CAMEL MILK AND URINE BASED NANOBODIES: PROMISING AVENUE TO OVERCOME WORLD EMERGING DISEASES

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ABSTRACT

Cancer, HIV infections, SARS, Lyme disease, *Escherichia coli* 157:H7 hantavirus, dengue fever, West Nile virus, corona virus (SARS) and Zika virus are a major health concern and accounts for one of the foremost causes of death worldwide. Innovative tactics are needed to aid in the diagnosis and treatment of different types of diseases. In the earlier published study, it has been found the small size property makes nanobodies capable of penetrating efficiently into tissues and distinguishing cryptic antigens. Robust antigen affinity and constancy in the gastrointestinal tract permit them to be used via oral administration. Recently, there has been an evolving interest in operating nanobodies of camel origin as therapeutic tools against several diseases. The current review highlights the potential use of camel milk and urine based nanobodies in the development of nanotechnology-based therapy for treating different world emerging diseases. Moreover, in view of importance of camel nanobodies can overcome the problem of decreasing world-wide camel populations are also illustrated.

Keywords: Nanobodies, camel, milk, urine, cancers, emerging diseases and camel papulation

Introduction

Population issues such as urbanization, socio-economic and environmental areas are driving forces for emerging and re-emerging zoonotic diseases in the world. Use of antibiotics in emerging diseases cause anti-microbial resistance which is a big threat towards world economy. Nanobodies are stated as a class of next-generation antibodies. Nanobodies allow the construction of multivalent formats that may achieve ultra-high neutralization potency and then may prevent mutational escape and can neutralize a wide range of SARS-CoV-2 variants. Animals belonging to this family are well adapted to live in harsh environments, such as deserts or high altitudes (Bo-kyung *et al.,* 2023). Camelid nanobodies are articulated only in conjunction with dedicated IgG2 and IgG3, which splice out the CH1 exon during transcription (Muyldermans *et al.,* 2013). Camelids create both conventional heterotetrametric antibodies and homodimeric heavy-chain only antibodies (Xu *et al.,* 2021). The antigen-binding region of such homodimeric heavy-chain solitary antibodies consists of one single domain, called VHH. Nanobodies (Nbs) are the slightest functional antibody fragments known in nature and have multiple submissions in biomedicine or environmental monitoring. Consequent successes in shaping the crystal structure of the VHH domain of the HCAbs, as well as proving the feasibility of selecting, identifying and recombinantly expressing the VHH domain alone have spurred on this field beyond the scope of trypanosome infections (Ehsani *et al.,* 2003; Dooley *et al.,* 2005; Fowler *et al.,* 1998). World faces risks and potential health threats from an increasing range of hazards, including infectious diseases, HIV and other threats associated with climate change including extreme weather events and deforestation. In recent decades, the number of outbreaks of diseases transmissible from animals to humans has been on the rise. For the most recent data available in 2018, the total number of camels recorded in the world was 35,525,270 (FAOSTAT, 2020) which is decreasing decades per decades. In statistic, the use of nanobodies as inhalant can be directly delivered to the mark organ, conferring high pulmonary drug concentrations and low systemic drug concentrations with minimal systemic side effects. Nanobodies permit the construction of multivalent formats that may achieve ultra-high neutralization potency and then may prevent mutational escape and can neutralize a wide range of emerging prevalent variants in world. In this review, we focus on the camelid milk and urine based nanobodies as a revolutionary therapy that can guide efforts to discover novel drugs opportunities for the controlling of world emerging diseases. Nanobodies are now not only extensively used in many research fields but they are also the subject of curiosity to companies for their various diagnostic and therapeutical applications.

Current treatment strategies of world emerging diseases

Camel overcome SARS-CoV-2 mutants (corona virus)

Severe acute respiratory syndrom is a viral respiratory illness triggered by a coronavirus, called SARS-associated coronavirus. Severe acute respiratory syndrom was first labelled in Asia in February 2003. With the worldwide spread of SARS-CoV-2, several variants that carry mutations have emerged that increase transmissibility or allow escape from antibody neutralization of particular interest is the B.1.1.7 variant (which contains an N501Y substitution) that caused an upsurge in COVID-19 cases in the UK (Kupferschmidt *et al*., 2021). A second variant of concern is B.1.351, which combines N501Y with two additional RBD substitutions (K417N and E484K). P.1, a third variant that spread rapidly in Brazil, shows changes similar to those of B.1.1.7 and B.1.351: N501Y, K417T and E484K (Faria *et al*., 2021). All of these mutations have been

