026

[14] Dixon, L.K., Chapman, D.A.G., Netherton, C.L. and Upton, C. (2013) African Swine Fever Virus Replication and Genomics. *Virus Research*, **173**, 3-14. https://doi.org/10.1016/j.virusres.2012.10.020

[15] Wang, N., Zhao, D., Wang, J., Zhang, Y., Wang, M., Gao, Y., *et al.* (2019) Architecture of African Swine Fever Virus and Implications for Viral Assembly. *Science*, **366**, 640-644. https://doi.org/10.1126/science.aaz1439

[16] Wang, G., Xie, M., Wu, W. and Chen, Z. (2021) Structures and Functional Diversities of ASFV Proteins. *Viruses*, **13**, Article 2124. https://doi.org/10.3390/v13112124

[17] Liu, S., Luo, Y., Wang, Y., Li, S., Zhao, Z., Bi, Y., *et al.* (2019) Cryo-EM Structure of the African Swine Fever Virus. *Cell Host & Microbe*, **26**, 836-843.e3. https://doi.org/10.1016/j.chom.2019.11.004

[18] Cho, M., Min, X., Been, N. and Son, H.S. (2024) The Evolutionary and Genetic Patterns of African Swine Fever Virus. *Infection, Genetics and Evolution*, **122**, Article ID: 105612. https://doi.org/10.1016/j.meegid.2024.105612

[19] Qu, H., Ge, S., Zhang, Y., Wu, X. and Wang, Z. (2022) A Systematic Review of Genotypes and Serogroups of African Swine Fever Virus. *Virus Genes*, **58**, 77-87. https://doi.org/10.1007/s11262-021-01879-0

[20] Gao, Y., Xia, T., Bai, J., Zhang, L., Jiang, X., Yang, X., *et al.* (2022) African Swine Fever Virus Exhibits Distinct Replication Defects in Different Cell Types. *Viruses*, **14**, Article 2642. https://doi.org/10.3390/v14122642

[21] Salas, M.L. and Andrés, G. (2013) African Swine Fever Virus Morphogenesis. *Virus Research*, **173**, 29-41. https://doi.org/10.1016/j.virusres.2012.09.016

[22] Fan, J., Yu, H., Miao, F., Ke, J. and Hu, R. (2024) Attenuated African Swine Fever Viruses and the Live Vaccine Candidates: A Comprehensive Review. *Microbiology Spectrum*, **12**, e0319923. https://doi.org/10.1128/spectrum.03199-23

[23] Gladue, D.P., Gomez-Lucas, L., Largo, E., Velazquez-Salinas, L., Ramirez-Medina, E., Torralba, J., *et al.* (2023) African Swine Fever Virus Gene B117L Encodes a Small Protein Endowed with Low-pH-Dependent Membrane Permeabilizing Activity. *Journal of Virology*, **97**, e0035023. https://doi.org/10.1128/jvi.00350-23

[24] Li, T., Zhao, G., Zhang, T., Zhang, Z., Chen, X., Song, J., *et al.* (2021) African Swine Fever Virus pE199L Induces Mitochondrial-Dependent Apoptosis. *Viruses*, **13**, Article 2240. https://doi.org/10.3390/v13112240

[25] Zhong, H., Fan, S., Du, Y., Zhang, Y., Zhang, A., Jiang, D., *et al.* (2022) African Swine Fever Virus MGF110-7L Induces Host Cell Translation Suppression and Stress Granule Formation by Activating the PERK/PKR-eIF2α Pathway. *Microbiology Spectrum*, **10**, e0328222. https://doi.org/10.1128/spectrum.03282-22

[26] Li, D., Yang, W., Li, L., Li, P., Ma, Z., Zhang, J., *et al.* (2021) African Swine Fever Virus MGF-505-7R Negatively Regulates cGAS-STING-Mediated Signaling Pathway. *The Journal of Immunology*, **206**, 1844-1857. https://doi.org/10.4049/jimmunol.2001110

[27] Li, D., Liu, Y., Qi, X., Wen, Y., Li, P., Ma, Z., *et al.* (2021) African Swine Fever Virus MGF-110-9L-Deficient Mutant Has Attenuated Virulence in Pigs. *Virologica Sinica*, **36**, 187-195. https://doi.org/10.1007/s12250-021-00350-6

[28] Li, J., Song, J., Zhou, S., Li, S., Liu, J., Li, T., *et al.* (2023) Development of a New Effective African Swine Fever Virus Vaccine Candidate by Deletion of the H240R and MGF505-7R Genes Results in Protective Immunity against the Eurasia Strain. *Journal of Virology*, **97**, e0070423. https://doi.org/10.1128/jvi.00704-23

[29] Hakizimana, J.N., Yona, C., Kamana, O., Nauwynck, H. and Misinzo, G. (2021) African Swine Fever Virus Circulation between Tanzania and Neighboring Countries: A Systematic Review and Meta-Analysis. *Viruses*, **13**, Article 306. https://doi.org/10.3390/v13020306

[30] Sauter-Louis, C., Conraths, F.J., Probst, C., Blohm, U., Schulz, K., Sehl, J., *et al.* (2021) African Swine Fever in Wild Boar in Europe—A Review. *Viruses*, **13**, Article 1717. https://doi.org/10.3390/v13091717

[31] Ambagala, A., Goonewardene, K., Kanoa, I.E., Than, T.T., Nguyen, V.T., Lai, T.N.H., *et al.* (2024) Characterization of an African Swine Fever Virus Field Isolate from Vietnam with Deletions in the Left Variable Multigene Family Region. *Viruses*, **16**, Article 571. https://doi.org/10.3390/v16040571

[32] Koh, E.Y., Tan, A.K.S., Yeo, D., Lau, C., Tan, L.Y., Ng, O.W., *et al.* (2023) Detection of African Swine Fever Virus from Wild Boar, Singapore, 2023. *Emerging Infectious Diseases*, **29**, 2580-2583. https://doi.org/10.3201/eid2912.230966