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COMPLEMENTARITY-DETERMINING REGION 3 (CDR3) OF THE HEAVY CHAIN ONLY ANTIBODIES: THERAPEUTIC PERSPECTIVES

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ABSTRACT

Neuroinfections are difficult and time-consuming to treat. The main bottleneck in therapy is drugs' inability to enter the central nervous system (CNS) via the blood-brain barrier (BBB). Because the diffusion of conventional antibodies in tissues is limited by their large size (150 kDa), it is necessary to find an antibody fragment that can cross the BBB and treat CNS infections. Camelid heavy chain only antibodies, also known as nanobodies, possess a unique Variable domain of a Heavy chain (VHH). The VHH could be used as an alternative to conventional antibodies and have enormous potential in a wide range of medical and biotechnology fields. The VHH with an average molecular weight of 15 kDa can diffuse nearly four times better in tissues and tumours than common antibodies. A VHH is made up of four framework regions (FR1–4) and three hypervariable complementarity-determining regions (CDR1–3), with CDR3 being particularly important in interaction with pathogen epitopes. Experimental results have shown that the CDR3 domain alone can bind to epitopes and may contribute to reduce the size of the antigen-recognizing molecule, allowing it to be used more effectively to diffuse much better in tissues, and as well as to cross the BBB and biodistributed in CNS.

Key words: blood-brain barrier; CDR3; central nervous system; nanobody; neuroinfections

GENERAL IMPLICATION OF NEUROINFECTIONS

Infections of the central nervous system (CNS) vary in their causes, symptoms, and prognoses. They can develop quickly and cause significant morbidity and mortality. The relatively isolated nature of the CNS and its protective mechanisms pose a unique clinical challenge. The blood-brain barrier is the most important protective feature, as it prevents pathogens and large molecules from entering the cerebrospinal fluid (CSF). Infections frequently involve a wide range of neurovascular space from the meninges to the encephalon. An infectious agent that causes meningitis can rapidly progress to encephalitis, also known as meningoencephalitis. Bacteria, viruses, fungi, and parasites can all cause neuroinfections. The infection spreads to the intracranial space haematogenously, through direct transfer, or via neuronal transmission. Vertical transmission can also happen in newborns through breast milk [6].

Meningitis can be caused by a variety of infectious and non-infectious conditions. The most common infectious causes are bacterial, viral, and fungal. Meningitis caused by a bacterial infection can result in significant morbidity

and mortality due to severe inflammation, oedema of the surrounding structures, and increased intracranial pressure. The main pathogenic bacteria involved in meningitis vary with age and degree of immunocompromise. The most common causes of meningitis in neonates are *Streptococcus agalactiae* and *E. coli*. Common agents in adolescents include *Neisseria meningitidis* and *Streptococcus pneumoniae*, while *Listeria monocytogenes* should also be considered, particularly in the elderly or immunocompromised individuals [35]. Viral meningitis is usually less severe than bacterial meningitis. The two most common neurotropic viruses that can cause disease are herpes simplex virus (HSV) and varicella-zoster virus. HSV-1 infection can cause severe encephalitis in adults, but HSV-2 infections are more serious in children. However, incidental and non-neurotropic viruses are responsible for the majority of viral meningitis cases. Nonpolio enteroviruses cause more than 85 % of all cases of viral meningitis [20]. Patients with meningitis commonly experience headaches, vomiting, and neck pain. Lumbar puncture is the most used diagnostic procedure for meningitis. Pleocytosis, or increased cell counts in the CSF, is a strong indicator of meningeal inflammation. The leukocyte differential can reveal additional information about the underlying cause. Viral aetiologies are more likely to produce lymphocytes, whereas bacterial aetiologies produce neutrophils. These trends and patterns in cell count and differential are still nonspecific, but there is evidence that marked pleocytosis is a sensitive indicator of bacterial meningitis. The first measurements reported from CSF analysis are typically CSF protein and glucose levels. While not diagnostic, protein and glucose levels can indicate whether the infection is viral, bacterial, or fungal. Polymerase chain reaction (PCR) methods enable rapid pathogen identification by amplifying and matching the pathogen's gene products. Rapid diagnosis is important because it can shorten the period of treatment and hospitalisation [22].