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shown to reduce the efficacy of serum antibodies elicited by the Moderna and Pfizer–BioNTech vaccines (Wang *et al.,* 2021; Wang *et al*., 2021)

Nanobodies that modulate immune function have been successfully created in immunized camelids. Several reports have been published in raising sdAbs directed in contradiction of cytokine by phage display technology (Nosenko *et al.,* 2017). For example, TNFα is a significant cytokine implicated in a number of chronic inflammatory disorders. TNFα-blocking sdAbs have been successfully inaccessible from a llama immunized with human and mouse TNFα which are more effective at neutralizing TNFα than the conventional TNFα-blocking antibodies Infliximab and Adalimumab (Coppieters *et al.,* 2006). Another Nb that binds to human IL-6-R and IL23 was produced for treatment of rheumatoid arthritis (Tillib *et al.,* 2015; Desmyter *et al.,* 2017; Danquah *et al.,* 2016).

Camel Nanobodies against various coronavirus species have been isolated and characterized to block interaction between host cell and virus. As specimen, Abs targeting RBD of MERS-CoV were recognized and selected from the immunized dromedary and it was revealed that camel/human chimeric HcAbs muddle to their target with picomolar affinity (Raj *et al.,* 2018).

According to a several current studies, VHHs showed high neutralization potency SARS-CoV-2. Humanized sdAbs-binding SARS-CoV-2 RBD proteins have been exposed in a synthetic sdAb phage display library (Chi *et al.,* 2020). The inhibition efficiency on SARS-CoV-2 pp and affinity kinetics was confirmed *in vitro*. These neutralizing sdAbs were isolated from immunized llamas and it was demonstrated that engineered bivalent nanobodies revelations cross-reactivity against SARS-CoV-1 RBD and SARS-CoV-2 RBD and able to neutralize SARS-CoV-2 S pseudoviruses with high affinity (Wrapp *et al.,* 2020). In their studies, Dong and collaborators verified that multi-specific antibodies displayed better affinity and avidity than separate monoclonal VHH-Fcs. Isolation of a monomeric Nb (Ty1) from immunised alpaca (Hanke *et al.,* 2020). The highly specificity and high-affinity binding of Ty1 to the RBD have been deep-rooted. Hanke and colleagues, recommend that the generation of homodimeric or trimeric formats is likely to further increase its neutralization activity. More lately, Schoof *et al.* (2020) have successfully isolated a panel of nanobodies, from synthetic nanobodies library, that bind to manifold epitopes on Spike. In a similar study, the documentation and characterization of two other high-affinity nanobodies (H11-D4 and H11-H4) have been conveyed (Huo *et al.,* 2020).

Increasing evidences have been reported that insufficient T cell responses could play a decisive role in clearance of SARS-CoV (Vellingiri *et al.,* 2020). Further, Duarte *et al.* (2016) showed that VHHs specific for these molecules enhanced immune responses in distinct dendritic cells (DCs) populations. So, VHH can be used to deliver proteins or peptides to APCs to trigger humoral immunity and to track inflammation to treat or prevent SARS-CoV-2 infection (Bessalah *et al.,* 2021). The virus can blowout when a person touches a surface or object contaminated with infectious droplets and then touches his or her mouth, nose, or eyes. In addition, it is possible that the SARS virus might spread more broadly through the air or by other ways that are not now known still.

