Case Study

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Nanobodies: A Novel Approach in Viral Disease Diagnosis and Therapeutics

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Abstract

The COVID-19 pandemic has led to the development of novel and improved prophylactic, diagnostic, and therapeutic tools. The epidemic has exposed some flaws in monoclonal antibodies production due to some restrictions throughout the world, despite of the fact that they have historically proven useful tools. Nanobodies can be used as a substitute for traditional monoclonal antibodies. Nanobodies are recombinant variable domains of heavy-chain-only antibodies, and they have many distinctive qualities that set them apart from other types of antibodies, including their small size, excellent solubility, superior stability, rapid blood clearance, and deep tissue penetration. Nanobodies are now a potentially useful tool for both diagnosis and treatment of many viruses and cancers.

Introduction

Nanobodies are only heavy chain antibodies derived from *Camelidae* family and in some cartilagenous fishes and sharks. Immune system of these camelids and sharks naturally lacks CH1 domain in IgG2 and IgG3 due to alternative splicing. As a result, the light chain of those immunoglobulins does not combine to the final antibody structure leading to formation of heavy chain antibodies (HCAbs). So, IgG2 and IgG3 antibodies depend only on heavy chain variable domain (VHH), it is about 15KDa which binds with specific antigen. When compared to nanobodies, size of conventional antibodies are 10 times more. The difference between conventional monoclonal antibodies and HCAbs is that, the HCAbs are encoded by single gene.

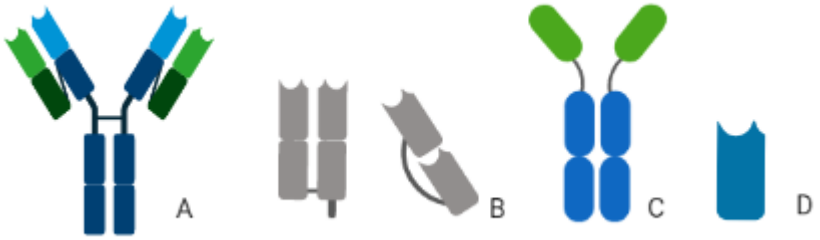

FIG. 1. A.Conventional Antibody, B. Antigen-binding fragments, C. Heavy chain antibody, D. Nanobody (Created in

Bond et al. (2003) found that nanobodies had remarkable penetrability and are soluble throughout a wide range of pH and temperatures. Some nanobodies can cross the blood-brain barrier, making them potentially

useful diagnostic and therapeutic agents for neurodegenerative diseases in the future. There are many advantages of nanobodies, among all one of the most outstanding is achieved through recombinant expression systems. Nanobodies can be efficiently produced in both prokaryotic and eukaryotic systems.