Encephalopathy, the second most common pathological condition, can cause an altered level of consciousness for at least 24 hours. This includes lethargy, irritability, or a change in personality or behaviour. Fever, focal neurological findings, seizures, CSF pleocytosis, electroencephalogram (EEG) abnormalities, and neuroimaging findings consistent with encephalitis all indicate CNS inflammation. The infection-induced CNS inflammation distinguishes encephalitis from other causes of encephalopathy [6]. The most common viral encephalitis aetiology is HSV, accounting for 50–75 % of all cases. However, there are several other causes of encephalitis, including viral, bacterial, fungal, mycoplasma, Rickettsia, protozoan, and autoimmune. The major components of any encephalitis diagnosis should include CSF analysis, neuroimaging, and EEG [10].

Despite decades of research into CNS disorders, many disease mechanisms remain unknown, owing in part to the brain's incredible complexity and vasculature. The brain requires a homeostatic environment to function properly. This protective environment is maintained by specialised endothelial cells found in the brain's vasculature, which form a tight blood-brain barrier (BBB). The BBB keeps large, polar substances and potentially neurotoxic compounds in the bloodstream from passively diffusing into the brain. Essential nutrients that cannot pass the BBB via diffusion, such as glucose, enter the brain through specialised influx transporters [39]. In contrast, harmful molecules are cleared from the brain via efflux transporters. Only a few molecules can pass passively through the BBB. Another route is via carrier-mediated transporters (for glucose or amino acids) or receptor-mediated endocytosis (for insulin or leptin). It is made up of the endothelial cells of brain capillaries, astrocyte end-feet that ensheathe the vessels, and pericytes embedded within the basement membrane (Figure 1) [36, 40]. Brain endothelial cells differ from endothelial cells in other parts of the body. Their main distinguishing feature is a high number of mitochondria, expression of endothelial tight junctions, the presence of very few caveolae in the luminal chamber, and a flattened appearance. Paracellular pathways are sealed by tight junctions between adjacent endothelial cells. Astrocytes are the most abundant cells in the central nervous system, and their foot processes connect astroglial end-feet to the superficial or perivascular basal lamina. They also carry out several functions, including neural parenchymal compartmentalization, extracellular ionic homeostasis maintenance, pH regulation, neurotransmitter uptake, and signal mediation from neurons to the vasculature. Pericytes are the BBB's least studied cellular component, but they appear to play an important role in angiogenesis, vessel structural integrity and differentiation, and endothelial tight junction formation. They share the same basal lamina as endothelial cells, and their phagocytic functions can help remove toxic metabolites. They also express receptors for vascular me-

diators like catecholamines, angiotensin I, endothelin-1, and vasopressin [19].

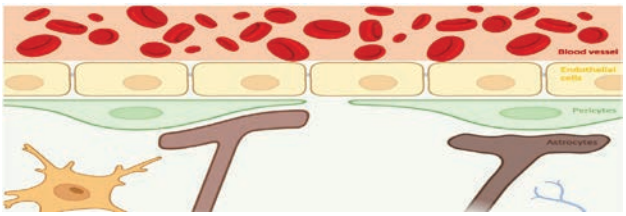

Fig. 1. Composition of the blood-brain barrier. The BBB is a selective semipermeable border that regulates the transfer of solutes and molecules between circulatory system and the central nervous system.
Source: Author (Created with BioRender.com)

It is believed that all the components of the BBB are essential for its normal function and stability. A healthy barrier provides effective but incomplete protection of CNS [2], which allows neuroinfections to cross the BBB. Neuropathogens can pass through the barrier and enter the CNS. An infection disrupts the BBB's functional homeostasis by altering permeability and triggering anti-inflammatory and pro-inflammatory innate immune responses. Viral entry can occur via three distinct pathways: transcellular, paracellular, and a Trojan horse mechanism that uses infected cells' diapedesis. Although pathogens penetrate the BBB, this strong line of defence prevents neurological drugs from moving across the barrier [37].

Antibodies are regarded as an attractive biomolecule for the diagnosis and antibody-based immunotherapy of neuroinfections. Although they are promising therapeutic tools, the major disadvantage is their size (approximately 150 kDa), which limits their penetration into the brain tissue [34]. In order to reduce the molecular size and preserve the properties, camelid antibodies with a significantly lower molecular weight were used for the preparation of recombinant proteins and peptides serving as therapeutics against various types of neuropathogens [24].