Nanobodies as potential HIV microbicides

Human immunodeficiency virus (HIV) is an infection that attacks the body's immune system that cause acquired immunodeficiency syndrome (AIDS) is the most advanced stage of the disease. HIV relics a major global public health issue, having claimed 40.1 million [33.6–48.6 million] lives so far with continuing transmission in all countries globally; with some countries reporting increasing trends in new infections when previously on the decline. For that, Nanobodies are suitable candidates for development as vaginal microbicides and for falling viral load after infection. It has been noted above that nanobodies are able to refold after denaturation especially when bound to their antigen (Dolk *et al.,* 2005). VHH A12 is also resilient against denaturation across a large range of pH values (Gorlani *et al.,* 2012). This broad pH stability at slightly acid conditions is useful because, while the normal pH in the vagina is 3.8–4.5, in sexual intercourse the ejaculation of semen normal pH (7.1–7.8) raises the overall pH towards neutral. Nanobodies readily penetrate mucosal surfaces to reach tissues (Kijanka *et al*., 2015), so their potential as microbicides merits further exploration. This small preclinical trial may reassure further testing leading to a human trial, possibly with a mixture of broadly neutralizing bi-specific nanobodies that raise the coverage from 96–100% of HIV-1 strains and increase potency (Strokappe *et al.,* 2019). Similarly, the versatility of nanobodies lends them to several applications in control of human pathogenic viruses (Togtmea *et al.,* 2019) alongside other single chain or mini-antibody constructs (Robin *et al.,* 2019). In HIV previous studies, they show particular promise as diagnostic and imaging agents, in vaccine design, and as microbicides. Neutralizing nanobodies have been beneficial in defining vulnerable neutralization sites on the HIV-1 envelope. Bivalent nanobodies binding to two distinct sites on gp120 and gp 41 show up to a 1400-fold increase in strength compared to either nanobody alone (Strokappe *et al.,* 2019). In addition to the expression of nanobodies in commensal lactic acid bacteria for vaginal delivery, the relatively small size of nanobodies and humanized nanobodies shows that several VHH can be easily incorporated into adenovirus-associated virus or other viral vectors as well as bacteria (Chowdhury *et al.,* 2019) for *in vivo* ontrol of infection. A newly published mouse model illustrates the potential use in vivo of a chronically secreted nanobody from bacteria binding to CD47, which is an anti-phagocytic receptor often over-expressed in human cancer (Chowdhury *et al.,* 2019).

Camel nanobodies against viral zoonosis

Viral Zoonoses are diseases transmissible from vertebrate animals, other than humans, to people. Mammals, birds, reptiles, and probably amphibians are reservoirs or magnifying hosts for viral zoonoses. Often, these viruses cause slight or no overt disease in their nonhuman vertebrate hosts. Due to their unusual properties, rapid progress has been made regarding the production of VHH domains for therapeutic and diagnostic applications (Lafaye & Li, 2018; Sanaei *et al.,* 2019; Chames & Rothbauer, 2020). It has been proved that these camel milk and urine based nanobodies can be easily

engineered without loss of functionality. At this time, several camel nanobodies produced by Ablynx, are in diverse clinical trials and with Caplacizumab, the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) permitted the first sdAb-based medicine for adults in November 2018 and in February 2019, respectively (Morrison, 2019; Jovèevska & Muyldermans, 2020). Viral neutralizing sdAb has been described as valuable biomolecules, with high potentials, able to perturb diverse steps in the viral life cycle with many specimens of viral neutralizing nanobodies (De Vlieger *et al.,* 2019; Sroga *et al.,* 2020).

For instance, sdAb specific for influenza viruses has been successfully isolated through selections against the nucleoprotein and M2 ion channel protein of Influenza A, the neuraminidase and trimeric spike protein and hemagglutinin of H5N1 and H1N1 (Cardoso *et al.,* 2014; Hufton *et al.,* 2014; Ashour *et al.,* 2015; Laursen *et al.,* 2018). An sdAb-specific viral protein (PV1) of poliovirus (PV) was recognized from the immunised phage display library. The mechanism by which these VHHs reduce viral masses is by blocking the ligand–receptor interactions (Strauss *et al.,* 2016). Human norovirus is classified among the principal cause of gastroenteritis worldwide. Several nanobodies against virus-like particles (VLPs) of norovirus have revealed potential for disease treatment (Koromyslova & Hansman, 2017).

Camelids could also be a source of bNAbs. Undeniably antibodies against MERS-Coronaviruses (Pere *et.al*.,2013; Reusken *et al.,* 2013), Crimean Congo hemorrhagic fever virus (CCHFV) (Mariner *et al*., 1995; Suliman *et al.,* 2017), Rift Valley fever (RFV), *Toxoplasma gondii* and Rickettsia sp. (Mentaberre *et al.,* 2013) have been found in the sera of infected camels, although antibodies against rabies virus, vesicular stomatitis virus, and FMD virus have been spotted in llamas (Rivera, 1987) and could lead to the likely isolation of specific broadly neutralizing VHHs. Currently available anti-viral therapeutic mAbs, at various stages of pre-clinical evaluation, typically target surface antigens that are frequently varied or variable in sequence. A substitute is the use of “broadly neutralizing antibodies (bNAb)”, which are antibodies found in infected mammals able to neutralize most strains of a given highly antigenically flexible pathogen. bNAbs have been isolated from infected humans against HIV (Schei *et al.,* 2009), influenza (Corti, 2017) and dengue viruses (Rouvinski *et.al*., 2015). Zoonosis emerging diseases are 100% preventable through vaccination even though it’s required much time but camel nanobodies are advance treatment with low cost and easily could be available for the public in less time.

