ISSN 1998 – 409X

REVIEW ARTICLE

Nanobody-Based Antiviral Strategies: From Passive Immunotherapy to Advanced Diagnostics

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Abstract

The emergence and continuous evolution of viral pathogens pose major challenges to human and veterinary medicine. Conventional vaccines and antiviral drugs may not provide immediate protection during outbreaks, whereas conventional monoclonal antibodies are associated with high production costs, relatively large molecular size, limited tissue penetration, and complex manufacturing requirements. Nanobodies, also known as camelid-derived single-domain antibodies or variable domains of heavy-chain antibodies, have emerged as promising next-generation biologics because of their small molecular size, high affinity, excellent solubility, remarkable thermal stability, and ability to recognize conserved or cryptic epitopes. These properties make nanobodies attractive candidates for passive antiviral immunotherapy and advanced diagnostic applications. Nanobodies can be generated through immunized camelid libraries, naïve or synthetic libraries, followed by phage, yeast, or ribosome display and recombinant expression. Their antiviral activity can involve inhibition of viral attachment, entry, membrane fusion, intracellular replication, assembly, and release. Protein engineering has further enabled multivalent, multispecific, Fc-fused, and albumin-binding nanobody formats to improve neutralization and pharmacokinetic properties. Nanobodies have been investigated against important human viruses, including severe acute respiratory syndrome coronavirus 2, influenza virus, respiratory syncytial virus, and HIV, as well as veterinary pathogens such as bovine viral diarrhea virus, foot-and-mouth disease virus, and Newcastle disease virus. Their integration into enzyme-linked immunosorbent assays, biosensors, molecular imaging, and lateral-flow diagnostic platforms further expands their applications. This review summarizes the structural characteristics, generation, antiviral mechanisms, diagnostic and therapeutic applications, advantages, limitations, and future prospects of nanobodies, with particular emphasis on their relevance to human and veterinary viral diseases.

Key words: Antiviral therapy, camelid antibodies, nanobodies, passive immunotherapy, single-domain antibodies, variable domains of heavy-chain antibodies, veterinary virology, viral diagnostics

INTRODUCTION

Viral diseases remain an important global challenge because of their high transmissibility, genetic variability, emergence of new variants, and considerable impact on human health and livestock production. Important human viral pathogens include severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza viruses, Ebola virus, and respiratory syncytial virus (RSV). In contrast, livestock production is affected by diseases such as foot-and-mouth disease (FMD), bovine viral diarrhea (BVD), Newcastle disease (ND), African swine fever (ASF), and porcine reproductive and respiratory syndrome (PRRS). Frequent mutations, particularly among RNA viruses, can facilitate immune escape and reduce the effectiveness of existing interventions.[1,2]

Vaccination remains the principal strategy for prevention of viral diseases; however, vaccines generally require time

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Received: 19-09-2026
Revised: 18-09-2026
Accepted: 27-09-2026

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to induce protective immunity, and their effectiveness can be reduced by antigenic variation. Antiviral drugs can also be limited by drug resistance, toxicity, and narrow target specificity. These limitations have stimulated increasing interest in passive immunotherapy, in which preformed antibodies are administered to provide immediate but temporary protection against infection.[3]

Conventional monoclonal antibodies have demonstrated considerable therapeutic potential, but their large molecular size of approximately 150 kDa, high manufacturing costs, limited tissue penetration, and relatively complex production systems can restrict their application. The discovery of heavy-chain-only antibodies in camelids provided an alternative antibody format. The antigen-binding variable domain of these antibodies, known as variable domains of heavy-chain antibodies (VHH) or nanobodies, is approximately 15 kDa and retains the antigen specificity of conventional antibodies.[4,5]

Nanobodies possess several properties that make them particularly attractive for antiviral applications. Their small size facilitates penetration into tissues and access to recessed epitopes, whereas their high stability and solubility facilitate recombinant production and storage. Furthermore, individual VHH domains can readily be engineered into multivalent and multispecific molecules. Consequently, nanobodies are increasingly being investigated as therapeutic, prophylactic, and diagnostic molecules against both established and emerging viral pathogens.[6,7]

BIOLOGY AND STRUCTURAL CHARACTERISTICS OF NANOBODIES

Nanobodies originated from the discovery of naturally occurring heavy-chain-only antibodies in camelids in 1993. Unlike conventional IgG molecules, camelid heavy-chain antibodies lack light chains and the CH1 domain. Their antigen-binding capacity is retained within a single variable domain, designated VHH, which subsequently became known as a nanobody.[4]