Antibodies: promising therapeutic molecules

The production of antibodies is central to the adaptive immune system's ability to identify and neutralise foreign objects (bacteria, viruses, parasites, and so on) in the body. They are large, Y-shaped proteins from the immunoglobulin (Ig) superfamily. Human antibodies are divided into five classes, often known as isotypes: IgA, IgD, IgE, IgG, and IgM (Fig. 2) [15].

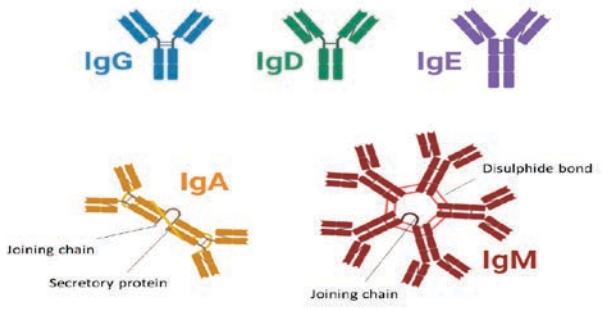

Fig. 2. Types of antibodies.
Source: Author (created with BioRender.com)

Antibodies have become the most promising natural biological molecules in a variety of biotechnological applications, including therapeutic development, due to their high affinity and specificity to their targets and the availability of technologies to engineer different antibodies to a wide range of targets [13]. Conventional antibodies (150 kDa) exist as a Y-shaped unit made up of four polypeptide chains. Each Y unit contains two identical copies of a heavy chain (H) and two identical copies of a light chain (L), with different sequences and lengths. The variable region (V), also known as the fragment antigen-binding (F(ab)) region, is located at the top of the Y shape (Fig. 3). The antibody base consists of constant domains (C) that form the fragment crystallizable region (Fc) [15].

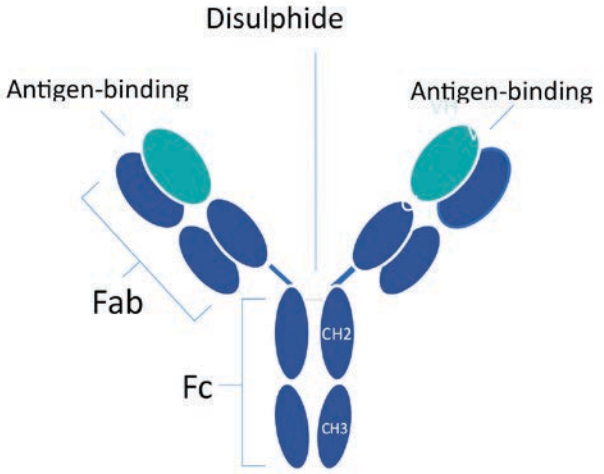

Fig. 3. Structure of immunoglobulin
Source: Author

In 1989, Professor Raymond Hamers-Casterman of the Vrije Universiteit Brussel made a serendipitous discovery, finding that *Camelidae* animals possess heavy-chain only antibodies (HCAb), that lack light chains and exist alongside conventional antibodies in their blood [11]. Ap-

proximately half of the antibodies in camelid blood are devoid of light chains. These antibodies bind to the antigen via a single domain known as the Variable domain of a Heavy Chain (VHH) or single domain antibody fragments (sdAb). They are also referred to as nanobodies due to their dimensions in the nanometer range (2.5 nm in diameter by 4 nm) [7]. This term nanobody was adapted by the company Ablynx and it led to create NANOBODY® Technology Platform (https://www.sanofi.com/en/magazine/our-science/nanobody-technology-platform). HCAb are also naturally produced by sharks, known as V_{NAR} (Variable domain of New Antigen Receptor) [30].

