Exploring lectin-glycan interactions to combat COVID-19: lessons acquired from other enveloped viruses

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Abstract

The emergence of a new human coronavirus (SARS-CoV-2) has imposed great pressure on the health system worldwide. The presence of glycoproteins on the viral envelope opens a wide range of possibilities for application of lectins to address some urgent problems involved in this pandemic. In this work, we discuss the potential contributions of lectins from non-mammalian sources in the development of several fields associated with viral infections, most notably COVID-19. We review the literature on the use of non-mammalian lectins as a

therapeutic approach against members of the *Coronaviridae* family, including recent advances in strategies of protein engineering to improve their efficacy. The applications of lectins as adjuvants for antiviral vaccines are also discussed. Finally, we present some emerging strategies employing lectins for the development of biosensors, microarrays, immunoassays and tools for purification of viruses from whole blood. Altogether, the data compiled in this review highlights the importance of structural studies aiming to improve our knowledge about the basis of glycan recognition by lectins and its repercussions in several fields, providing potential solutions for complex aspects that are emerging from different health challenges.

1. Introduction

The year of 2020 has been marked by the global dissemination of a new type of severe acute respiratory syndrome, defined as COVID-19 (Rothan, H.A. and Byrareddy, S.N. 2020, Yi, Y., Lagniton, P.N.P., et al. 2020). The novel human coronavirus (SARS-CoV-2 or 2019-nCoV) was first reported in Wuhan (China) with similarity to other types of *Betacoronavirus*: (i) SARS-CoV, the etiologic agent of an outbreak of severe acute respiratory syndrome in China (SARS, 2003); (ii) MERS-CoV, the virus identified as the cause of a severe respiratory syndrome in Middle East (MERS, 2012) (Zhang, Y.Z. and Holmes, E.C. 2020). However, SARS-CoV-2 has higher dissemination rates than MERS-CoV and SARS-CoV, imposing serious challenges to health systems worldwide (Requia, W.J., Kondo, E.K., et al. 2020, Sun, J., He, W.T., et al. 2020).

The initial symptoms of COVID-19 disease are similar to the common cold. However, this infection can progress to severe respiratory failure with impaired and deleterious immunological responses (Li, R., Tian, J., et al. 2020, Sun, S., Cai, X., et al. 2020, Xu, B., Fan, C.Y., et al. 2020). This state is characterized by increased ratios of monocytes and neutrophils and exacerbated release of inflammatory mediators (especially IL-6) that may contribute to dysfunction of multiple organs (Qin, C., Zhou, L., et al. 2020, Xu, B., Fan, C.Y., et al. 2020). Despite the occurrence of other coronavirus outbreaks, there is still no specific therapy or vaccine for COVID-19 (Alanagreh, L., Alzoughool, F., et al. 2020, Pastick, K.A., Okafor, E.C., et al. 2020).

Other important concern is the urgent need for simple and fast devices for viral detection in clinical and environmental samples (Liang, K.H., Chang, T.J., et al. 2020, Qiu, G., Gai, Z., et al. 2020). Particularly, the early detection of SARS-CoV-2 in asymptomatic and/or presymptomatic individuals is important to break the chain of transmission (Arons, M.M., Hatfield, K.M., et al. 2020, Furukawa, N.W., Brooks, J.T., et al. 2020). The

extracorporeal removal of SARS-CoV-2 from blood by plasmapheresis is also important for implementation of alternative therapies (Turgutkaya, A., Yavasoglu, I., et al. 2020). This complex panorama has imposed a race against time across different fields of knowledge (biomedical science, biotechnology, drug development, molecular analysis, etc.) to offer the maximum number of solutions to these and other issues raised by this pandemic (Venkatakrishnan, K., Yalkinoglu, O., et al. 2020).

SARS-CoV-2 has a lipoprotein envelope surrounding the infectious virion particles which is derived from the host cell during the budding (Bianchi, M., Benvenuto, D., et al. 2020, Sternberg, A. and Naujokat, C. 2020). The viral envelope has two layers of lipids whose composition depends on the type of membrane from which this structure is derived (Cosset, F.L. and Lavillette, D. 2011). The proteins are interspersed in the lipid layers and some of them are glycosylated by hots enzymes (Carbaugh, D.L. and Lazear, H.M. 2020, Cipollo, J.F. and Parsons, L.M. 2020). Like other enveloped viruses, the envelope glycoproteins of SARS-CoV-2 are involved in the process of viral adhesion and entry (Sternberg, A. and Naujokat, C. 2020, Verma, J., Subbarao, N., et al. 2020).