A typical nanobody has a molecular mass of approximately 15 kDa, nearly one-tenth that of conventional IgG. Despite its small size, it contains the three complementarity-determining regions (CDR1, CDR2, and CDR3) responsible for antigen recognition and four framework regions. The CDR3 region of nanobodies is often longer and more flexible than that of conventional antibodies. This structural feature allows VHHs to penetrate cavities, clefts, and cryptic antigenic sites that may be inaccessible to conventional antibodies.[5,6]

The framework regions of VHH domains contain structural adaptations that compensate for the absence of a light chain. Increased hydrophilicity reduces aggregation, whereas additional disulfide bonds may provide enhanced structural stability. Consequently, many nanobodies retain their functional activity under conditions that may destabilize conventional antibodies.[6]

The major characteristics of nanobodies include:

- Molecular mass of approximately 15 kDa;
- High antigen-binding specificity and affinity;
- Excellent solubility;
- High thermal and chemical stability;
- Improved tissue penetration;
- Ability to bind cryptic and conserved epitopes;
- Relatively simple recombinant production;
- Extensive molecular engineering flexibility; and
- Suitability for diagnostic and therapeutic applications.

These properties have established nanobodies as versatile biological platforms rather than merely antibody fragments.[2]

GENERATION AND ENGINEERING OF NANOBODIES

The conventional generation of nanobodies begins with the immunization of camelids such as llamas, alpacas, or camels, with purified antigens or inactivated pathogens. Following repeated immunization, peripheral blood lymphocytes are collected and VHH-encoding RNA is isolated. Reverse transcription and PCR amplification are then used to obtain VHH genes for library construction.[8]

Nanobody libraries can broadly be classified as immune, naïve, and synthetic libraries. Immune libraries are generated from immunized animals and are enriched for antigen-specific VHHs. Naïve libraries are obtained from non-immunized animals and contain broad VHH diversity. Synthetic libraries are constructed by introducing designed sequence diversity, particularly within complementarity-determining regions, allowing nanobodies to be selected without animal immunization. Synthetic approaches are especially useful for emerging or poorly immunogenic pathogens.[9]

Following library construction, antigen-specific nanobodies are identified using display technologies. Phage display remains one of the most widely used approaches. In this method, VHH molecules are displayed on bacteriophage particles and subjected to repeated rounds of antigen-based selection or biopanning. Yeast surface display permits quantitative screening and fluorescence-activated cell sorting, whereas ribosome display provides a cell-free system capable of accommodating very large libraries.[8,9]

Selected VHH genes are subsequently cloned into recombinant expression vectors. *Escherichia coli* is commonly used because of its rapid growth, relatively low production cost, and ease of genetic manipulation. Yeast systems such as *Pichia pastoris* may provide advantages in protein folding and secretion, whereas mammalian systems may be required for complex fusion molecules.[8]

Further engineering can improve the performance of nanobodies. Humanization may reduce immunogenicity for human therapeutic applications, whereas affinity maturation can increase binding strength and specificity. Linking two or more VHHs produces multivalent constructs, whereas combining VHHs recognizing different epitopes generates multispecific molecules. Fc fusion, albumin-binding domains, and other modifications can increase serum persistence and improve pharmacokinetics.[5,6]

MECHANISMS OF ANTIVIRAL ACTION

Nanobodies can interfere with different stages of the viral life cycle. Their antiviral effects primarily result from high-affinity binding to viral proteins and disruption of essential virus–host interactions.

Inhibition of viral attachment and entry

Viral attachment to host-cell receptors represents an important target for nanobody-mediated neutralization. Nanobodies directed against receptor-binding regions can prevent interaction between viral surface proteins and cellular receptors. For example, SARS-CoV-2-specific nanobodies targeting the spike receptor-binding domain can interfere with ACE2 receptor binding and prevent viral entry.[10,11]

Nanobodies can also target viral fusion proteins. Binding to these proteins may prevent the conformational changes required for membrane fusion. ALX-0171, an inhaled nanobody directed against the RSV fusion protein, demonstrated potent antiviral activity in experimental studies.[12]

Inhibition of intracellular viral replication

The small size of nanobodies allows them to be engineered for intracellular expression as intrabodies. Intrabodies can recognize viral proteins involved in replication, transcription, or other intracellular processes. An important example is an NS5B-specific intracellular nanobody developed against BVD virus. This nanobody inhibited viral replication in MDBK cells by targeting the viral RNA-dependent RNA polymerase.[13]

Interference with viral assembly and release

Nanobodies may also target structural or regulatory proteins required for virion assembly. Binding to capsid or nucleoproteins can interfere with formation of infectious viral particles. Similarly, targeting proteins involved in viral budding and release may reduce dissemination of progeny virions.[2]

Multivalent and multispecific neutralization

Viral mutation and antigenic variation can reduce the activity of monovalent antibodies. Multivalent and multispecific nanobodies address this problem by simultaneously recognizing multiple epitopes. Structure-guided multivalent SARS-CoV-2 nanobodies have demonstrated strong neutralization and reduced mutational escape in experimental studies.[11]

APPLICATIONS IN VIRAL DIAGNOSTICS

The biochemical stability and high specificity of nanobodies make them useful diagnostic reagents. Their small size allows dense immobilization on diagnostic surfaces and rapid interaction with target antigens.

