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REVIEW ARTICLE

Defective viral genomes for antiviral therapeutics, vector control, spillover prevention, and vaccine development

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Abstract

Defective viral genomes (DVGs) are altered forms of viral genomes generated during error-prone replication, particularly in RNA viruses. This review examines the current understanding of DVG biology, its mechanisms of action, and its translational potential as an antiviral agent, in vector control strategies, zoonotic disease spillover prevention, and vaccine development. DVGs modulate virus-host interactions by interfering with the replication of full-length viruses and/or activating innate immune responses. Both naturally occurring and synthetic DVGs could suppress viral loads, reduce disease severity, and enhance the efficacy of inactivated and live attenuated vaccines. DVG-derived particles, such as defective interfering particles and therapeutic interfering particles, have shown broad-spectrum antiviral activity against numerous viruses *in vitro* and *in vivo*. By reducing viral replication in both insect and animal hosts, DVGs may block the vector transmission cycle and prevent spillover between species, thus playing a key role in controlling arthropod-borne viruses and zoonotic diseases. However, DVGs could contribute to viral persistence, which may hamper their clinical application. Additional challenges include standardizing DVGs production, understanding their effects on adaptive immunity, and ensuring their safety profile as vaccines or vaccine adjuvants.

Keywords: Defective viral genomes; Defective interfering particles; Therapeutic interfering particles; One Health; Antiviral; Vaccine

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1. Introduction

Conventional antiviral therapies, including small molecules, monoclonal antibodies, and vaccines, have demonstrated efficacy against specific viruses. However, the emergence of drug-resistant strains, viral mutations, and the complexity of viral-host interactions limit the effectiveness of these antiviral therapies. The rapid evolutionary and adaptive capabilities of viruses pose significant barriers to the innovative development of treatments. Novel antiviral strategies, such as defective viral genomes (DVGs) that leverage the intricacies of viral replication and host immune responses, hold promise for improving viral control. The objective of this review is to provide a comprehensive overview of research on DVGs in antiviral therapy, vector-borne disease control, zoonotic spillover, and vaccine development, along with the associated challenges.

An advanced search was performed on PubMed to compile the existing research on the applications of DVGs in the control of viral infections, utilizing the following search strategy: "defective viral genome [tiab] OR defective interfering particle [tiab] OR therapeutic interfering particle [tiab] OR defective interfering genome [tiab]." Based on the search results, we compiled summaries of viruses that generate DVGs (Table S1) and the applications of DVGs and defective interfering particles (DIPs) as antivirals, vector control strategies, spillover prevention, and vaccine components (Table S2). Section 4 presents several key applications of DVGs based on selected viruses, as shown in Table S2.

2. Different types of DVGs and their mechanisms of inhibition

2.1. Definition of DVGs

A naturally occurring DVG is a modified genome variant produced spontaneously during virus replication.[1] DVGs are commonly derived from RNA viruses due to the lack of proofreading and low fidelity of the RNA-dependent RNA polymerase. Synthetic DVGs are artificially engineered analogs of naturally occurring DVGs, sharing the same structural and functional properties but with enhanced design features, such as homogeneity and stability, for therapeutic applications. DVGs are termed DIPs when packaged into virions. Natural DIPs may not efficiently inhibit the full-length virus replication, but when synthetically engineered as a therapeutic interfering particle (TIP), they are specifically optimized to suppress replication and provide therapeutic benefits. A helper virus is a full-length parental virus that provides what is lacking in the DVG to initiate a complete viral replication cycle.[2] For example, natural DVGs generated as byproducts of Zika virus (ZIKV) replication depend on the full-length parental ZIKV as the helper virus to provide the essential replication and packaging machinery required for their propagation.[3] When DIP is optimally engineered, it can serve as TIP with therapeutic potential.

2.2. DVGs and defective interfering particle generation

DVGs are modified viral genomes that arise due to the errors of viral polymerase during high-titer viral replication. Under replicative stress, the polymerase may dissociate prematurely or reinitiate at a downstream site. This template switching or misalignment results in atypical viral genomes, which are then encapsulated by viral proteins to form DIPs and released from cells (Figure 1). DVGs often retain essential *cis*-acting elements and 5' and 3' ends, allowing them to be replicated and packaged into DIPs in the presence of a helper virus.[4]

The molecular mechanisms behind the generation of DVGs remain inadequately understood. Since DVGs arise from polymerase errors during replication, which are typically random, various studies suggest that DVG generation is a random event. High-resolution long-read sequencing has identified the generation of a large and seemingly random deletion DVG population during Flock house virus infections *in vitro*.[5] In addition, attempts to re-isolate a particular severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) DVG failed,[6] further supporting the hypothesis that DVG generation is a random and non-reproducible event.

Conversely, several studies also showed that DVG generation is non-random. For example, a specific copyback DVG (cbDVG) derived from Sendai virus (SeV) was consistently detected across multiple independent infections,[7-9] and similar DVG generation was observed in respiratory syncytial virus (RSV)[8] and parainfluenza virus infections.[10] Conserved genomic motifs in RSV act as hotspots for polymerase breakage and rejoining, promoting DVG formation. Mutation of these hotspots significantly reduced DVG production, indicating that their formation depends on specific viral RNA sequences rather than random polymerase errors. This phenomenon has been observed both in cell culture and patient samples, supporting the existence of a conserved mechanism for DVG generation.[11] Similarly, in SARS-CoV-2, specific deletion DVGs were generated with recombination sites clustered in genomic hotspots, suggesting a non-random process facilitated by RNA secondary structures at these regions.[12] Similar influenza A virus (IAV) DVGs were also isolated from different human patients and independent *in vitro* cultures.[13] Taken together, these findings indicate that the generation of DVGs is predictable when the viral genome contains specific signals promoting recombination, whereas in their absence, the process becomes random.

2.3. Types of DVGs

Figure 2A depicts the unmodified full-length genome and antigenome, which do not contain any modifications associated with DVG formation. The first type of DVGs is deletions, shortened forms of the original viral genome, typically retaining the 3' and 5' ends of the parental virus for replication and packaging in the presence of a helper virus.[14,15] Deletion DVGs are formed when the viral polymerase dissociates from the original template, then rebinds at a downstream position (Type 1) or to a different template while remaining attached to the nascently synthesized strand (Type 2), resulting in the loss of a segment of the genome (Figure 2B).[16-19] Deletion DVGs are the most abundant DVGs isolated from various viruses, including the lymphocytic choriomeningitis virus

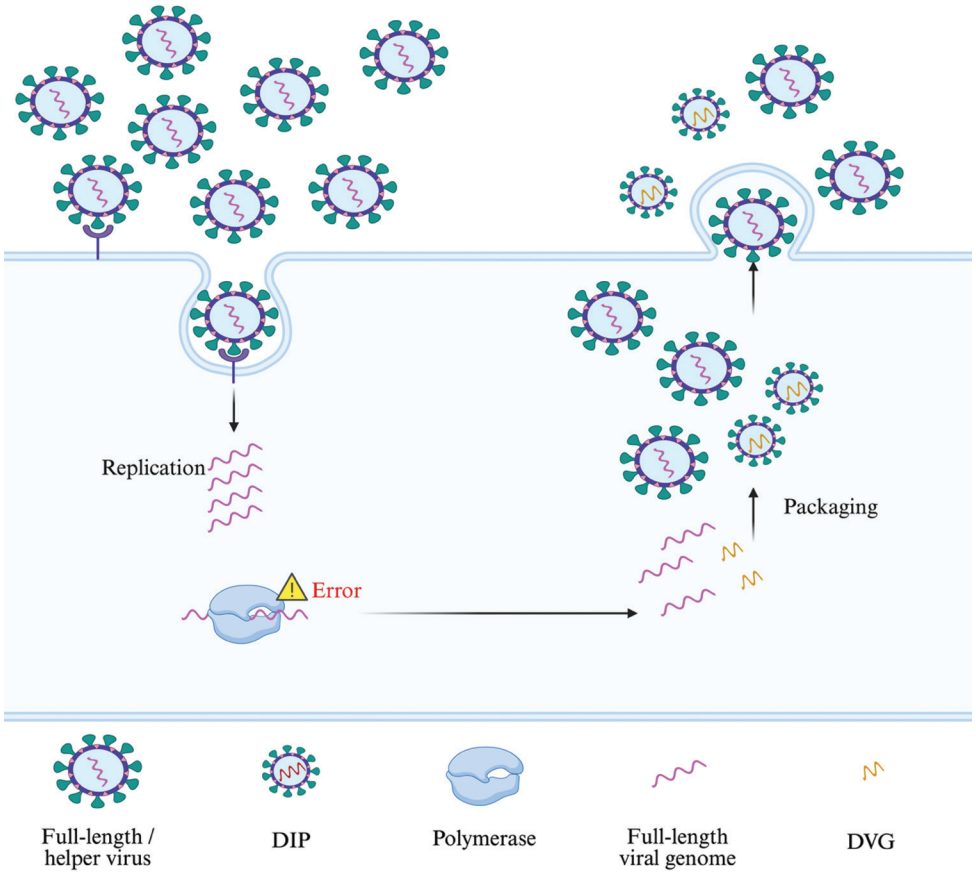

Figure 1. DVGs and DIPs generation during high-titer viral replication. Created with BioRender. Chan, Y.F. (2025) https://BioRender.com/olyyo33.
Abbreviations: DIP: Defective interfering particles; DVG: Defective viral genome.