For instance, the envelope of SARS-CoV-2 exhibits glycoproteins such as spike (S-protein) and membrane (M-protein) proteins (Ahmed, S.F., Quadeer, A.A., et al. 2020, Shajahan, A., Supekar, N.T., et al. 2020) (Supplementary Figure 1A). These structural proteins (specially S-protein) exert central roles in viral pathogenesis and are pointed as important targets for neutralizing antibodies, vaccine and drug design (Ahmed, S.F., Quadeer, A.A., et al. 2020, Bagdonaite, I. and Wandall, H.H. 2018). Besides, non-structural proteins are also found in glycosylated forms, such as the 3a protein, which plays an essential role in SARS-CoV-2 virulence (Fung, T.S. and Liu, D.X. 2018, Issa, E., Merhi, G., et al. 2020). The presence of glycoproteins in viral envelope opens a wide range of possibilities for application

of carbohydrate binding agent, such as lectins, to address some urgent problems involved in this pandemic situation.

Lectins comprises a large class of proteins sharing the ability to recognize specific types of carbohydrates residues (Coelho, L.C., Silva, P.M., et al. 2017). They are ubiquitously distributed in the organisms where participate in sugar storage, immune defense systems and other physiological processes (Brown, G.D., Willment, J.A., et al. 2018, Prado Acosta, M. and Lepenies, B. 2019, Ribeiro, A.C., Monteiro, S.V., et al. 2014). Moreover, lectins from pathogenic agents (including viruses, bacteria and fungi) are involved in their virulence mechanisms through binding to glycans present on host cell surfaces (Landi, A., Mari, M., et al. 2019, Patra, D., Mishra, P., et al. 2014, Van Breedam, W., Pohlmann, S., et al. 2014).

The recognition of glycans allow the use of lectins for several biotechnological applications (de Oliveira Figueiroa, E., Albuquerque da Cunha, C.R., et al. 2017, Hamorsky, K.T., Kouokam, J.C., et al. 2019, Swanson, M.D., Boudreaux, D.M., et al. 2015). Importantly, these agglutinins are pointed as broad-spectrum inhibitors of viral invasion, since they could target sugar moieties in surface proteins and block the adhesion to host cells (Gondim, A.C.S., Roberta da Silva, S., et al. 2019, Mitchell, C.A., Ramessar, K., et al. 2017, Wang, D., Tang, J., et al. 2015). The interaction with glycoproteins also allows the use of lectins in the development of devices for identification and characterization of glycoproteins in viral envelope or alterations in host glycoproteins during viral infection (Andrade, C.A., Oliveira, M.D., et al. 2011, Koch, B., Schult-Dietrich, P., et al. 2018, Simao, E.P., Silva, D.B.S., et al. 2020).

Herein, we discuss the potential contributions of non-mammalian lectins in the development of different fields of viral infections research, using COVID-19 disease as background. Given the recent emergence of the SARS-CoV-2, we review some lessons acquired from previous studies on closely related coronaviruses (SARS-CoV, MERS-CoV)

and other enveloped viruses. In this regard, the possible applications of lectins include: (i) design of leading molecules for antiviral therapy; (ii) development of adjuvant compounds for immunization; (iii) establishment of approaches to characterize glycosylation of viruses or infected host protein structures; (iv) development of devices for viral detection and purification. We also discuss challenges and limitations for each target application.

2. Overview of glycosylation of viral proteins

Glycosylation is the most common type of post-translational modification (PTM), which significantly regulates the structure (folding and stability) and functions of proteins (Cipollo, J.F. and Parsons, L.M. 2020, Ohyama, Y., Nakajima, K., et al. 2020). Glycans are involved in viral adhesion and epitope shielding from antibody recognition, therefore glycosylation sites may be under selective pressure (Vankadari, N. and Wilce, J.A. 2020, Watanabe, Y., Bowden, T.A., et al. 2019). The synthesis, folding and glycosylation (as other PTMs) of viral proteins depend on host organelles (ribosomes, endoplasmic reticulum and Golgi apparatus) and enzymes (glycotransferases and glycosidases) (Carbaugh, D.L. and Lazear, H.M. 2020, Cipollo, J.F. and Parsons, L.M. 2020).

The current experimental data regarding the glycosylation of viral proteins is dependent on the carbohydrate processing enzymes available in the biological systems used to propagate the viral strain. In this sense, our knowledge about the natural pattern of viral glycosylation is very limited (Bagdonaite, I., Vakhrushev, S.Y., et al. 2018, Cipollo, J.F. and Parsons, L.M. 2020). It is also important to consider that viral proteins may follow different pathways than those observed from host glycoproteins (Watanabe, Y., Bowden, T.A., et al. 2019).