The VHH has a molecular weight of 12–15 kDa and retains full antigen binding capacity. They have been identified as the naturally occurring smallest single domain capable of serving as a fully functional antibody. Their molecular weight gives them an advantage, as they diffuse in tissues and tumours nearly four times better than common antibodies. Their morphological structure enables the identification of otherwise non-immunogenic epitopes as well as epitopes hidden within antigen caveats. They exhibit low immunogenicity and high affinity for the target protein [12, 32, 34]. Despite their high solubility, they are extremely stable and can withstand extended thermal incubation (37 °C/200 hours) with little loss of affinity. Additionally, they can withstand a wide temperature range, high pressure (500–750 MPa), pH changes (3–9), and chemical denaturants (3M guanidium chloride, 6–8 M urea) [7, 9]. They refold, and their monomeric structure and lack of post-translational modifications enable their expression in simple microbial systems (*Escherichia coli, Saccharomyces cerevisiae,* and *Pichia pastoris*), making them more affordable and easier to produce [16]. Although some of their properties are advantageous in comparison to conventional antibodies, they are foreign to humans and thus may elicit an immune response [1]. VHH lacks certain effector functions associated with the absence of the Fc region. Another complication is their rapid elimination from the blood stream. Their molecular weight is far below the renal cut-off for glomerular filtration (60 kDa), so they are cleared from the blood much faster than full-sized immunoglobulins [14]. These issues could be resolved by fusing the Fc region or modifying it with other molecules to stabilise VHH in the body [23].

A typical VHH consists of four framework regions (FR1–4) and three hypervariable complementary determining regions (CDR1–3; see Figure 4). Q i u and colleagues described a truncated version of the VHH with a CDR3 loop flanked by 30 amino acids from FR3 and 10 amino acids from FR4 alone. This truncated VHH had a nanomolar affinity for its epitope. These results highlight the possibility that flanking the CDR3 with 10 amino acids from FR3 and FR4 would result in the maintenance of antigen recognition, despite its relatively short sequence [29]. Furthermore, the longer CDR3 loop plays a key role in the interaction with epitopes, and it can bind with high affinity to small hidden epitopes in the concave antigen surface or in the antigen gap [29].

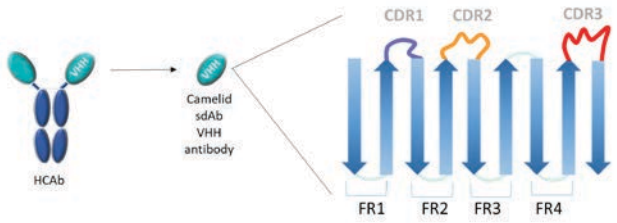

Fig. 4. Structure of camelid heavy-chain only antibody (HCAb). Three CDRs and four framework regions in VHH.
Source: Author

VHH has a longer CDR3 than VH in conventional antibodies. This enables the formation of a convex ring structure that can penetrate into difficult-to-reach crevices of the antigen surface, increasing the contact surface area and thus improving binding affinity [38]. The CDR3 loop of camelid VHH has a much wider range of lengths (3–28 amino acids) than human VH domains (8–15 amino acids) [25]. The VHH's long CDR3 increases its potential interaction surface with the target antigen and is significantly more variable than the VH, partially compensating for the absence of the VL domain. The C-terminal part of a long CDR3 loop frequently folds over onto the side of the VHH domain that is opposite the side of a VH domain facing a VL domain. This allows for binding to unique epitopes that are not accessible to conventional antibodies, whose antigen-binding interface is generally flat [3]. VHH are appropriate for use in a variety of clinical settings due to their numerous benefits. They have been used to treat cancer in clinical practice for several decades with the goal of blocking or neutralising growth factor receptors, opsonizing cancer cells that will be subsequently removed by the immune system, modifying apoptosis and other biological processes, or delivering chemotherapeutics [18]. There is

also a potential for use in the fight against viral, bacterial, parasitic, and fungal diseases, in pathogen detection, and, due to their size, in the treatment of neuroinfections [34].