(LCMV), Pichinde virus, equine arteritis virus, porcine reproductive and respiratory syndrome virus, and others (Table S1).

The second type, cbDVGs, consists of rearranged genomes with reverse-complementary 5' and 3' ends, forming a panhandle structure due to the absence of viral nucleoproteins. They arise when the viral polymerase detaches from the template strand and reattaches to the nascently synthesized strand, continuing elongation through the 5' end (Figure 2C).[20-22] CbDVGs are commonly detected in Ebola virus, canine distemper virus (CDV), measles virus (MeV), mumps virus, and others (Table S1).

The third DVG, snapback DVGs, represent another class characterized by their unique self-complementary structure. They arise when the viral polymerase initiates synthesis on one strand of the genome, then prematurely switches direction and copies back along the same strand in reverse orientation. This results in a covalently linked, double-stranded RNA molecule with two identical sequences arranged in opposite orientations, forming a hairpin structure (Figure 2D).[23] Snapback DVGs are found in human metapneumovirus, vesicular stomatitis virus (VSV), and adeno-associated virus (Table S1).

A lesser-known category involves substitution mutation DVGs. They are formed when the polymerase introduces numerous substitutions into the newly synthesized strand. These DVGs retain genome lengths similar to those of the full-length virus but harbor clusters of point mutations that can impair their replication competency (Figure 2E).[24,25] Examples of substitution DVGs are isolated from the dengue virus (DENV), IAV, and foot-and-mouth disease virus (Table S1).

Another distinct type of DVGs, known as mosaic DVGs, emerges from intricate recombination events that involve non-contiguous regions of the original viral genome. These genomes often incorporate a combination of internal deletions, duplications, and substitution mutations, producing hybrid structures with altered functions (Figure 2F).[25-27] Examples of mosaic DVGs have been detected in

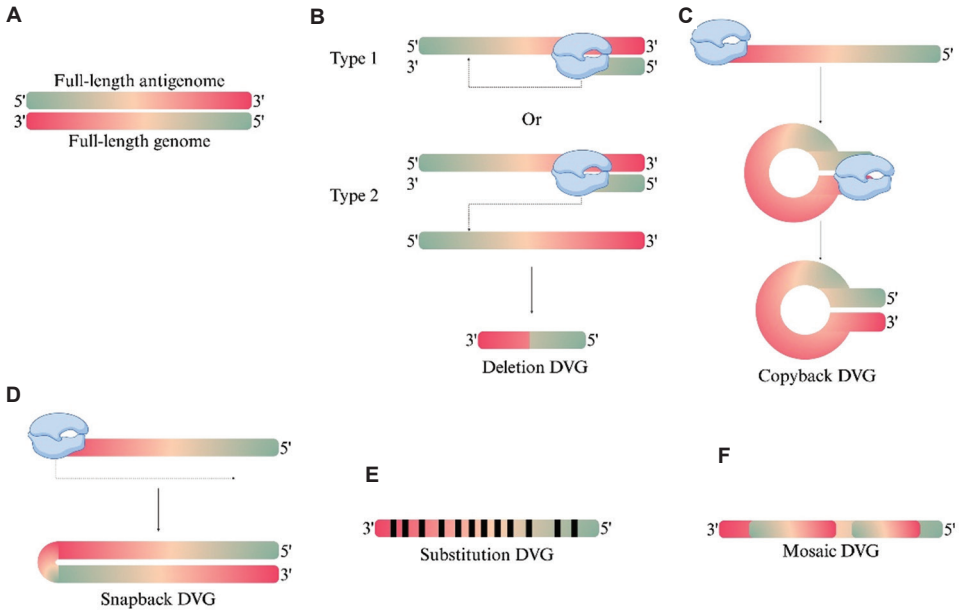

Figure 2. Different types of DVG. The black regions indicate substitution mutations caused by polymerase errors. (A) Full-length viral genome and anti-genome. (B) Deletion DVG. (C) Copyback DVG. (D) Snapback DVG. (E) Substitution DVG. (F) Mosaic DVG. Created with BioRender. Chan, Y.F. (2025) https://BioRender.com/olyyo33.
Abbreviation: DVG: Defective viral genome.

coronavirus, mouse hepatitis virus, and flock house virus (Table S1).

2.4. Methods to detect DVGs

Accurate detection and characterization of DVGs are essential to understanding their mechanisms of generation, biological functions, and translational potential. Density gradient ultracentrifugation has been used to separate DIPs from standard virus particles based on their altered buoyant density and sedimentation properties compared to the full-length virus.[2,28-30] This approach enables the physical enrichment of particles carrying DVGs from viral cultures, which can then be characterized by next-generation sequencing or functional assays, including infectivity tests, viral infection interference, innate immune activation, or the ability to facilitate persistent infection.

High-throughput sequencing has become the most widely employed strategy for DVG discovery.[31,32] Short-read sequencing platforms, such as Illumina,[3,12,33-41] provide exceptional sensitivity for mapping deletion junctions, but rely on assembly algorithms that can fragment or misrepresent larger structural rearrangements. Long-read sequencing technologies, including Nanopore[5] and PacBio, enable the theoretical near full-length resolution of DVGs and can uncover complex recombination patterns; however, their elevated error rates necessitate complementary validation.

The bioinformatic analysis of the sequencing results is equally important. Bioinformatic tools, such as ViReMa,[12,38,41,42] DI-tector,[34,43] DVG-profiler,[44,45] DVGfinder,[36,46] and VODKA2,[47] can systematically identify and quantify DVGs from raw sequencing reads. These pipelines implement algorithms to detect non-contiguous alignments, classify deletion junctions, and estimate relative abundance, while filtering the sequencing artifacts. Reproducibility remains a challenge with differences in read-mapping strategies, threshold cutoffs, and filtering parameters. Pipelines, such as CallVariants[3] or DG-seq,[37] demonstrate how analytical design choices (alignment stringency or reference genome completeness) directly influence the decision on DVG calling.

Future advances will require integrative bioinformatics pipelines incorporating unbiased sequencing, including direct RNA sequencing and single-cell sequencing, to distinguish DVGs from sequencing artifacts.

3. Roles of DVGs

3.1. DVGs outcompete full-length viral genomes for replication resources

Several studies have highlighted the capacity of DVGs to inhibit the production of full-length viruses. Gradient centrifugation data reveal that DIPs are physically smaller than parent viral particles, attributed to the shorter sequence length of DVGs within the DIPs.[48,49] This reduced length is thought to confer replication advantages that

indirectly inhibit the replication of the full-length viral genome.[2] Early work by Portner and Kingsbury[50] provided foundational evidence that DIPs mediate homologous interference by suppressing viral RNA synthesis intracellularly. Kolakofsky[51] demonstrated that cbDVGs with retained terminal promoter elements of the viral genome are capable of attenuating the production of full-length virus. Furthermore, Re and Kingsbury[52] identified that the complementation of the 5' and 3' termini of cbDVGs significantly enhances replication efficiency by improving promoter activity. As a result, DVGs replicate and accumulate rapidly, depleting a substantial portion of the available viral replication machinery.

DVGs can exhibit preferential packaging with structural proteins over full-length viral genomes.[53-55] DVGs lacking essential *cis*-acting packaging signals tend to be poorly maintained, as demonstrated in SARS-CoV-2, human immunodeficiency virus (HIV), and ZIKV.[40,56] Mechanistically, cbDVGs increase binding affinities for viral RNA polymerase and nucleocapsid proteins due to the complementation at both 5' and 3' termini. This enhanced binding competes with the full-length viral RNA for efficient replication and encapsidation.[57] Some DVGs also express truncated proteins that can modulate the replication of full-length viruses. For example, SARS-CoV-2 DVGs can encode fusion proteins (Nsp1-10) that interfere with the replication of the full-length virus, although the exact mechanism remains unclear.[6] Such interactions highlight the diverse mechanisms through which DVGs modulate virus infection, beyond merely competing for replication machinery.

3.2. DVGs stimulate innate immune responses

Although DVGs and DIPs are replication-incompetent without helper viruses, they play critical roles in modulating host immunity, such as stimulating innate immune responses, particularly type I[58-60] and type III interferon (IFN),[60,61] which are central to antiviral defense. DVGs are sensed by cellular pattern recognition receptors, such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), triggering signaling cascades that result in the production of IFN-α and IFN-β, as depicted in Figure 3.

Virus stocks with DVGs produce a stronger IFN-I response than those without DVGs, as demonstrated in SeV.[7] The DVGs act as viral pathogen-associated molecular patterns, promoting the sustained activation of transcription factors, such as IFN regulatory factor 3 and nuclear factor kappa-light-chain-enhancer of activated B cells, which drive the activation of IFN-I. Intriguingly, IFN-β expression was observed only in cells harboring DVGs. This selective activation highlights DVGs as key triggers of antiviral immunity, functioning as early danger signals during the infection process.

DVGs stimulate the activation of the IFN-III pathway, particularly through the induction of IFN-λ.[60,61] The presence of DVGs in infected cells triggers the rapid nuclear accumulation of IFN regulatory factor 1, which is essential for IFN-λ1 expression and mediated by the mitochondrial antiviral signaling protein pathway. The detection of DVGs in infected tissues and cell cultures correlates with an increased expression of antiviral genes, including IFNλ, as shown in RSV and MeV.