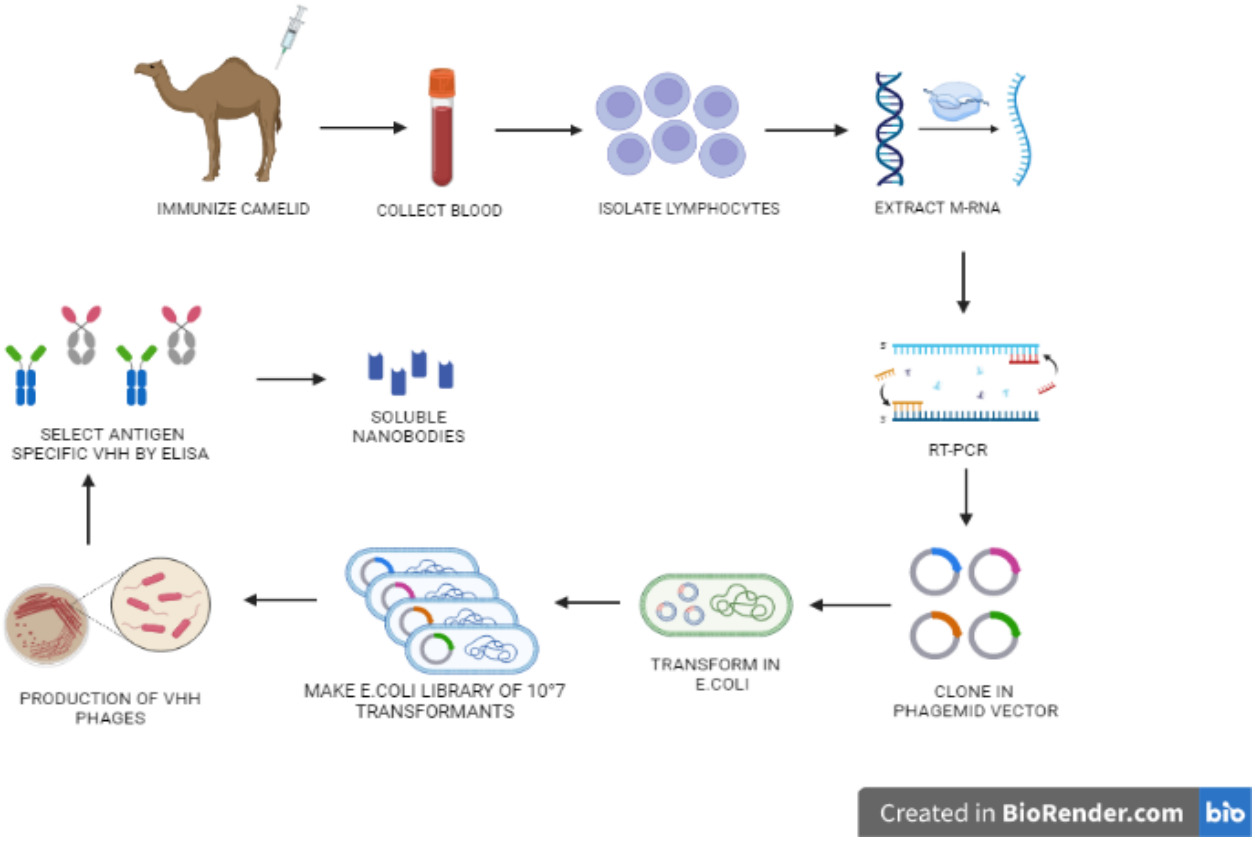

FIG. 2 Schematic Overview Of Nanobody Generation (Created in Biorender app)

Applications of Nanobodies

Molecular Tools

When expressed in the cytoplasm of living cells, nanobodies have a special property that allows them to take on a native structure. This feature enables us to target intracellular proteins and interfere with their function by harnessing the selectivity and binding affinity of nanobodies. This is particularly intriguing for virology since it makes it possible to examine unaltered, wild-type viral proteins throughout the replication cycle and provides a great alternative to genetic alterations such as direct fusions with fluorescent proteins. Surprisingly, a specific nanobody can be applied to a variety of complimentary studies, enabling an integrative examination of the same process or protein.

Track and Visualize Viral Protein

Nanobodies are excellent immunostaining agents when they are combined with fluorescent protein or dye. It can be used in many applications in place of conventional IgGs. In comparison to conventional IgG staining techniques, the use of directly conjugated nanobodies decreases sample preparation time and signal-to-noise ratio. The species restriction for multiple co-staining is further eliminated. Viral proteins in

infected cells have been found using nanobodies coupled to various fluorophores. The 3–4 nm nanobody length results in a lower linkage error compared to traditional antibodies, improving image resolution. This is especially true for advanced microscopic methods like cryo-correlative light and electron microscopy or super-resolution imaging (SRM).

Live Cell Imaging

Nanobodies that are produced intracellularly can detect viral infection in cells. A nanobody-based reporting system (or "biosensor") was created by Cao et al. for cells that can detect influenza A virus(IAV) infection. Two IAV nucleoprotein-specific nanobodies are expressed in tandem for this biosensor, one of which is coupled to a DNA binding domain (DBD), and the other to a VP64 transactivation domain (AD). A sandwich scaffold is created when both bind to the IAV nucleoprotein and enable the production of a fluorescent reporter gene. This technology shows how nanobodies can be used in novel viral detection methods and has the potential to be used in procedures to test antiviral medications or assess vaccine-induced neutralizing antibodies. Notably, this method does not call on prior viral manipulation, and given The Largely Conserved

Intracellular Expression and Antiviral Activity

The ability to prevent HIV-1 replication and the production of late viral RNAs was demonstrated by a nanobody made against the HIV-1 Rev protein. The HIV-1 protein Nef, which is highly conserved, is crucial for the virus infectiousness and the development of the disease. It is an intriguing target for antiviral intervention since it obstructs the trafficking of numerous transmembrane proteins. Nef function was hampered by a Nef-specific nanobody in a number of ways, including the internalisation of CD4 and the suppression of actin cytoskeleton rearrangements.

When produced intracellularly, nanobodies against the nucleoprotein of Influenza A Virus or vesicular stomatitis virus (VSV) have been found to extremely efficiently shield cells from viral infection. Nanobodies can be used to target proteins for destruction in addition to directly affecting a protein's ability to function**.** Intracellularly produced nanobodies can deplete the target protein via proteasomal destruction when fused to F-box containing ubiquitin ligases.

Protein Structure Determination

Protein structures are determined through X-ray crystallography by stabilising the target, trapping the protein in specific conformations, and enhancing crystal packing, antibodies abd chaperone function makes it possible. Nanobodies are no exception, and numerous complex viral structures have been solved using particular nanobodies. For instance, Garza and colleagues created four nanobodies against the Marburg virus nucleoprotein, which encouraged the crystallisation of the viral protein's C-terminal region and helped to determine its structure.

Cryo-EM, or electron cryo-microscopy, is particularly well suited for determining the protein structure of larger proteins and protein complexes. By securing these compounds in particular conformations and producing more homogeneous samples, nanobodies can help in this situation. Particle orientation is a common problem in single-particle CryoEM. Nanobodies were introduced into scaffold protein loops by Uchánski et al. to produce rigid molecules of larger size. These "megabodies" are visible as protrusions on the protein of interest and aid in particle orientation, allowing the reconstruction of three-dimensional structures.

Diagnostic Applications

The detection limit of the swine influenza virus (SIV)-specific nanobody-based ELISA test created by Du and colleagues was much lower than that of an ELISA kit purchased from a vendor. As seen with the nanobody-based ELISA for the foot-and-mouth disease virus (FMDV), which saw a false-positive rate drop from 16 to 7%, other tests report fewer false-positive results. Additionally, nanobodies are simple to modify, which can speed up production and minimise the labor-intensive labelling required by conventional methods, which could help with commercialization. Mu and associates created a nanobody-based competitive ELISA using a PCV2 specific nanobody that was genetically linked to an HRP enzyme.

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