Antibody fragments: plausible therapeutics targeting the brain infections

In homeostasis, the integrity of the BBB may make it difficult for conventional antibodies to cross the barrier spontaneously due to Fc-receptor-mediated efflux into the bloodstream. The permeability of the BBB is limited to receptor-specific ligands or molecules that show both lipophilic solubility and a molecular weight smaller than 400 Da, hindering therapeutic concentrations in the brain [26]. Under these restrictive conditions, only 0.01–0.4 of blood proteins can enter the CNS via passive diffusion, including some therapeutic antibodies, which are frequently IgG isotype (150 kDa). Despite the controversy, some authors believe that the Fc region's binding to Fc receptors in the BBB could represent a potential pathway for brain access and/or exit. Furthermore, the neonatal Fc receptor (FcRn), located in the brain microvascular endothelium, actively mediates the reverse transcytosis of IgG from the brain to blood across the barrier [31]. Temporary disruption of the BBB with techniques such as focused ultrasound or pharmacological agents can temporarily increase its permeability, allowing the smaller antibody fragments like VHH or CDR3 to pass more easily. This approach requires careful modulation to minimise potential negative effects on brain function [28]. Another mechanism exploited mainly for VHH to cross the BBB is the receptor-mediated transcytosis (RMT). Transferrin and insulin receptors are the most commonly used receptors for delivering conventional antibodies into the brain, while lipoprotein-related proteins and insulin-like growth factor 1 receptors have also been reported [33]. Engineering the CDR3 to bind to receptors expressed on brain microvascular endothelial cells could allow for efficient transport into the brain via receptor-mediated transcytosis. It was hypothesized that *Camelid* CDR3 can bypass the barrier by mimicking the receptors' natural ligands [28]. Angiopep-2 (TFFYGGSRGKRNNFKTEEY, 2.4 kDa) is a ligand that binds to LRP1, the low-density lipoprotein receptor-related protein. This 19-amino-acid oligopeptide belongs to the angiopep peptide family, which was derived from aprotinin's Kunitz domain. These peptides can cross the blood-brain barrier and have been used to help deliver pharmacological agents to the brain. [8]. Angiopep-2 has demonstrated increased transcytosis and parenchymal accumulation because it interacts with LRP1, which is thought to promote RMT delivery. LRP1 is highly expressed on the endothelial cells of the BBB vascular system and is also overexpressed on certain types of tumour cells (for example, glioblastomas) [27]. This fact qualifies angiopep-2 as a candidate molecule for the development of new brain delivery systems for pharmaceuticals. One such delivery system is dendrimers, which have already been conjugated to angiopep-2 for the delivery of therapeutic agents to treat brain disorders (tumours, brain injury, stroke, epilepsy, fungal infections, Alzheimer's disease, Parkinson's disease) [4].

Many studies have shown that delivery systems, also known as nanocarriers, are effective in treating brain diseases. Using them as carriers for CDR3 could improve stability and make transport easier. Surface modifications using ligands that target BBB receptors can improve their uptake and transport into the brain parenchyma [17]. Among several nanocarriers, dendrimers are the most commonly tested carriers. They are artificial macromolecules that are radially symmetrical and hyperbranched, with a compact structure and numerous functional groups. The structure is made up of a central core, attached branches (dendrons), and a terminal functional group (Fig. 5). The branches frequently bridge among themselves, resulting in an organised, radially concentric, layer-based geometric structure known as "generations" [5]. Surface modification, such as the addition of the angiopep-2 moiety, is fairly simple. For example, polyamidoamine (PAMAM), a starburst dendrimer, has demonstrated good molecular size, structure, and brain barrier penetrating properties [21]. Furthermore, dendritic architectures are highly controllable, making them suitable carriers in biomedical applications. Nanotechnologies have improved BBB permeability, regional targeting, drug stability, and delivery. Furthermore, RMT has shown initial promises in delivering macromolecular biopharmaceuticals and small molecules, respectively, across the BBB [4].