TIP derived from the Newcastle Disease virus[62,63] and SeV[64] have been shown to promote dendritic cell (DC) maturation mediated by IFN-I responses. *MDA5* was essential for the recognition of DVGs to activate DCs, as MDA5-deficient DCs display significantly impaired maturation.[64,65] The production of cbDVGs is also necessary for potent immune activation, as ultraviolet (UV)-inactivated cbDVGs failed to stimulate DCs. When UV damages the secondary structure of the cbDVGs and impairs production, these structures are not sufficiently amplified to activate immune signaling. This highlights that the mere presence of DVGs does not trigger immune sensing but requires their active production within host cells to accumulate in quantities high enough to initiate immune activation.

DVGs can act as molecular decoys, undermining viral immune evasion strategies. The V protein encoded by SeV is known to suppress IFN-I activation by inhibiting MDA5.[64] Despite the presence of such antagonistic viral proteins, cbDVGs retain the ability to trigger strong IFN-I responses by preferentially binding to MDA5 compared to the V protein. This suggests that DVGs can override viral mechanisms to evade host defenses. Thus, DVGs serve a dual function: acting as pathogen-associated molecular patterns that are highly visible to host sensors and simultaneously bypassing viral immune evasion to initiate an antiviral innate immune cascade.

3.3. DVGs possess genetic barriers to viral resistance

Any mutation in the virus that would confer resistance to the DVGs could simultaneously impair the virus's ability to replicate effectively, thus imposing a genetic barrier to resistance. As DVGs rely on the presence of the full-length virus for replication, the evolution of viral resistance to DVGs is constrained by the fact that they share critical elements with the full-length virus. The viral evolution may still allow for certain levels of adaptation, particularly in cases of high viral mutation rates, which could eventually diminish the efficacy of DVGs. As the

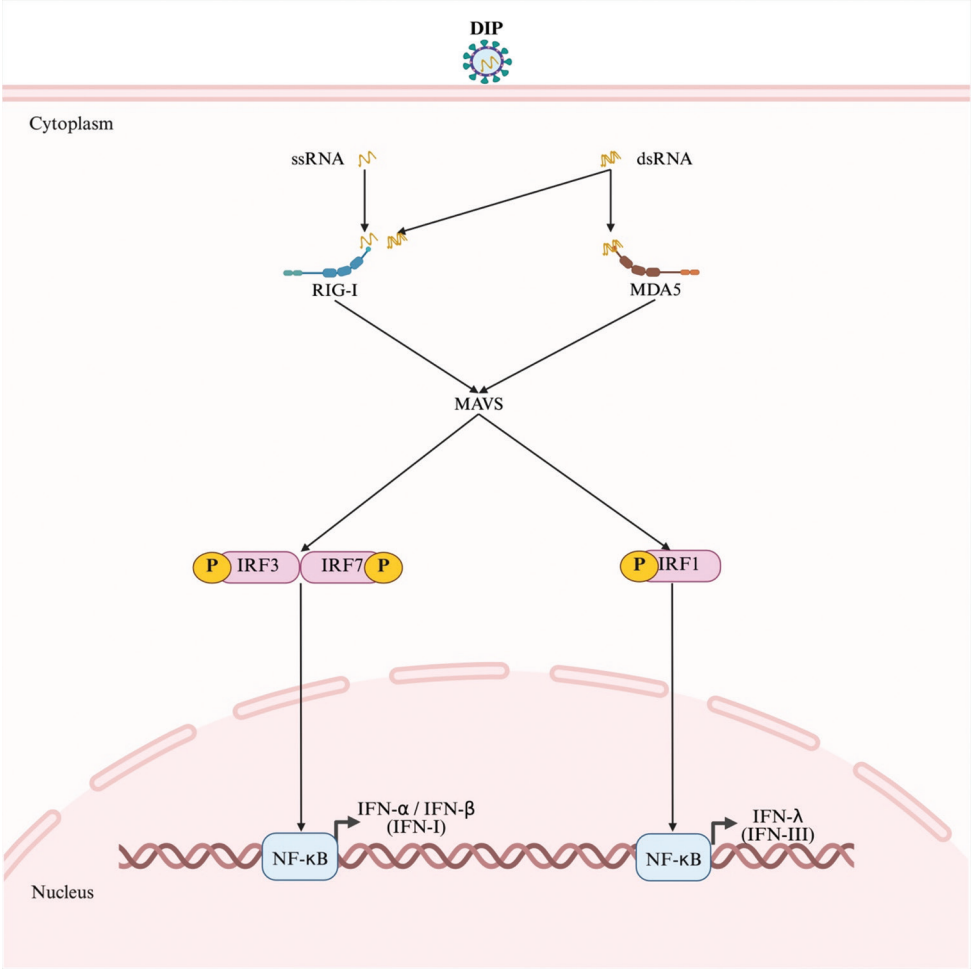

Figure 3. DVG-mediated IFN activation. Ss- and ds-DVG RNA activate *RIG-I* and *MDA5* receptors, leading to the activation of MAVS, IRF1, IRF3, IRF7, and NF-κB, which in turn leads to the activation of IFN-α, IFN-β, and IFN-λ. Created with BioRender. Chan, Y.F. (2025) https://BioRender.com/olyyo33. Abbreviations: DIP: Defective interfering particles; DVG: Defective viral genome; Ds: Double-stranded; IFN: Interferon; IRF: Interferon regulatory factor; MAVS: Mitochondrial antiviral signaling protein; MDA5: Melanoma differentiation-associated gene 5; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; P: Phosphate; RIG-I: Retinoic acid-inducible gene I; Ss: Single-stranded.

virus continues to evolve, it may develop mechanisms to minimize the impact of DVGs; however, competition for replication machinery may still present significant barriers to resistance. This dynamic sets the stage for DVGs to maintain long-term effectiveness, potentially. The evolutionary trajectory of both the virus and DVGs must be closely studied to understand their sustainability in viral control strategies.[66,67]

3.4. DVG/defective interfering particle promotes viral persistence

Although DVGs are known for their ability to suppress viral replication and stimulate host antiviral responses, they can also play a paradoxical role in promoting viral persistence. This complex behavior is known as the Von Magnus effect, a dynamic interplay in which asynchronous cycling between DIPs and their helper viruses results in fluctuating replication outcomes (Figure 4).[2,48] High titers of full-length virus promote the generation of DVGs, which then interfere with viral replication and reduce the population of full-length virus. However, because DIPs rely on the full-length virus for their replication, a subsequent drop in full-length virus levels limits DIP amplification. Infections with either DIPs alone, resulting in non-productive infection, or with the full-length virus alone, which can reignite the viral replication cycle, enable long-term, low-level infections in host tissues. Thus, the outcome of infection often depends on the delicate balance between full-length viral genomes and their defective counterparts.

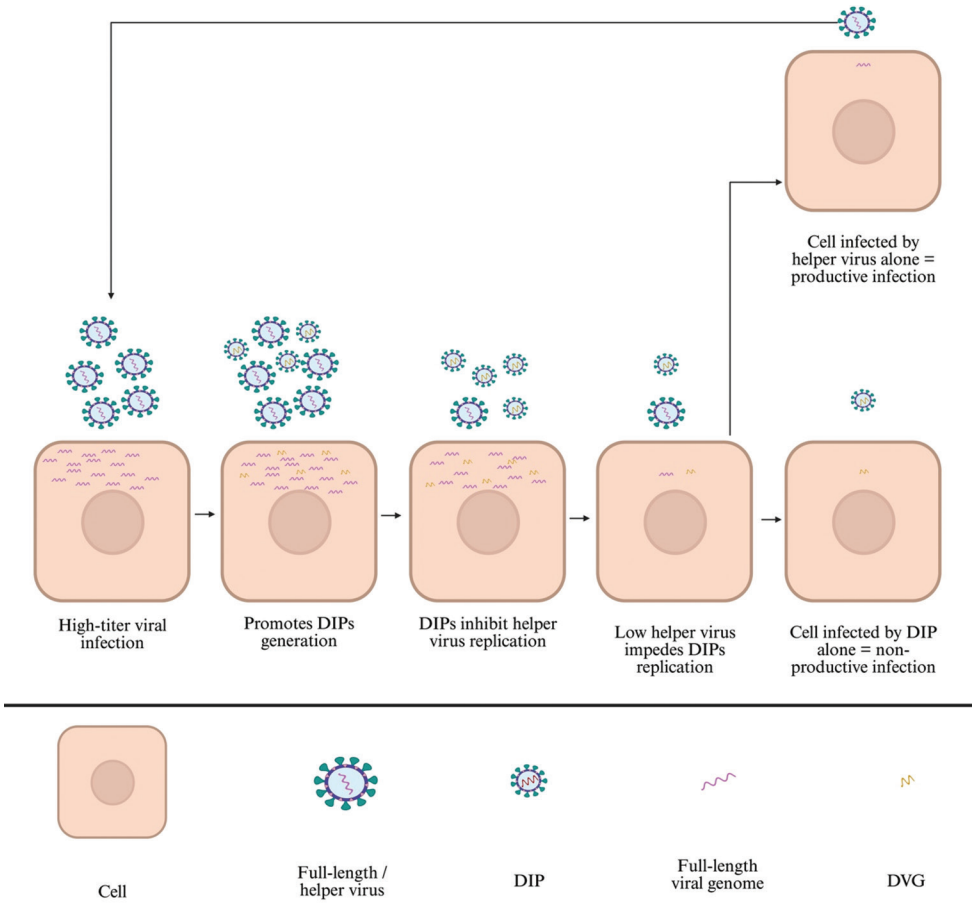

Figure 4. DIP-mediated viral persistence (von Magnus effect). Created with BioRender. Chan, Y.F. (2025) https://BioRender.com/olyyo33.
Abbreviations: DIP: Defective interfering particles; DVG: Defective viral genome.