The covalent addition of glycans in viral proteins occurs mainly through two pathways: (i) N-glycosylation, when the glycan is connected to asparagine (Asn) residues;

and (ii) O-glycosylation, when the modification is performed in oxygen atoms of serine (Ser) and threonine (Thr) residues [sometimes tyrosine (Tyr) residues are used] (Watanabe, Y., Bowden, T.A., et al. 2019). The mucin-type O-glycosylation, also known as N-acetylgalactosamine (GalNAc)-type, is the most commonly observed in viral proteins (Chen, N., Kong, X., et al. 2020, Hargett, A.A. and Renfrow, M.B. 2019).

The process of N-glycosylation of viral proteins in mammalian cells starts in parallel with their synthesis. In the cytoplasmatic side of endoplasmatic reticulum (ER), the glycan precursor is synthesized containing three glucose (Glc), nine mannose (Man), and two N-acetylglucosamine (GlcNAc) ($Glc_3Man_9GlcNAc_2$) glycosylation sites (Hargett, A.A. and Renfrow, M.B. 2019). This precursor is transferred to the ER lumen and modified by the addition of monosaccharides (Fung, T.S. and Liu, D.X. 2018).

The mature glycan structure is then added, by an oligosaccharyltransferase, to the Asn residue of an Asn-X-Ser/Thr motif ('X' in any amino acid, except proline) in the nascent protein chain (Carbaugh, D.L. and Lazear, H.M. 2020). Following, other enzymes from the ER and Golgi apparatus (glucosidases, mannosidases, galactosyl-, fucosyl- and sialyl-transferases) modify the structure, which results in the diversification of glycan molecules that are allocated in the classes: oligomannose, hybrid, and complex-type N- glycan structures (Supplementary Figure 1B) (Fung, T.S. and Liu, D.X. 2018, Watanabe, Y., Bowden, T.A., et al. 2019).

The mucin-type O-glycosylation can be considered more complex than *N*-glycosylation. The first takes place in the Golgi apparatus, where a group of GalNAc-transferases mediates the insertion of GalNAc monosaccharide to the appropriated amino acids residues (Ser, Thr or Tyr) (Cipollo, J.F. and Parsons, L.M. 2020, Hargett, A.A. and Renfrow, M.B. 2019). Next, glycosyltransferases can process the O-linked glycan generating the eight types of cores (cores 1 to 8) (as shown in Supplementary Figure 1C). The core types

1 to 4 are the most common in mammalian systems. During the passage through the Golgi apparatus, these structures are further altered, which leads to the diversification of mucin-type O-linked glycans (Hargett, A.A. and Renfrow, M.B. 2019, Watanabe, Y., Bowden, T.A., et al. 2019).

3. Glycosylation profile of the Spike protein from SARS-CoV-2

Glycoproteins of SARS-CoV-2 are involved in cell adhesion and invasion, morphogenesis and modulation immune response processes (Fung, T.S. and Liu, D.X. 2018, Issa, E., Merhi, G., et al. 2020, Walls, A.C., Park, Y.J., et al. 2020). Although other SARS-CoV-2 proteins have predictable glycosylation sites (such as M-protein, E-protein), the majority of experimental data currently available is based on the S-protein (Supplementary Figure 1D) (Andersen, K.G., Rambaut, A., et al. 2020, Watanabe, Y., Allen, J.D., et al. 2020). This is a trimeric protein that mediates the viral adhesion through binding to the human angiotensin-converting enzyme 2 (hACE2) and also interacts with the host immune defense (Ou, X., Liu, Y., et al. 2020, Vankadari, N. and Wilce, J.A. 2020, Walls, A.C., Park, Y.J., et al. 2020).

The S-protein from SARS-CoV-2 has two functional subunits (S1 and S2) with 22 and 3 potential sites for N-glycosylation and O-glycosylation, respectively (Andersen, K.G., Rambaut, A., et al. 2020, Watanabe, Y., Allen, J.D., et al. 2020). Despite the similarity with SARS-CoV spike (approximately 87.2%), some differences in the glycosylation sites repertoire and known epitopes have been reported for SARS-CoV-2 spike (Kumar, S., Maurya, V.K., et al. 2020, Vashi, Y., Jagrit, V., et al. 2020). For instance, it exhibits an unusual cleavage site for the furin protease between the S1/S2 subunits, which is not observed in SARS-CoV. The N-linked glycans were recently mapped by cryoelectron microscopy in 16

amino acid residues of the S-protein expressed in HEK293F cells (from human embryonic kidney) (Walls, A.C., Park, Y.J., et al. 2020).