Nanobodies overcome Ebola/Zika virus mutants

Ebola virus disease (EVD) is a lethal disease with infrequent outbreaks that arise mostly on the African continent. EVD most usually affects people and nonhuman primates (such as monkeys, gorillas, and chimpanzees). Ebola virus (EBOV) is tremendously virulent and causes fatal hemorrhagic fever in ~ 50% of the cases. The interaction between viral envelope glycoprotein GP and host cell receptors plays a serious role in pathogenicity of this virus. An immune sdAb phage show library derived from a llama immunized with killed EBOV and recombinant GP was made and SdAbs specific for Ebola GP were selected and evaluated for their affinity and thermal stability (Liu *et al.,* 2017). Likewise, monovalent, bivalent and trivalent VHHs targeting the glycoproteins, gp120 and gp41 of VIH have been created to disrupt virus entry into cluster of differentiation 4 $(CD4)^+$ T cells (Strokappe *et al.,* 2012; Weiss & Verrips, 2019). Five anti-CHIKV sdAbs have been produced in immunized llamas. These viral neutralizing sdAbs have been successfully isolated through selections compared to the CHIKV VLPs (Liu *et al.,* 2019).

Nanobodies have been described as panted bio-therapeutics for lung diseases. Human respiratory syncytial virus (RSV) goes to the Pneumoviridae family and signifies the most important cause of lower respiratory tract infections in young infants. Fruitful phase I/IIa clinical trials were completed for an anti-RSV VHH domain called ALX-0171 (Detalle *et al.,* 2015; Van Heeke *et al.,* 2017; Wilken & McPherson, 2018).

Nanobodies settled against the main antigen of hepatitis B (HBcAg) have an effect on the viral life cycle in HBV-transfected hepatocytes (Serruys et al. 2010). Furthermore, VHHs focused against rotavirus serotype G3 reduce morbidity of rotavirus-induced diarrhea in vivo (Van der Vaart *et al.,* 2006). In another instance, it has been revealed by Terryn *et al.,* (2016) that multi-merization of VHH domains significantly expands protection of mice from lethal rabies infection. These VHHs can be concocted in several ways to improve their diagnostic andor therapeutic properties and efficacy (Lafaye and Li**,** 2018). In addition, VHHs voluntarily and rapidly penetrate tissues, even the brain, and it is prospective that specific VHH-based paradigms will be developed that can neutralize agents tangled in brain infections, such as influenza (Ashour *et al.,* 2015), Zika, or rabies virus. Overall, VHHs or VHH-based molecules are potentially valuable diagnostic and therapeutic reagents to treat ZDs. Zoonotic diseases (ZDs) are contagions that can be naturally blowout between vertebrate animals and humans. Numerous recent outbreaks, such as Ebola and Zika have highlighted the serious impact of these diseases on human health (Qualio *et al.,* 2016).

Nanobodies overcome *Escherichia coli* and infectious diseases

Infectious diseases are disorders caused by organisms such as bacteria, viruses, fungi or parasites. Many organisms animate in and on our bodies. Conflicting to the wide range of nanobodies contenders for oncology and autoimmune disorders, a superficial search through the National Library of Medicine’s clinical trial database vintages next to no therapeutic contenders against infectious diseases instigated by viruses, bacteria and parasites. However, there are a few reports of nanobodies therapeutics against infectious diseases such as VHH batch 203027 targeting for the treatment of diarrhea and ALX-0171 for treating respiratory syncytial virus lower respiratory tract infections (Sarker *et al.,* 2013; Cunningham, *et al.,* 2021). Inappropriately, no updates on the former have been released since 2013 and the latter contender failed to produce significant results in a phase 2b trial (Sarker *et al*., 2013; Cunningham *et al.,* 2021). A more recent therapeutic nanobodies contender, LMN-101, for the treatment of *Campylobacter jejuni* infections has been stated and is presently experiencing phase 2 trials (Lumen Bioscience, 2023).

Initially, it was discovered how a chimeric construct

between an anti-trypanosome Nb and a truncated kind of the human trypanolytic protein APOL1 was able to employ a potent anti-parasite effect in vivo (Bo-kyung *et al.,* 2023). Furthermore, it has been revealed that the use of large doses of high affinity nanobodies against trypanosome variant surface glycoprotein (VSG) can persuade trypanocidal effects (Caljon *et al*., 2012; Hempelmann *et al.,* 2021). This tactic was able to diminish the obligatory curative dose multiple-fold (Arias *et al.,* 2015, Unciti-Broceta *et al.,* 2015). Finally, two high affinity nanobodies against Pfs230, a surface protein articulated during the erotic phase of the malaria-causing *Plasmodium falciparum*, were able to decrease flagellation and disrupt the sexual reproduction stage of the male microgametes-thus opening up the possibility of using nanobodies in the development of a transmission-blocking malaria vaccine (Dietrich *et al.,* 2022).