For instance, the Ebola virus containing DVGs continues to shed infectious virus for months during serial passaging in tissue culture.[68] DVGs derived from equine herpesvirus type 1 (EHV-1) could retain and express functional *UL2* gene, with expression levels even exceeding those observed in infections without DVGs. This elevated expression of *UL2* is associated with the establishment of persistent EHV-1 infection *in vitro*.[69] Furthermore, DIP-mediated persistence has been documented in numerous viruses, as summarized in Table S1. While often linked to attenuation, the ability of DVGs to maintain persistent infection underscores the importance of understanding their regulatory dynamics, particularly in therapeutic contexts where persistence may be undesirable.

In persistent HIV infection, defective proviruses rapidly emerge during acute infection and persist despite long-term therapy, constituting the majority of the HIV reservoir.[70,71] Although replication-incompetent, many of these proviruses remain transcriptionally active, producing viral RNAs and proteins that sustain chronic immune activation.[72,73] The defective viral products promote chronic immune activation and the activation of T helper (Th) 17 cells, which play a significant role in chronic inflammation.[74,75] The defective proviral products generated during HIV infection are often incomplete or qualitatively distinct from those of intact viruses, resulting in suboptimal antigenic stimulation that fails to fully activate cytotoxic T lymphocyte responses.[71,72,76] In parallel, CD8+ T cell activity is further constrained by persistent expression of inhibitory natural killer cell receptors during highly active antiretroviral therapy.[77] Collectively, weakened cytotoxic T lymphocyte stimulation by defective proviruses, suppression of CD8+ T cells in highly active antiretroviral therapy, and activation of Th17 cells further exacerbate chronic inflammation that sustains HIV persistence.[74]

4. Applications of DVGs/defective interfering particles for controlling viral infection

4.1. DVG/defective interfering particle as an antiviral therapeutic

As of July 20, 2025, no clinical trials involving DVGs as antiviral therapeutics have been registered on ClinicalTrials.gov. However, extensive *in vitro* and *in vivo* studies highlight the therapeutic potential of DVGs against various human viruses, demonstrating their ability to interfere with viral replication and modulate immune responses.

A specific synthetic DIP derived from the IAV A/PR/8/34 (H1N1) strain, known as 244/PR8, can effectively inhibit the replication of the IAV in human lung diploid fibroblasts, bronchial epithelial cells, and primary nasal basal cells in a dose-dependent manner.[78] This DIP has also shown a dose-dependent *in vivo* protection, not only against IAV, but also displays broad-spectrum antiviral efficacy against influenza B virus (IBV) and a mouse-adapted RSV.[79-83] This cross-protection is primarily attributed to the ability of the DIP to stimulate IFN-I responses.[38] Mice given the UV-inactivated 244/PR8 all succumbed to the IBV challenge, consistent with the idea that active DVG production is required to elicit innate immune responses, as mentioned previously.[83] Pre-treatment of mice with 244/PR8 resulted in reduced IBV infectivity in the lungs and conferred complete protection against lethal IBV challenge. This protection elicited from the pre-treatment of DIP was also observed in the ferret model, providing supporting evidence that the protection of this DIP is not host-specific.[13,80]

Engineered SARS-CoV-2 TIPs have shown substantial potential as an antiviral intervention against SARS-CoV-2.[6,40] Single administration of TIPs inhibits *in vitro* SARS-CoV-2 replication by up to 100-fold, with efficacy extending to different variants that are resistant to antibody neutralization. The TIPs demonstrate a high barrier to resistance, primarily due to their competition with the full-length virus for essential viral trans-acting elements, nucleocapsid protein, and RNA-dependent RNA polymerase that are required for viral replication and packaging. By occupying these key components, TIPs effectively limit the virus's ability to replicate and evolve, reducing the likelihood of the development of new variants of concern. In Syrian golden hamsters, a single intranasal administration of TIPs resulted in a durable suppression of SARS-CoV-2 in the lungs, reducing viral load and preventing pulmonary edema. In addition, TIPs were found to suppress the production of pro-inflammatory cytokines, thereby mitigating the severe inflammation commonly associated with severe COVID-19. These findings were further corroborated by the absence of viral escape in long-term culture studies, with TIPs maintaining efficacy across extended serial passaging, demonstrating a high barrier to resistance. Remarkably, TIPs not only protect against the virus but also lower the risk of further transmission.

Xiao *et al.*[84] described an enteroviral-based TIP1 (eTIP1) derived from poliovirus as a promising antiviral with broad-spectrum efficacy against several viruses, including coxsackievirus B3, enterovirus A71, enterovirus D68, rhinovirus 16, rhinovirus 1A, IAV virus, and SARS-CoV-2. When administered intranasally or intraperitoneally, eTIP1 induces robust IFN-I responses, providing both prophylactic and therapeutic protection by inhibiting viral replication and stimulating the production of neutralizing antibodies, offering long-lasting immunity. However, a critical aspect of eTIP1's functionality lies in its ability to elicit neutralizing antibodies against SARS-CoV-2, despite being derived from poliovirus, which lacks the structural proteins of SARS-CoV-2, such as the spike protein targeted by most neutralizing antibodies. This unexpected ability is likely due to eTIP1's activation of IFN-I responses, which enhance the DCs' maturation to recognize viral antigens from SARS-CoV-2. This, in turn, could facilitate the induction of B-cell maturation to produce specific neutralizing antibodies against SARS-CoV-2. However, further research is needed to fully understand these cross-reactive immune responses and optimize eTIP1 for use against a broader range of viral pathogens.

Holland and Villarreal[85] provide robust evidence supporting the *in vivo* production and replication of DIPs in newborn mice infected with VSV and rabies virus. Their findings show that, in contrast to adult mice, newborn mice generate substantial quantities of DIPs in response to these viral infections. These DIPs, which replicate efficiently across serial intracerebral passages in the brains of newborn mice, effectively interfere with viral replication. While these VSV-derived DIPs specifically target and inhibit VSV replication, they do not exhibit a broad-spectrum effect, as they do not interfere with the replication of a neurotropic IAV (NWS strain). Importantly, these DIPs do not establish persistent infections, avoiding the long-term complications associated with chronic infections. The *in vivo* production of DIPs, particularly in newborn mice, highlights the importance of age-related differences in the immune response, suggesting that DIP-based therapies might need to be tailored to account for these variations across different age groups.

The potential of DIPs as antiviral agents is hindered by various challenges. The absence of clinical trials

involving DVGs leaves their safety, efficacy, and long-term effectiveness in humans largely untested. DIPs have shown efficacy primarily in acute infections; however, their effectiveness in chronic or persistent infections remains to be determined. DIPs do not necessarily eliminate the viral reservoir, leaving the possibility of reactivation. The broad-spectrum antiviral efficacy of DVGs is notable; however, several studies have found that DVGs may not consistently offer protection against other strains or viral families, and the exact mechanisms by which DVGs exhibit broad-spectrum activity remain unclear. The production and delivery of DIPs also remain unresolved, with challenges related to scalability, stability, and consistency of production for clinical use.

4.2. DVG as a vector control strategy

The application of DVGs in vector control represents an innovative and sustainable approach to controlling insect-transmitted viruses, such as DENV, ZIKV, chikungunya (CHIKV), and the West Nile virus (WNV), which remain major public health threats globally. By targeting the viral replication cycle within the vector, DVGs can reduce viral titers in insect populations, thereby decreasing the likelihood of virus transmission to humans.

Chikungunya DVGs generated through high-titer infection passages in mammalian and mosquito cells can significantly reduce viral replication *in vitro,* showing their potential efficacy in limiting CHIKV spread within both human and mosquito hosts. CHIKV DVGs have also demonstrated broad-spectrum activity against other arboviruses, including O'nyong'nyong and Sindbis viruses. In *Aedes aegypti*, which transmits CHIKV, DVGs effectively blocked CHIKV dissemination.[33,86]

The research on ZIKV DVGs also reveals their significant potential as a vector control strategy. ZIKV DVG interfered with ZIKV replication in both vertebrate (Vero) and mosquito cells (C6/36), reducing viral load by competing for viral replication machinery.[3] To further examine the *in vivo* therapeutic application of this DVG, Rezelj *et al.*[3] established a packaging system to encapsidate the DVG into virus-like particles, forming a TIP for *in vivo* delivery. The ZIKV TIP-treated AG129 mice (deficient in α/β and γ receptors) demonstrated significantly lower weight loss and reduced viremia compared to the full-length ZIKV-infected mice. In ZIKV TIP-treated immunocompetent C57BL/6 mice, viral load in ovaries and brains was significantly lower. The safety profile of the TIPs was demonstrated by their lack of persistence and dissemination to distal organs in the absence of full-length ZIKV, as it was degraded and undetectable within a day of administration. In the *Aedes aegypti*, ZIKV DVG was detectable in the midgut and carcass for at least 10 days post-transfection, even in the absence of full-length ZIKV, suggesting its role in controlling persistent infection. The dissemination rate of the full-length ZIKV dropped by 90% when the mosquitoes were pre-treated with ZIKV DVG, suggesting its role as vector control by limiting virus dissemination and transmission.