Nanobodies have been incorporated into enzyme-linked immunosorbent assays (ELISA) for detection of viral antigens. They can serve as capture or detection reagents and may recognize conserved epitopes that remain accessible despite antigenic variation.[2]

Another important application is in biosensors. Nanobodies can be coupled to electrochemical, optical, and surface plasmon resonance platforms. Their small size permits high-density immobilization and may facilitate rapid interaction with target molecules, supporting development of portable and real-time diagnostic systems.[14]

Nanobodies have also been investigated for molecular imaging. Fluorescent or radionuclide-labeled VHHs can be used to visualize specific antigens. Their rapid tissue penetration and clearance can provide favorable imaging characteristics.[2]

Their high stability also makes nanobodies suitable for lateral-flow and point-of-care diagnostic platforms. Such systems may be particularly valuable in veterinary field settings where refrigeration and sophisticated laboratory infrastructure are limited.

THERAPEUTIC APPLICATIONS IN HUMAN VIRAL DISEASES

SARS-CoV-2

The COVID-19 pandemic stimulated extensive development of nanobody-based antiviral strategies. Many SARS-CoV-2 nanobodies target the spike protein receptor-binding domain. Multivalent and biparatopic formats have demonstrated enhanced neutralization and improved resistance to viral escape.[10,11]

Influenza virus

Influenza viruses undergo continuous antigenic variation. Nanobodies directed against conserved regions of hemagglutinin, particularly the HA stem, have demonstrated broad neutralizing activity against different influenza subtypes. Multidomain antibody formats can increase breadth and potency.[15]

RSV

RSV is an important respiratory pathogen. ALX-0171, directed against the RSV F protein, was developed as an inhaled nanobody therapy. Local respiratory delivery is particularly attractive because it can produce high concentrations of the antiviral molecule at the site of infection.[12]

HIV and emerging viruses

Nanobodies directed against conserved regions of HIV-1 gp120 have demonstrated cross-subtype neutralizing activity.[16] Nanobody discovery is also being explored for pathogens such as Ebola virus, MERS-CoV, and Nipah virus, where rapid generation of synthetic or display-based libraries may facilitate development of candidate therapeutics.

APPLICATIONS IN VETERINARY VIRAL DISEASES

Nanobodies have considerable potential in veterinary medicine because viral diseases cause substantial economic losses through mortality, reduced productivity, reproductive failure, trade restrictions, and control costs. Their stability, relatively economical recombinant production and adaptability to field diagnostics are particularly relevant to livestock health management.

BVD virus

BVDV is an economically important cattle pathogen associated with respiratory disease, reproductive problems, and persistent infection. Duan *et al*.[13] demonstrated that an intracellular NS5B-specific nanobody could suppress BVDV replication in MDBK cells. This study illustrates the potential of intrabodies for targeting intracellular viral enzymes.

FMD virus

FMD is highly contagious and affects cattle, buffaloes, pigs, and other cloven-hoofed animals. Llama-derived single-domain antibody fragments have demonstrated antiviral activity against FMDV. Harmsen *et al*.[17] reported that passive immunization with llama antibody fragments reduced FMDV transmission between experimentally infected pigs. Multivalent formats may provide increased neutralizing activity compared with monovalent VHH molecules.

ND Virus

ND is a major viral disease of poultry. Nanobodies directed against the nucleocapsid protein have demonstrated inhibitory activity against NDV replication in DF-1 cells.[18] Because the nucleoprotein is an important viral component, such nanobodies may have both therapeutic and diagnostic applications.

Nanobody technology is also being explored for other veterinary pathogens, including ASFV, PRRSV, avian influenza virus, bluetongue virus and rabies virus. However, for many of these applications, evidence remains primarily experimental, and further animal studies are required.