Manifold collections were able to account the development of nanobodies specific to the spike protein of the receptor binding domain of the virus, which is the supreme contender for the development of therapeutics (Huo *et al.,* 2020; Ma *et al.,* 2021). Moreover, nanobodies were revealed to be feasible for the gratitude of hidden spike protein epitopes of five various strains, highlighting their potential in the protracted fight against SARS-CoV-2 (Maeda *et al.,* 2022). Other recent promising nanobodies against viruses include a Nb–single chain variable fragment conjugate booming siRNA against the human immunodeficiency virus and a bivalent Nb construct that exhibited extraordinary binding affinity to the influenza H7N9 virus (Cunha-Santos *et al.,* 2020; Gaiotto *et al.,* 2021). As an illustration, three high affinity nanobodies against the internalin B surface protein were revealed to be able to neutralize *Listeria monocytogenes in vitro* King *et al*., 2018). In addition, two bivalent Nb constructs, one bivalent for *E. coli* Shiga toxin-2a B subunit and the other bispecific for *E. coli* Shiga toxin-2a B subunit and cell surface protein intimin, were hypothesized to be innovative neutralizers for the said *E. coli* virulence factors (Bernedo-Navarro *et al.,* 2018; Lu *et al.,* 2022). It has also been revealed that *Lactococcus lactis* bacteria-modified to show on their surface nanobodies specific to the fimbrial adhesins of *E. coli* were able to reduce fecal *E.* coli shedding when orally fed to piglets (Okello *et al.,* 2021).

Camel nanobodies overcome novel challenges for a sustainable development

Though, the camel population is not declining at the world level, even if its position is variable from one country to another, from its frightening deterioration in India to its astounding growth in the Horn of Africa and Sahelian nations. This is in malice of its marginal number (27 million in 2014 according to FAO) compared with other domestic farm animals and the steady decline in the proportion of the human population living in the desert areas.Apparently, sustainable development is not merely a challenge for camelids, but as “the animal of the desert” (camel) or “the animal of the Andean highlands” (small camelids), their responsibility is particular high because they contribute strappingly to the conservation of rural activities, nanobodies and the household economy among the more remote places of our earth. The apparent impacts of the climatic change on the camel stock include the enlargement of the geographical dispersal of the species, the use of the camel with its higher amalgamation in mixed crop-livestock systems and the increased risk of emerging diseases (Faye *et al.*, 2012; Megersa *et al.,* 2012), as the current outbreak of MERS-Coronavirus has shown (Gossner *et al.,* 2016); The strengthening of their farming is not the inevitable way, but hereafter, camelid breeders have simultaneously to contribute to maintain camel diversity, to improve the management of their resources in order to deliver products with high added value as expected by the municipal population and to reserve the future of their animals. Nevertheless, in many countries, the appearance of small or large camel dairy plants, scientific lab and diagnosis and milk processing, allowing new camel dairy products such as pasteurized milk, cheese, yogurt, or ice cream and milk and urine based nanobodies open the path of treatment of emerging diseases and play an important role in reduction of antimicrobial resistance and use of antibiotics have led to significant changes in the added value chains of camel milk as well as camel population (Faye *et al.,* 2014).

Conclusion

In this review, it can be provided evidence for the possible use of camel milk and urine based nanobodies as valuable biomolecules for the diagnosis and treatment of world emerging diseases counting bacterial, parasitic and viral zoonosis. Camel nanobodies provide numerous advantages over conventional antibodies and presently used antibody-based fragments. The eruption of VHH application in the last decades extremely seems. Various VHH arrangements were developed for their use in therapeutic and/or diagnostic applications. Subsequently there were peculiar appearances like small size, low immunogenicity and high affinity and constancy, combined with their easy countenance as recombinant proteins, nanobodies can be transported to the infection site via inhalation. Selected camel milk and urine based nanobodies represent an interesting approach to combat the world emerging diseases in the future. Hereafter, this field is set to cultivate further in the near future. Moreover, the potential for camel nanobodies therapeutics against infectious diseases has also been exposed to clearly exist but still entails much research and development. Later, it is certain that nanobodies will play a significant role in the development of next generation diagnostic tools and therapeutics in the years to come.

Conflict of interest

The authors declare that they have no conflict of interest in the publication.

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