Research into DIPs in DENV infections yields compelling insights into their role in viral dynamics, immune modulation, and potential therapeutic applications. Li *et al.*[87] successfully developed a transgenic HEK 293T cell line that can continuously produce DENV DIPs, termed DI290. The purified DIPs displayed broad-spectrum activity by inhibiting the replication of all DENV serotypes in Huh7 cells in a dose-dependent manner. Nonetheless, the efficacy of the DIPs decreased tremendously after UV inactivation. In another experiment, Lin *et al.*[88] demonstrated that both DI290 DIPs and lipid nanoparticles loaded with the DI290 RNA (LNP-290) effectively suppressed DENV infection in human primary monocyte-derived macrophages, THP-1 macrophages, and fibroblasts. Interestingly, DI290 did not compete in RNA replication; instead, it is a potent inducer of innate immune responses. LNP-290 demonstrated tremendously greater DENV inhibition compared to DI290 by activating IFN-III responses when IFN-I signaling was inhibited. Another study using DENV DIP, termed DIP-296, inhibited the replication of all DENV serotypes in a dose-dependent manner by activating RIG-I and MDA5, resulting in increased expression of IFN-β and the ubiquitin-like protein ISG15.[89] Although DENV DVGs have shown promising antiviral efficiency against all DENV serotypes, there is still a lack of *in vivo* experiments using *Aedes aegypti* to determine the effects of DIPs on blocking DENV transmission.

The study of DIPs in WNV has also provided valuable insights into replication dynamics, population diversity, and the potential role in controlling the transmission of WNV. WNV isolated from lorikeets contained abundant DIPs that suppressed the production of full-length WNV in Vero cells.[90] These DIPs were able to reduce infection and dissemination rates of WNV in *Culex pipiens quinquefasciatus* mosquitoes. Nonetheless, the DIPs did not protect WNV-infected mice from morbidity and mortality. This suggests DIPs may have host specificity, or a different dose may be required to reduce WNV in mosquitoes and mice. In C3H/RV cells derived from mice resistant to WNV, DIPs were more abundant and significantly inhibited virus replication.[91] In contrast, in susceptible C3H/HE cells, DIPs were less effective, indicating that their

interference with virus replication was context-dependent and influenced by host factors. Collectively, these findings show that the antiviral effectiveness of DIPs against WNV is not universal, but rather shaped by specific host genetic factors.

It is critical to address the challenges associated with using DVGs in vector control strategies. The inability of DIPs to replicate independently presents challenges in their use as a long-term solution for viral control, particularly in environments where viral transmission is seasonal. Thus, while DIPs offer a novel tool for reducing vector-borne transmission in the short term, their role in the long-term management of mosquito-borne viruses remains uncertain. There are also ecological concerns regarding the release of genetically modified viral particles into the environment.

4.3. DVG as a barrier for zoonotic diseases and spillover

Preventing zoonotic spillover of viruses from animals to humans has become a central focus in global One Health programs. RNA viruses exhibit remarkable adaptability across diverse environments and hosts, primarily due to the high error rate of their RNA-dependent RNA polymerase. This high mutation rate enables RNA viruses to evolve rapidly, facilitating their ability to overcome host immune defenses, adapt to new hosts, and exploit various ecological niches. RNA viruses are particularly prone to spillover events, in which they transfer from animal reservoirs to humans, leading to the emergence of infectious diseases with pandemic potential.

CDV is a zoonotic morbillivirus closely related to MeV, and it poses a spillover risk due to its broad host range and capacity to infect both dogs and other mammals. Findings demonstrate that DVGs derived from CDV are not only produced spontaneously during infection but can also persist and function effectively in both *in vitro* and *in vivo*.[92] In cell culture, DIPs robustly suppressed full-length CDV replication in a dose-dependent manner. In the ferret model, DIPs maintained detectable replication for at least 14 days in the presence of co-infecting full-length virus and were recovered from lymphoid tissues, which are key sites for CDV amplification and dissemination. Since lymphoid tissues are also the key sites for B- and T-cells activation, the persistence of DVG at these immunologically active sites implies that DVGs may also serve as potent immunogenic antigens that can engage adaptive immune responses.

LCMV is an arenavirus capable of establishing persistent and asymptomatic infections in rodents, while posing a significant risk of severe illness in humans. During infection, LCMV rapidly generates DIPs, facilitating a non-cytolytic, persistent state in infected cells by suppressing the cytopathic effects typically induced by full-length virus replication.[93] DIPs also inhibit the presentation of LCMV antigens on the cell surface, thereby evading antibody and T-cell recognition. As a result, host cells are spared from full-length virus-induced cell death, allowing for long-term cellular survival despite the ongoing presence of the virus. Similarly, LCMV-infected mice displayed low antigenic presentation on the cellular surface, protecting the infected cells from adaptive immune responses while facilitating viral persistence. In short, DIPs derived from LCMV can protect the infected cells from the cytopathic effect and immune recognition but permit persistent infection at the same time. However, a successful antiviral therapy against LCMV must overcome the virus-induced suppression of antigen presentation, which may be achieved by enhancing the expression of major histocompatibility complex class I/II on infected cells for better immune recognition and clearance.

Another significant zoonotic potential virus is the Bunyamwera virus (BUNV), a representative of the *Bunyaviridae* family, which includes several emerging pathogens of public health concern. Serial high-titer passage of BUNV in BHK-21 cells consistently leads to the production of DIPs that can suppress full-length virus replication in a cell-type-dependent manner mediated by viral replication interference.[94] The interference was more pronounced in BHK and Vero cell lines compared to Madin-Darby bovine kidney and *Aedes albopictus* cells.[94,95] The observed variability in interference across different cell types further suggests that host cellular factors modulate the efficacy of DIP.

Collectively, these examples highlight that DVG-mediated antiviral activity extends beyond human-infecting viruses and is relevant across a range of zoonotic viruses. Nonetheless, the first limitation is that DVGs are replication-defective and require co-infection with a competent helper virus. Moreover, the window of maximal DVG efficacy remains uncertain and appears to vary widely between viruses; although some studies suggest early intervention may be more effective. In some viruses, DVGs have even been associated with enhanced cytopathogenicity rather than attenuation,[96-99] potentially exacerbating disease rather than mitigating it. Finally, ecological uncertainties, such as how DVGs persist or transmit in natural reservoirs, and technical hurdles related to their delivery in wildlife and livestock populations, present significant implementation challenges. While DVGs offer conceptual appeal for spillover control, their real-world utility demands refined delivery systems and careful risk-benefit assessment.

4.4. DVG in vaccine development

One of the most direct applications of DVGs and DIPs is the development of live attenuated and inactivated vaccines, based on their ability to elicit both innate and adaptive immune responses through the structural proteins they carry. In live attenuated vaccines, DIPs can be engineered or enriched in viral stocks to create replication-defective or attenuated virus preparations. They can express viral proteins and are safe since DVGs fail to propagate inherently. Although no DVG-based vaccine or vaccine adjuvant has yet entered clinical trials, DVGs have been identified in several inactivated virus vaccine preparations. Their presence often results from the virus propagation process, suggesting that DVGs may already contribute to the immunogenicity or attenuation of some vaccines. Early evidence of DVG presence in vaccines comes from McLaren and Holland,[100] who identified DIPs in oral poliovirus vaccine preparations. These DIPs were found to interfere with the replication of full-length viruses in cell culture. Despite their replication defect, the DIPs retained antigenicity, suggesting a potential contribution to the immunogenicity and attenuation of the vaccine. Similarly, Bellocq *et al.*[101] analyzed nine MeV vaccine preparations and found that six of these vaccines contained MeV DIPs. The consistent presence of DIPs in these vaccines, regardless of manufacturer, suggests that they likely arose during virus propagation and may contribute to both the attenuation of the vaccine and its immunogenicity.

The beneficial effects of DVGs in live attenuated vaccines also depend on their abundance. While moderate levels may enhance safety and immunogenicity, excessive accumulation can impair vaccine performance. This was evident in a study of the Fluenz Tetra live attenuated influenza vaccine, which found unexpectedly high levels of many unique DVG species from both IAV and IBV, resulting in viral stocks with very low infectivity.[102] While this likely enhanced safety by suppressing viral replication, it may have inadvertently reduced immunogenic potency. Since live attenuated vaccines rely on controlled viral replication to produce antigen and stimulate adaptive responses, overly abundant DVGs may curtail antigen availability and limit immune system stimulation. This highlights the dual-edged nature of DVGs in vaccine formulations, which offer both attenuation and immunostimulation, but require careful control to maintain a balance between safety and immunogenicity.

DVGs have also gained attention as immune potentiators in inactivated vaccine formulations. DVG, with its inherent ability to activate innate immune responses, is an attractive candidate for vaccine adjuvants. DVGs derived from SeV are potent innate immune stimulants that drive IFN-I activation and natural adjuvants for vaccination.[103] The *in vitro*-transcribed SeV DVG RNA co-administered with an inactivated H1N1 influenza vaccine induced significant anti-hemagglutinin IgG and IgA in mice compared to those that received the vaccine alone. The anti-hemagglutinin IgG titer elicited by the DVG as an adjuvant was comparable to that of AddaVax and poly(I·C) adjuvants. The DVG triggered robust RIG-I-mediated signaling, promoting a strong IFN-I response and Th1-biased immunity. Similarly, in another study, mice receiving the SeV DVG cocktail with a formalin-inactivated influenza A vaccine exhibited elevated IgG2c levels, increased CD8+ T-cell responses, and a potent Th1-polarized immune response.[104] Together, these findings underscore the potential of DVGs as potent adjuvants capable of enhancing both innate and adaptive immunity in the context of inactivated influenza vaccines.