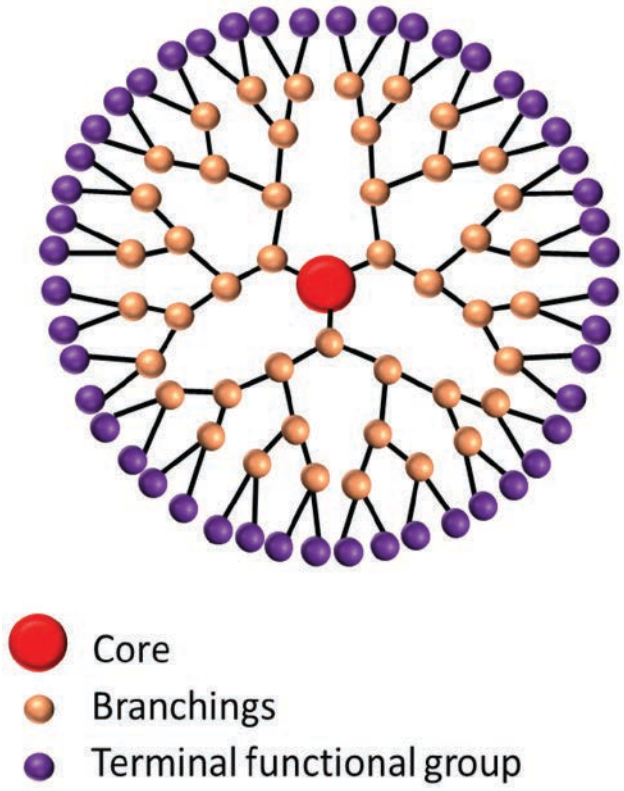

Fig. 5. General structure of dendrimer.
Source: Author

CONCLUSIONS

The exploration of camelid CDR3 offers a promising avenue for the development of novel therapeutic strategies for neuroinfections. Camelid antibodies are attractive candidates for targeting pathogens that can infiltrate the CNS. Researchers are actively investigating the potential of camelid CDR3 in combating neuroinfections. One distinguishing feature is its plausible ability to cross the blood-brain barrier, when coupled with BBB homing peptide. Camelid CDR3 can also be engineered to penetrate and target pathogens in the brain parenchyma through a variety of mechanisms, including nanoparticle-mediated delivery, and temporary BBB disruption. These strategies have the potential to significantly improve the efficacy of treatments. Furthermore, the specificity of camelid CDR3 allows for precise antigen targeting, which reduces off-target effects and the risk of adverse reactions. This targeted approach may result in more effective and safer treatments against neuroinfections, with fewer side effects than traditional therapies.

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REFERENCES

1. **Ackaert, C., Smiejkowska, N., Xavier, C., Sterckx, Y. G. J., Denies, S., Stijlemans, B., et al., 2021:** Immunogenicity risk profile of nanobodies. *Front. Immunol*., 12, 632–687. DOI: 10.3389/fimmu.2021.632687.
2. **Ballabh, P., Braun, A., Nedergaard, M., 2004:** The blood-brain barrier: An overview: Structure, regulation, and clinical implications. *Neurobiol. Dis*., 16, 1, 1–13. DOI: 10.1016/j.nbd.2003.12.016.
3. **Bannas, P., Hambach, J., Koch-Nolte, F., 2017:** Nanobodies and nanobody-based human heavy chain antibodies as antitumor therapeutics. *Front. Immunol*., 8, 1603. DOI: 10.3389/fimmu.2017.01603.
4. **Bhunia, S., Kolishetti, N., Vashist, A., Yndart Arias, A., Brooks, D., Nair, M., 2023:** Drug delivery to the brain: Recent advances and unmet challenges. *Pharmaceutics.,* 15, 12, 2658. DOI: 10.3390/pharmaceutics15122658.
5. **Birdhariya, B., Kesharwani, P., Jain**, **N. K., 2015:** Effect of surface capping on targeting potential of folate decorated poly (propylene imine) dendrimers. *Drug Dev. Ind. Pharm.,* 41, 8, 1393–1399. DOI: 10.3109/03639045.2014.954584.
6. **Bowers, K. M., Mudrakola, V., 2020:** Neuroinfections: Presentation, diagnosis, and treatment of meningitis and encephalitis. *EMJ Neurology*, 8, 1, 9–102. DOI: 10.33590/emjneurol/20-00063.
7. **De Vos, J., Devoogdt, N., Lahoutte, T., Muyldermans, S., 2013:** Camelid single-domain antibody-fragment engineering for (pre)clinical in vivo molecular imaging applications: adjusting the bullet to its target. *Expert Opin. Biol. Ther*., 13, 8, 1149–1160. DOI: 10.1517/14712598.2013.800478.
8. **Demeule, M., Currie, J. C., Bertrand, Y., Ché, C., Nguyen, T., Régina, A., et al., 2008:** Involvement of the low-density lipoprotein receptor-related protein in the transcytosis of the brain delivery vector angiopep-2. *J. Neurochem*., 106, 4, 534–44. DOI: 10.1111/j.1471-4159.2008.05492.x.
9. **Dumoulin, M., Conrath, K., Van Meirhaeghe, A., Meersman, F., Heremans, K., Frenken, L. G., et al., 2002:** Sin-

gle-domain antibody fragments with high conformational stability. *Protein Sci*., 11, 3, 500–515. DOI: 10.1110/ps.34602.