ADVANTAGES AND LIMITATIONS

Nanobodies offer several potential advantages over conventional monoclonal antibodies. Their approximately 15-kDa molecular size facilitates tissue penetration and access to sterically restricted epitopes. They are generally soluble, stable, and amenable to recombinant production in microbial systems. Their modular structure also permits generation of multivalent, multispecific, and fusion constructs.[5,6]

However, their small size also creates an important limitation: rapid renal clearance. Because of their low molecular mass, unmodified nanobodies can have short serum half-lives. Fc fusion, PEGylation, albumin-binding domains, and multimerization have therefore been investigated to improve pharmacokinetics.[3]

The absence of an Fc region also means that conventional nanobodies do not naturally provide Fc-dependent functions such as antibody-dependent cellular cytotoxicity, antibody-dependent phagocytosis, and complement activation. Fc fusion can be used where such functions are desirable.

Other limitations include potential anti-drug antibody responses, viral escape mutations, manufacturing standardization, and limited clinical validation. Although nanobody technology has progressed substantially, relatively few antiviral nanobody candidates have reached advanced clinical development, and veterinary applications remain largely experimental.[7,19]

FUTURE PERSPECTIVES

Future development of antiviral nanobodies will likely focus on improving durability, breadth, delivery, and scalability. Multispecific nanobodies targeting several conserved epitopes

may provide broader antiviral protection and reduce the probability of escape. Fc fusion and albumin-binding strategies may increase systemic persistence, while inhalable formulations may be particularly useful for respiratory viral diseases.

Integration with nanoparticles, liposomes, mRNA-based expression systems, and viral vectors may provide alternative methods for delivering nanobodies directly to sites of infection. Artificial intelligence and computational protein design are also expected to accelerate identification of suitable epitopes and optimization of VHH sequences.[20]

In veterinary medicine, field-deployable nanobody-based diagnostic systems could support rapid surveillance and early detection of infectious diseases. This may be particularly important for livestock viral diseases, where rapid identification and containment are essential for limiting transmission and economic losses.

The clinical approval of nanobody-based products such as caplacizumab has demonstrated that VHH-based molecules can progress from experimental antibody formats to approved biological therapeutics. Although caplacizumab is not an antiviral drug, its regulatory success provides an important precedent for nanobody development. Continued investigation of antiviral candidates, including inhaled and multispecific formats, will determine their future clinical utility.[14]

RESEARCH GAPS

Important research gaps remain before nanobodies can be widely adopted as antiviral therapeutics. Long-term safety, repeated-dose immunogenicity, pharmacokinetics, and efficacy in target animal species require additional evaluation. In veterinary medicine, controlled field trials are particularly important because laboratory and experimental-animal results may not always predict performance under field conditions.

Standardization of nanobody production, purification, and quality control is another important requirement. Harmonized regulatory approaches and Good Manufacturing Practice-compliant production will be necessary for commercial translation.[19]

Future research should therefore emphasize:

1. Development of broad-spectrum and multispecific nanobodies
2. Improvement of serum half-life;
3. Optimized delivery systems;
4. Large-scale recombinant production;
5. Evaluation in target livestock species;
6. Long-term safety and immunogenicity studies;
7. Integration with rapid diagnostic technologies; and
8. AI-assisted nanobody discovery and engineering.

CONCLUSION

Nanobodies represent an important next-generation antibody platform with considerable potential for antiviral passive immunotherapy and advanced diagnostics. Their small molecular size, high stability, excellent solubility, ability to recognize cryptic epitopes, and remarkable engineering flexibility distinguish them from conventional monoclonal antibodies. Nanobodies can interfere with several stages of the viral life cycle, including attachment, entry, fusion, intracellular replication, assembly, and release.

Research against SARS-CoV-2, influenza virus, RSV, HIV, BVDV, FMDV, and ND virus demonstrates the broad applicability of this technology. In parallel, nanobody-based ELISA, biosensor, molecular imaging, and point-of-care diagnostic platforms provide opportunities for rapid detection of viral pathogens.

Nevertheless, rapid renal clearance, limited Fc-mediated immune functions, possible immunogenicity, viral escape, manufacturing requirements, and limited clinical validation remain important challenges. Multivalent and multispecific engineering, Fc fusion, albumin binding, advanced delivery technologies, and computational design are providing strategies to address these limitations.

Overall, continued multidisciplinary research involving virology, immunology, molecular biology, biotechnology, veterinary medicine, and clinical sciences will be essential for translating nanobody technology into practical antiviral therapeutics and diagnostics. Their potential integration into One Health approaches could contribute to improved preparedness against emerging and re-emerging viral diseases in both humans and animals.

ACKNOWLEDGMENT

The authors are grateful to the Maharashtra Animal & Fishery Sciences University, Nagpur, Associate Dean, Krantisinh Nana Patil College of Veterinary Science, Shirwal, for providing necessary facilities and providing the financial assistance to undertake the present study.

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Source of Support: Nil. **Conflicts of Interest:** None declared.