DVGs also face significant limitations when used as live attenuated or inactivated vaccine formulations. DVG's genomic instability, especially when large deletions are involved, can result in the degradation or loss of the DVGs during replication, complicating their manufacturing and storage. DVGs can interfere unpredictably with full-length viruses during infection, potentially reducing the yield of vaccine production. The efficient delivery of DVGs to the correct anatomical sites, which can efficiently transduce target cells, requires intensive testing. Addressing these limitations will require advancements in synthetic biology and delivery systems for DVG-based vaccines and adjuvants.

5. Conclusion

DVGs and their associated particles (DIPs/TIPs) have immense potential in antiviral therapy, vector control strategy, zoonotic disease spillover prevention, and vaccine development. Far from being inert byproducts of replication, DVGs actively shape infection outcomes by interfering with full-length virus replication and activating robust innate immune responses. Their unique ability to engage the immune system makes them valuable as adjuvants and antivirals. However, significant gaps remain, particularly in elucidating how DVGs influence adaptive immunity, ensuring consistent manufacturing, and preventing potential persistence. As research continues to bridge these gaps, DVGs may form the basis of next-generation vaccine enhancement and broad-spectrum antiviral strategies.

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Conflict of interest

The authors declare they have no competing interests.

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All data are available in the supplementary file of this article.

References

1. Poirier EZ, Vignuzzi M. Virus population dynamics during infection. *Curr Opin Virol*. 2017;23:82-87.

   doi: 10.1016/j.coviro.2017.03.013

2. Huang AS, Baltimore D. Defective viral particles and viral disease processes. *Nature*. 1970;226(5243):325-327.

   doi: 10.1038/226325a0

3. Rezelj VV, Carrau L, Merwaiss F, *et al*. Defective viral genomes as therapeutic interfering particles against flavivirus infection in mammalian and mosquito hosts. *Nat Commun*. 2021;12(1):2290.

   doi: 10.1038/s41467-021-22341-7

4. Li Q, Tong Y, Xu Y, Niu J, Zhong J. Genetic analysis of serum-derived defective hepatitis C virus genomes revealed novel viral *cis* elements for virus replication and assembly. *J Virol*. 2018;92(7):e02182-17.

   doi: 10.1128/jvi.02182-17

5. Jaworski E, Routh A. Parallel ClickSeq and nanopore sequencing elucidates the rapid evolution of defective-interfering RNAs in Flock house virus. *PLoS Pathog*. 2017;13(5):e1006365.

   doi: 10.1371/journal.ppat.1006365

6. Girgis S, Xu Z, Oikonomopoulos S, *et al*. Evolution of naturally arising SARS-CoV-2 defective interfering particles. *Commun Biol*. 2022;5(1):1140.

   doi: 10.1038/s42003-022-04058-5

7. Tapia K, Kim WK, Sun Y, *et al*. Defective viral genomes arising *in vivo* provide critical danger signals for the triggering of lung antiviral immunity. *PLoS Pathog*. 2013;9(10):e1003703.

   doi: 10.1371/journal.ppat.1003703

8. Strahle L, Garcin D, Kolakofsky D. Sendai virus defective-interfering genomes and the activation of interferon-beta. *Virology*. 2006;351(1):101-111.

   doi: 10.1016/j.virol.2006.03.022

9. Sanchez-Aparicio MT, Garcin D, Rice CM, Kolakofsky D, Garcia-Sastre A, Baum A. Loss of sendai virus C protein leads to accumulation of RIG-I immunostimulatory defective interfering RNA. *J Gen Virol*. 2017;98(6):1282-1293.

   doi: 10.1099/jgv.0.000815

10. Killip MJ, Young DF, Gatherer D, *et al*. Deep sequencing analysis of defective genomes of parainfluenza virus 5 and their role in interferon induction. *J Virol*. 2013;87(9):4798-4807.

    doi: 10.1128/JVI.03383-12

11. Sun Y, Kim EJ, Felt SA, *et al*. A specific sequence in the genome of respiratory syncytial virus regulates the generation of copy-back defective viral genomes. *PLoS Pathog*. 2019;15(4):e1007707.

    doi: 10.1371/journal.ppat.1007707

12. Zhou T, Gilliam NJ, Li S, *et al*. Generation and functional analysis of defective viral genomes during SARS-CoV-2 infection. *mBio*. 2023;14(3):e0025023.

    doi: 10.1128/mbio.00250-23

13. Saira K, Lin X, DePasse JV, *et al*. Sequence analysis of *in vivo* defective interfering-like RNA of influenza a H1N1 pandemic virus. *J Virol*. 2013;87(14):8064-8074.

    doi: 10.1128/jvi.00240-13

14. Li D, Aaskov J. Sub-genomic RNA of defective interfering (D.I.) dengue viral particles is replicated in the same manner as full length genomes. *Virology*. 2014;468-470:248-255.

    doi: 10.1016/j.virol.2014.08.013

15. Duhaut S, Dimmock NJ. Approximately 150 nucleotides from the 5' end of an influenza a segment 1 defective virion RNA are needed for genome stability during passage of defective virus in infected cells. *Virology*. 2000;275(2):278-285.

    doi: 10.1006/viro.2000.0502

16. Nomoto A, Jacobson A, Lee YF, Dunn J, Wimmer E. Defective interfering particles of poliovirus: Mapping of the deletion and evidence that the deletions in the genomes of DI(1), (2) and (3) are located in the same region. *J Mol Biol*. 1979;128(2):179-196.

    doi: 10.1016/0022-2836(79)90125-6

17. Perrault J, Semler BL. Internal genome deletions in two distinct classes of defective interfering particles of vesicular stomatitis virus. *Proc Natl Acad Sci U S A*. 1979;76(12):6191-6195.

doi: 10.1073/pnas.76.12.6191

18. Davis AR, Hiti AL, Nayak DP. Influenza defective interfering viral RNA is formed by internal deletion of genomic RNA. *Proc Natl Acad Sci U S A*. 1980;77(1):215-219.

doi: 10.1073/pnas.77.1.215

19. Brennan JW, Sun Y. Defective viral genomes: Advances in understanding their generation, function, and impact on infection outcomes. *mBio*. 2024;15(5):e0069224.

doi: 10.1128/mbio.00692-24

20. Lazzarini RA, Keene JD, Schubert M. The origins of defective interfering particles of the negative-strand RNA viruses. *Cell*. 1981;26(2 Pt 2):145-154.

doi: 10.1016/0092-8674(81)90298-1

21. Perrault J. Origin and replication of defective interfering particles. *Curr Top Microbiol Immunol*. 1981;93:151-207.

doi: 10.1007/978-3-642-68123-3_7

22. Dimmock NJ, Easton AJ. Defective interfering influenza virus RNAs: Time to reevaluate their clinical potential as broad-spectrum antivirals? *J Virol*. 2014;88(10):5217-5227.

doi: 10.1128/JVI.03193-13

23. Schubert M, Lazzarini RA. Structure and origin of a snapback defective interfering particle RNA of vesicular stomatitis virus. *J Virol*. 1981;37(2):661-672.

doi: 10.1128/JVI.37.2.661-672.1981

24. Kupke SY, Riedel D, Frensing T, Zmora P, Reichl U. A novel type of influenza A virus-derived defective interfering particle with nucleotide substitutions in its genome. *J Virol*. 2019;93(4):e01786-18.

doi: 10.1128/jvi.01786-18

25. O'Hara PJ, Nichol ST, Horodyski FM, Holland JJ. Vesicular stomatitis virus defective interfering particles can contain extensive genomic sequence rearrangements and base substitutions. *Cell*. 1984;36(4):915-924.

doi: 10.1016/0092-8674(84)90041-2

26. Hillman BI, Carrington JC, Morris TJ. A defective interfering RNA that contains a mosaic of a plant virus genome. *Cell*. 1987;51(3):427-433.

doi: 10.1016/0092-8674(87)90638-6

27. Molenkamp R, Rozier BC, Greve S, Spaan WJ, Snijder EJ. Isolation and characterization of an arterivirus defective interfering RNA genome. *J Virol*. 2000;74(7):3156-3165.

doi: 10.1128/jvi.74.7.3156-3165.2000

28. Von Magnus P. Incomplete forms of influenza virus. *Adv Virus Res*. 1954;2:59-79.

doi: 10.1016/s0065-3527(08)60529-1

29. Cole CN, Smoler D, Wimmer E, Baltimore D. Defective interfering particles of poliovirus. I. Isolation and physical properties. *J Virol*. 1971;7(4):478-485.

doi: 10.1128/jvi.7.4.478-485.1971

30. Maeda A, Suzuki Y, Matsumoto M. Isolation and characterization of defective interfering particle of newcastle disease virus. *Microbiol Immunol*. 1978;22(12):775-784.

doi: 10.1111/j.1348-0421.1978.tb00431.x

31. Pitchai FNN, Tanner EJ, Khetan N, *et al*. Engineered deletions of HIV replicate conditionally to reduce disease in nonhuman primates. *Science*. 2024;385(6709):eadn5866.

doi: 10.1126/science.adn5866

32. Munoz-Sanchez JC, Olmo-Uceda MJ, Oteo JA, Elena SF. Quantifying defective and wild-type viruses from high-throughput RNA sequencing. *Bioinformatics*. 2024;40(11):btae651.