10. **Ellul, M., Solomon, T., 2018:** Acute encephalitis – diagnosis and management. *Clin. Med.,* 18, 2, 155–159. DOI: 10.7861/clinmedicine.18-2-155.
11. **Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E. B., et al., 1993:** Naturally occurring antibodies devoid of light chains. *Nature,* 363, 6428, 446–448. DOI: 10.1038/363446a0.
12. **Henry, K. A., MacKenzie, C. R., 2018:** Antigen recognition by single-domain antibodies: structural latitudes and constraints. *MAbs*., 10, 6, 815–826. DOI: 10.1080/19420862.2018.1489633.
13. **Hillman, Y., Lustiger, D., Wine, Y., 2019:** Antibody-based nanotechnology. *Nanotechnology,* 30, 28, 282001. DOI: 10.1088/1361-6528/ab12f4.
14. **Holliger, P., Hudson, P. J., 2005:** Engineered antibody fragments and the rise of single domains. *Nat. Biotechnol*., 23, 9, 1126–1136. DOI: 10.1038/nbt1142.
15. **Hořejší, V., Bartůňková, J., Brdlička, T., Špíšek, R., 2017:** *Basics of Immunology* (In Czech). 6th edn., Triton, Prague, 316 pp.
16. **Hruškovicová, J., Bhide, K., Petroušková, P., Tkáčová, Z., Mochnáčová, E., Čurlík, J., et al., 2022:** Engineering the single domain antibodies targeting receptor binding motifs within the domain III of West Nile virus envelope glycoprotein. *Front. Microbiol.,* 13, 801466. DOI: 10.3389/fmicb.2022.801466.
17. **Jin, S., Ye, K., 2007:** Nanoparticle-mediated drug delivery and gene therapy. *Biotechnol. Prog.,* 23, 1, 32–41. DOI: 10.1021/bp060348j.
18. **Jovčevska, I., Muyldermans, S., 2020:** The therapeutic potential of nanobodies. *BioDrugs,* 34, 1, 11–26. DOI: 10.1007/s40259-019-00392-z.
19. **Kadry, H., Noorani, B., Cucullo, L., 2020:** A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids Barriers CNS,* 17, 1, 69. DOI: 10.1186/s12987-020-00230-3.
20. **Leber, A. L., Everhart, K., Balada-Llasat, J. M., Cullison, J., Daly, J., Holt, S., et al., 2016:** Multicenter evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J. Clin. Microbiol.,* 54, 9, 2251–2261. DOI: 10.1128/JCM.00730-16.
21. **Li, Z., Liu, T., Yang, N., Han, D., Mi, X., Li, Y., et al., 2020:** Neurological manifestations of patients with COVID-19: potential routes of SARS-CoV-2 neuroinvasion from the periphery to the brain. *Front. Med*., 14, 5, 533–541. DOI: 10.1007/s11684-020-0786-5.
22. **Lyons, T. W., McAdam, A. J., Cohn, K. A., Monuteaux, M. C., Nigrovic, L. E., 2012:** Impact of in-hospital enteroviral polymerase chain reaction testing on the clinical management of children with meningitis. *J. Hosp. Med*., 7, 7, 517–520. DOI: 10.1002/jhm.1947.
23. **Masserini, M., 2013:** Nanoparticles for brain drug delivery. *ISRN Biochem*., 238428. DOI: 10.1155/2013/238428.
24. **Minatel, V. M., Prudencio, C. R., Barraviera, B., Ferreira, R.S., 2024:** Nanobodies: A promising approach to treatment of viral diseases. *Front. Immunol*., 14. DOI:10.3389/fimmu.2023.1303353.
25. **Muyldermans, S., 2013:** Nanobodies: Natural single-domain antibodies. *Annu. Rev. Biochem.,* 82, 775–797. DOI: 10.1146/annurev-biochem-063011-092449.
26. **Pardridge, W. M., 2012:** Drug transport across the blood-brain barrier. *J. Cereb. Blood Flow Metab.,* 32, 11, 1959–1972. DOI: 10.1038/jcbfm.2012.126.
27. **Pethő, L., Oláh-Szabó, R., Mező, G., 2023:** Influence of the drug position on bioactivity in Angiopep-2—Daunomycin conjugates. *Int. J. Mol. Sci.,* 24, 4, 3106. DOI: 10.3390/ijms24043106.
28. **Pothin, E., Lesuisse, D., Lafaye, P., 2020:** Brain delivery of single-domain antibodies: A focus on VHH and VNAR. *Pharmaceutics,* 12, 10, 937. DOI: 10.3390/pharmaceutics12100937.
29. **Qiu, L., Feng, Y., Ma, X., Li, J., 2017:** A camel anti-lysozyme CDR3 only domain antibody selected from phage display VHH library acts as potent lysozyme inhibitor. *Acta Biochim. Biophys. Sin.,* 49, 6, 513–519. DOI: 10.1093/abbs/gmx037.
30. **Roux, K. H., Greenberg, A. S., Greene, L., Strelets, L., Avila, D., McKinney, E. C., et al., 1998:** Structural analysis of the nurse shark (new) antigen receptor (NAR): Molecular convergence of NAR and unusual mammalian immunoglobulins. *Proc. Natl. Acad. Sci. U.S.A.,* 95, 20, 11804–11809. DOI: 10.1073/pnas.95.20.11804.
31. **Ruiz-López, E., Schuhmacher, A. J., 2021:** Transportation of single-domain antibodies through the blood-brain barrier. *Biomolecules,* 11, 8, 1131. DOI: 10.3390/biom11081131.
32. **Salvador, J. P., Vilaplana, L., Marco, M. P., 2019:** Nanobody: Outstanding features for diagnostic and therapeutic applications. *Anal. Bioanal. Chem*., 411, 9, 1703–1713. DOI: 10.1007/s00216-019-01633-4.