doi: 10.1093/bioinformatics/btae651

33. Levi LI, Madden EA, Boussier J, *et al*. Chikungunya virus RNA secondary structures impact defective viral genome production. *Microorganisms*. 2024;12(9):1794.

doi: 10.3390/microorganisms12091794

34. Johnson RI, Boczkowska B, Alfson K, *et al*. Identification and characterization of defective viral genomes in Ebola virus-infected rhesus macaques. *J Virol*. 2021;95(17):e0071421.

doi: 10.1128/jvi.00714-21

35. Welch SR, Tilston NL, Lo MK, *et al*. Inhibition of Nipah virus by defective interfering particles. *J Infect Dis*. 2020;221(Suppl 4):S460-s470.

doi: 10.1093/infdis/jiz564

36. Hillung J, Olmo-Uceda MJ, Munoz-Sanchez JC, Elena SF. Accumulation dynamics of defective genomes during experimental evolution of two betacoronaviruses. *Viruses*. 2024;16(4):644.

doi: 10.3390/v16040644

37. Boussier J, Munier S, Achouri E, *et al*. RNA-Seq accuracy and reproducibility for the mapping and quantification of influenza defective viral genomes. *RNA*. 2020;26(12):1905-1918.

doi: 10.1261/rna.077529.120

38. Ghorbani A, Ngunjiri JM, Rendon G, Brooke CB, Kenney SP, Lee CW. Diversity and complexity of internally deleted viral genomes in influenza A virus subpopulations with enhanced interferon-inducing phenotypes. *Viruses*. 2023;15(10):2107.

doi: 10.3390/v15102107

39. Mendes M, Russell AB. Library-based analysis reveals segment and length dependent characteristics of defective influenza genomes. *PLoS Pathog*. 2021;17(12):e1010125.

doi: 10.1371/journal.ppat.1010125

40. Chaturvedi S, Vasen G, Pablo M, *et al*. Identification of a therapeutic interfering particle-A single-dose SARS-CoV-2 antiviral intervention with a high barrier to resistance. *Cell*. 2021;184(25):6022-6036.e18.

doi: 10.1016/j.cell.2021.11.004

41. Alnaji FG, Holmes JR, Rendon G, *et al*. Sequencing framework for the sensitive detection and precise mapping of defective interfering particle-associated deletions across influenza A and B viruses. *J Virol*. 2019;93(11):e00354-19.

doi: 10.1128/JVI.00354-19

42. Routh A, Johnson JE. Discovery of functional genomic motifs in viruses with ViReMa-a virus recombination mapper-for analysis of next-generation sequencing data. *Nucleic Acids Res*. 2014;42(2):e11.

doi: 10.1093/nar/gkt916

43. Beauclair G, Mura M, Combredet C, Tangy F, Jouvenet N, Komarova AV. DI-tector: Defective interfering viral genomes' detector for next-generation sequencing data. *RNA*. 2018;24(10):1285-1296.

doi: 10.1261/rna.066910.118

44. Bosma TJ, Karagiannis K, Santana-Quintero L, *et al*. Identification and quantification of defective virus genomes in high throughput sequencing data using DVG-profiler, a novel post-sequence alignment processing algorithm. *PLoS One*. 2019;14(5):e0216944.

doi: 10.1371/journal.pone.0216944

45. De Jongh HH, Brasseur R, Killian JA. Orientation of the alpha-helices of apocytochrome C and derived fragments at membrane interfaces, as studied by circular dichroism. *Biochemistry*. 1994;33(48):14529-14535.

doi: 10.1021/bi00252a020

46. Olmo-Uceda MJ, Munoz-Sanchez JC, Lasso-Giraldo W, Arnau V, Diaz-Villanueva W, Elena SF. DVGfinder: A metasearch tool for identifying defective viral genomes in RNA-seq data. *Viruses*. 2022;14(5):1114.

doi: 10.3390/v14051114

47. Achouri E, Felt SA, Hackbart M, Rivera-Espinal NS, Lopez CB. VODKA2: A fast and accurate method to detect non-standard viral genomes from large RNA-seq data sets. *RNA*. 2023;30(1):16-25.

doi: 10.1261/rna.079747.123

48. Von Magnus P. *Studies on Interference in Experimental Influenza. I. Biological Observations*. Stockholm: Almqvist and Wiksell; 1947.

49. Huang AS, Greenawalt JW, Wagner RR. Defective T particles of vesicular stomatitis virus. I. Preparation, morphology, and some biologic properties. *Virology*. 1966;30(2):161-172.

doi: 10.1016/0042-6822(66)90092-4

50. Portner A, Kingsbury DW. Homologous interference by incomplete Sendai virus particles: Changes in virus-specific ribonucleic acid synthesis. *J Virol*. 1971;8(4):388-394.

doi: 10.1128/JVI.8.4.388-394.1971

51. Kolakofsky D. Studies on the generation and amplification of Sendai virus defective-interfering genomes. *Virology*. 1979;93(2):589-593.

doi: 10.1016/0042-6822(79)90263-0

52. Re GG, Kingsbury DW. Nucleotide sequences that affect replicative and transcriptional efficiencies of Sendai virus deletion mutants. *J Virol*. 1986;58(2):578-582.

doi: 10.1128/JVI.58.2.578-582.1986

53. Duhaut SD, Dimmock NJ. Defective segment 1 RNAs that interfere with production of infectious influenza A virus require at least 150 nucleotides of 5' sequence: Evidence from a plasmid-driven system. *J Gen Virol*. 2002;83(Pt 2):403-411.

doi: 10.1099/0022-1317-83-2-403

54. Odagiri T, Tashiro M. Segment-specific noncoding sequences of the influenza virus genome RNA are involved in the specific competition between defective interfering RNA and its progenitor RNA segment at the virion assembly step. *J Virol*. 1997;71(3):2138-2145.

doi: 10.1128/JVI.71.3.2138-2145.1997

55. Duhaut SD, McCauley JW. Defective RNAs inhibit the assembly of influenza virus genome segments in a segment-specific manner. *Virology*. 1996;216(2):326-337.

doi: 10.1006/viro.1996.0068

56. Notton T, Glazier JJ, Saykally VR, Thompson CE, Weinberger LS. Randel-seq: A high-throughput method to map viral cis- and trans-acting elements. *mBio*. 2021;12(1):e01724-20.

doi: 10.1128/mBio.01724-20

57. Rao DD, Huang AS. Interference among defective interfering particles of vesicular stomatitis virus. *J Virol*. 1982;41(1):210-221.

doi: 10.1128/jvi.41.1.210-221.1982

58. Marcus PI, Sekellick MJ. Defective interfering particles with covalently linked [+/-]RNA induce interferon. *Nature*. 1977;266(5605):815-819.

doi: 10.1038/266815a0

59. Van den Hoogen BG, Van Boheemen S, De Rijck J, *et al*. Excessive production and extreme editing of human metapneumovirus defective interfering RNA is associated with type I IFN induction. *J Gen Virol*. 2014;95(Pt 8):1625-1633.

doi: 10.1099/vir.0.066100-0

60. Shivakoti R, Siwek M, Hauer D, Schultz KL, Griffin DE.

Induction of dendritic cell production of type I and type III interferons by wild-type and vaccine strains of measles virus: Role of defective interfering rnas. *J Virol*. 2013;87(14):7816-7827.

doi: 10.1128/JVI.00261-13

61. Sun Y, Jain D, Koziol-White CJ, *et al*. Immunostimulatory defective viral genomes from respiratory syncytial virus promote a strong innate antiviral response during infection in mice and humans. *PLoS Pathog*. 2015;11(9):e1005122.

doi: 10.1371/journal.ppat.1005122

62. Honda K, Sakaguchi S, Nakajima C, *et al*. Selective contribution of IFN-alpha/beta signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. *Proc Natl Acad Sci U S A*. 2003;100(19):10872-10877.

doi: 10.1073/pnas.1934678100

63. Martinez-Sobrido L, Gitiban N, Fernandez-Sesma A, *et al*. Protection against respiratory syncytial virus by a recombinant newcastle disease virus vector. *J Virol*. 2006;80(3):1130-1139.

doi: 10.1128/JVI.80.3.1130-1139.2006

64. Yount JS, Gitlin L, Moran TM, Lopez CB. MDA5 participates in the detection of paramyxovirus infection and is essential for the early activation of dendritic cells in response to sendai virus defective interfering particles. *J Immunol*. 2008;180(7):4910-4918.

doi: 10.4049/jimmunol.180.7.4910

65. Fuller FJ, Marcus PI. Interferon induction by viruses. Iv. Sindbis virus: Early passage defective-interfering particles induce interferon. *J Gen Virol*. 1980;48(1):63-73.

doi: 10.1099/0022-1317-48-1-63

66. Metzger VT, Lloyd-Smith JO, Weinberger LS. Autonomous targeting of infectious superspreaders using engineered transmissible therapies. *PLoS Comput Biol*. 2011;7(3):e1002015.

doi: 10.1371/journal.pcbi.1002015

67. Weinberger LS, Schaffer DV, Arkin AP. Theoretical design of a gene therapy to prevent AIDS but not human immunodeficiency virus type 1 infection. *J Virol*. 2003;77(18):10028-10036.

doi: 10.1128/jvi.77.18.10028-10036.2003

68. Calain P, Monroe MC, Nichol ST. Ebola virus defective interfering particles and persistent infection. *Virology*. 1999;262(1):114-128.

doi: 10.1006/viro.1999.9915

69. Harty RN, Holden VR, O'Callaghan DJ. Transcriptional and translational analyses of the *UL2* gene of equine herpesvirus 1: A homolog of UL55 of herpes simplex virus type 1 that is maintained in the genome of defective interfering particles. *J Virol*. 1993;67(4):2255-2265.

doi: 10.1128/JVI.67.4.2255-2265.1993

70. Ho YC, Shan L, Hosmane NN, *et al*. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell*. 2013;155(3):540-551.

doi: 10.1016/j.cell.2013.09.020

71. Bruner KM, Murray AJ, Pollack RA, *et al*. Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat Med*. 2016;22(9):1043-1049.

doi: 10.1038/nm.4156

72. Pollack RA, Jones RB, Pertea M, *et al*. Defective HIV-1 proviruses are expressed and can be recognized by cytotoxic T lymphocytes, which shape the proviral landscape. *Cell Host Microbe*. 2017;21(4):494-506.e4.

doi: 10.1016/j.chom.2017.03.008

73. Kuniholm J, Coote C, Henderson AJ. Defective HIV-1 genomes and their potential impact on HIV pathogenesis. *Retrovirology*. 2022;19(1):13.

doi: 10.1186/s12977-022-00601-8

74. Murdaca G, Colombo BM, Puppo F. The role of Th17 lymphocytes in the autoimmune and chronic inflammatory diseases. *Intern Emerg Med*. 2011;6(6):487-495.

doi: 10.1007/s11739-011-0517-7

75. Elhed A, Unutmaz D. Th17 cells and HIV infection. *Curr Opin HIV AIDS*. 2010;5(2):146-150.

doi: 10.1097/coh.0b013e32833647a8

76. Lian X, Gao C, Sun X, *et al*. Signatures of immune selection in intact and defective proviruses distinguish HIV-1 elite controllers. *Sci Transl Med*. 2021;13(624):eabl4097.

doi: 10.1126/scitranslmed.abl4097

77. Costa P, Rusconi S, Mavilio D, *et al*. Differential disappearance of inhibitory natural killer cell receptors during HAART and possible impairment of HIV-1-specific CD8 cytotoxic T lymphocytes. *AIDS*. 2001;15(8):965-974.

doi: 10.1097/00002030-200105250-00004

78. Smith CM, Scott PD, O'Callaghan C, Easton AJ, Dimmock NJ. A defective interfering influenza RNA inhibits infectious influenza virus replication in human respiratory tract cells: A potential new human antiviral. *Viruses*. 2016;8(8):237.

doi: 10.3390/v8080237

79. Scott PD, Meng B, Marriott AC, Easton AJ, Dimmock NJ. Defective interfering virus protects elderly mice from influenza. *Virol J*. 2011;8:212.

doi: 10.1186/1743-422x-8-212

80. Dimmock NJ, Dove BK, Scott PD, *et al*. Cloned defective interfering influenza virus protects ferrets from pandemic 2009 influenza A virus and allows protective immunity to be established. *PLoS One*. 2012;7(12):e49394.

doi: 10.1371/journal.pone.0049394

81. Dimmock NJ, Dove BK, Meng B, *et al*. Comparison of the protection of ferrets against pandemic 2009 influenza A virus (H1N1) by 244 DI influenza virus and oseltamivir. *Antiviral Res*. 2012;96(3):376-385.

doi: 10.1016/j.antiviral.2012.09.017

82. Easton AJ, Scott PD, Edworthy NL, Meng B, Marriott AC, Dimmock NJ. A novel broad-spectrum treatment for respiratory virus infections: Influenza-based defective interfering virus provides protection against pneumovirus infection *in vivo*. *Vaccine*. 2011;29(15):2777-2784.

doi: 10.1016/j.vaccine.2011.01.102

83. Scott PD, Meng B, Marriott AC, Easton AJ, Dimmock NJ. Defective interfering influenza A virus protects *in vivo* against disease caused by a heterologous influenza B virus. *J Gen Virol*. 2011;92(Pt 9):2122-2132.

doi: 10.1099/vir.0.034132-0

84. Xiao Y, Lidsky PV, Shirogane Y, *et al*. A defective viral genome strategy elicits broad protective immunity against respiratory viruses. *Cell*. 2021;184(25):6037-6051.e14.

doi: 10.1016/j.cell.2021.11.023

85. Holland JJ, Villarreal LP. Purification of defective interfering T particles of vesicular stomatitis and rabies viruses generated *in vivo* in brains of newborn mice. *Virology*. 1975;67(2):438-449.

doi: 10.1016/0042-6822(75)90445-6

86. Levi LI, Rezelj VV, Henrion-Lacritick A, *et al*. Defective viral genomes from chikungunya virus are broad-spectrum antivirals and prevent virus dissemination in mosquitoes. *PLoS Pathog*. 2021;17(2):e1009110.

doi: 10.1371/journal.ppat.1009110

87. Li D, Lin MH, Rawle DJ, *et al*. Dengue virus-free defective interfering particles have potent and broad anti-dengue virus activity. *Commun Biol*. 2021;4(1):557.

doi: 10.1038/s42003-021-02064-7

88. Lin MH, Maniam P, Li D, *et al*. Harnessing defective interfering particles and lipid nanoparticles for effective delivery of an anti-dengue virus RNA therapy. *Mol Ther Nucleic Acids*. 2025;36(1):102424.

doi: 10.1016/j.omtn.2024.102424

89. Wang S, Chan KWK, Naripogu KB, Swarbrick CMD, Aaskov J, Vasudevan SG. Subgenomic RNA from dengue virus type 2 suppresses replication of dengue virus genomes and interacts with virus-encoded NS3 and NS5 proteins. *ACS Infect Dis*. 2020;6(3):436-446.

doi: 10.1021/acsinfecdis.9b00384

90. Pesko KN, Fitzpatrick KA, Ryan EM, *et al*. Internally deleted WNV genomes isolated from exotic birds in new mexico: Function in cells, mosquitoes, and mice. *Virology*. 2012;427(1):10-17.

doi: 10.1016/j.virol.2012.01.028

91. Brinton MA. Analysis of extracellular west nile virus particles produced by cell cultures from genetically resistant and susceptible mice indicates enhanced amplification of defective interfering particles by resistant cultures. *J Virol*. 1983;46(3):860-870.

doi: 10.1128/jvi.46.3.860-870.1983

92. Tilston-Lunel NL, Welch SR, Nambulli S, *et al*. Sustained replication of synthetic canine distemper virus defective genomes *in vitro* and *in vivo*. *mSphere*. 2021;6(5):e0053721.

doi: 10.1128/mSphere.00537-21

93. Welsh RM, Oldstone MB. Inhibition of immunologic injury of cultured cells infected with lymphocytic choriomeningitis virus: Role of defective interfering virus in regulating viral antigenic expression. *J Exp Med*. 1977;145(6):1449-1468.

doi: 10.1084/jem.145.6.1449

94. Kascsak RJ, Lyons MJ. Bunyamwera virus. II. The generation and nature of defective interfering particles. *Virology*. 1978;89(2):539-546.

doi: 10.1016/0042-6822(78)90195-2

95. Patel AH, Elliott RM. Characterization of Bunyamwera virus defective interfering particles. *J Gen Virol*. 1992;73(Pt 2):389-396.

doi: 10.1099/0022-1317-73-2-389

96. Kosmidou A, Buttner M, Meyers G. Isolation and characterization of cytopathogenic classical swine fever virus (CSFV). *Arch Virol*. 1998;143(7):1295-1309.

doi: 10.1007/s007050050376

97. Aoki H, Ishikawa K, Sakoda Y, *et al*. Characterization of classical swine fever virus associated with defective interfering particles containing a cytopathogenic subgenomic RNA isolated from wild boar. *J Vet Med Sci*. 2001;63(7):751-758.

doi: 10.1292/jvms.63.751

98. Meyers G, Thiel HJ. Cytopathogenicity of classical swine fever virus caused by defective interfering particles. *J Virol*. 1995;69(6):3683-3689.

doi: 10.1128/jvi.69.6.3683-3689.1995

99. Aoki H, Ishikawa K, Sekiguchi H, Suzuki S, Fukusho A. Pathogenicity and kinetics of virus propagation in swine infected with the cytopathogenic classical swine fever virus containing defective interfering particles. *Arch Virol*. 2003;148(2):297-310.

doi: 10.1007/s00705-002-0907-2

100. McLaren LC, Holland JJ. Defective interfering particles from

poliovirus vaccine and vaccine reference strains. *Virology*. 1974;60(2):579-583.

doi: 10.1016/0042-6822(74)90352-3

101. Bellocq C, Mottet G, Roux L. Wide occurrence of measles virus subgenomic RNAs in attenuated live-virus vaccines. *Biologicals*. 1990;18(4):337-343.

doi: 10.1016/1045-1056(90)90039-3

102. Gould PS, Easton AJ, Dimmock NJ. Live attenuated influenza vaccine contains substantial and unexpected amounts of defective viral genomic RNA. *Viruses*. 2017;9(10):269.

doi: 10.3390/v9100269

103. Martinez-Gil L, Goff PH, Hai R, Garcia-Sastre A, Shaw ML, Palese P. A Sendai virus-derived RNA agonist of RIG-I as a virus vaccine adjuvant. *J Virol*. 2013;87(3):1290-1300.

doi: 10.1128/JVI.02338-12

104. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. *Vaccine*. 2018;36(28):4039-4045.

doi: 10.1016/j.vaccine.2018.05.100