33. **Stanimirovic, D., Kemmerich, K., Haqqani, A. S., Farrington, G. K., 2014:** Engineering and pharmacology of blood-brain barrier-permeable bispecific antibodies. *Adv. Pharmacol.,* 71, 301–335. DOI: 10.1016/bs.apha.2014.06.005.
34. **Steeland, S., Vandenbroucke, R. E., Libert, C., 2016:** Nanobodies as therapeutics: Big opportunities for small antibodies. *Drug Discov. Today,* 21, 7, 1076–1113. DOI: 10.1016/j.drudis.2016.04.003.
35. **Tanski, M. E., Ma, O. J., 2020:** Central nervous system and spinal infections. In *Tintinalli's Emergency Medicine: A Comprehensive Study Guide.* 9th edn., McGraw-Hill Education: New York, NY. Available online at https://accessmedicine.mhmedical.com/content.aspx?
36. **Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., et al., 2020:** Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv.,* 12, 21. DOI: 10.1101/2020.12.21.20248640.
37. **Tyagi, K., Rai, P., Gautam, A., Kaur, H., Kapoor, S., Suttee, A., et al., 2023:** Neurological manifestations of SARS-CoV-2: complexity, mechanism and associated disorders. *Eur. J. Med. Res.,* 28, 1, 307. DOI: 10.1186/s40001-023-01293-2.
38. **Wesolowski, J., Alzogaray, V., Reyelt, J., Unger, M., Juarez, K., Urrutia, M., et al.,** 2009: Single domain antibodies: Promising experimental and therapeutic tools in infection and immunity. *Med. Microbiol. Immunol*., 198, 3, 157–174. DOI: 10.1007/s00430-009-0116-7.
39. **Wevers, N. R., De Vries, H. E., 2023:** Microfluidic models of the neurovascular unit: A translational view. *Fluids Barriers CNS,* 20, 1, 86. DOI: 10.1186/s12987-023-00490-9.
40. **Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., et al., 2020:** A new coronavirus associated with human respiratory disease in China. *Nature,* 579, 7798, 265–269. DOI: 10.1038/s41586-020-2008-3.

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