More details on N-glycosylation profiles of the 22 sites were unraveled using recombinant proteins expressed in HEK293F cells. The oligomannose-type glycans were predominant in two sites (N234 and N709). Complex-type glycans were predominantly exhibited in 14 amino acid residues (N17, N74, N149, N165, N282, N331, N343, N616, N657, N1098, N1134, N1158, N1173, N1194); while six sites showed a mixture of oligomannose- and complex-type glycans (N61, N122, N603, N717, N801 and N1074). The most common configuration of oligomannose-type glycans was $Man_5GlcNAc_2$. Afucosylated and fucosylated hybrid-type glycans were detected in at least 9 sites. The authors highlighted that the glycosylation profile of the SARS-CoV-2 S-protein was different from those observed for host glycoproteins or for other enveloped viruses (Watanabe, Y., Allen, J.D., et al. 2020).

Other experimental study evaluated the N-glycosylation and O-glycosylation of spike protein subunits also using HEK293-based expression system. The authors have solved the structures of N-linked glycans in 17 predicted sites and reported the presence of three classes of N-glycans. Importantly, this study revealed O-glycosylation modifications on two residues (Thr323 and Ser325) present in the receptor binding domain (RBD) of the S1 subunit (Shajahan, A., Supekar, N.T., et al. 2020). Recently, the characterization of the glycosylation profile of the S-protein expressed in BTI-Tn-5B1-4 insect cells was reported showing the presence of high-mannose N-glycans in all 22 predicted sites. Interestingly, these glycans cover almost all of the RBD area (Zhou, D., Tian, X., et al. 2020).

4. Lectins can target envelope glycoproteins and inhibit the adhesion of coronavirus to host cell receptors.

As previously mentioned, the S-protein of SARS-CoV-2 has a crucial role in viral adhesion by binding to hACE2. Therefore, the disruption of this interaction is considered an attractive target for antiviral therapy (Batlle, D., Wysocki, J., et al. 2020, Ou, X., Liu, Y., et al. 2020). Some non-mammalian derived lectins (from plants and bacteria) are pointed as alternative antiviral agents against enveloped viruses due to their ability to recognize the glycans present in the structural proteins and to impair the initial steps of viral pathogenesis (Mitchell, C.A., Ramessar, K., et al. 2017) (Supplementary Figure 2A and 2B).

Given the recent emergence of SARS-CoV-2, only the lectin isolated from *Lablab purpureus* (FRIL) has been reported so far as an antiviral agent against this virus. FRIL (Flt3 Receptor Interacting Lectin) is a glucose/mannose lectin also known as DLL-I. This protein completely inhibited the cytopathic effect of SARS-CoV-2 (strain hCoV-19/Taiwan/NTU04/2020) towards Vero cells at concentrations higher or equal than 6.25 µg/mL. The PRNT50 (50% plaque reduction neutralization test) and microneutralization EC50 (half maximal effective concentration) values for FRIL against hCoV-19/Taiwan/NTU04/2020 were 0.71 µg/mL and 0.80 µg/mL, respectively. This action was correlated with the ability of FRIL to bind to SARS-CoV-2 S-protein harboring complex-type N-glycans (as shown by ELISA-lectin assay). The authors also showed that FRIL treatment impairs the synthesis of SARS-CoV-2 N-protein and SARS-CoV-2 S-protein. Furthermore, FRIL has *in vitro* and *in vivo* action against influenza virus (Liu, Y.M., Shahed-Al-Mahmud, M., et al. 2020).

Besides these insights given by the anti-SARS-CoV-2 action of FRIL, other important lessons can be learned from other SARS-CoV and related viruses (as summarized in Table I). For instance, a study evaluated the *in vitro* antiviral activity of 33 plant lectins towards coronaviruses (SARS-CoV and feline infectious peritonitis virus). Mannose-binding agglutinins showed the highest anti-SARS-CoV effects. Among the studied lectins, the higher

selective indexes (SI) were found for the ones isolated from *Allium porrum* (APA; SI >222.2), *Morus nigra* (Morniga M II; SI >62.5) and *Epipactis helleborine* (EHA; SI >55.5). *Urtica dioica* (UDA) and *Nicotiana tabacum* agglutinins (NICTABA), both specific for GlcNAc, also showed promising activity (SI >76.9 and >58.8, respectively) (Keyaerts, E., Vijgen, L., et al. 2007). NICTABA and UDA have also shown inhibitory activity against other enveloped viruses including influenza A/B, Dengue virus type 2 (DENV-2), herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and human immunodeficiency viruses (HIV-1/2) (Gordts, S.C., Renders, M., et al. 2015).

The *in vivo* activity of UDA was reported in murine models of lethal SARS-CoV (Day, C.W., Baric, R., et al. 2009, Kumaki, Y., Wandersee, M.K., et al. 2011). Initially, the potential effects of UDA was associated to the reduction of IL-6 levels in the lungs (Day, C.W., Baric, R., et al. 2009). Following, the UDA mechanism of action was further detailed and revealed that UDA-treated mice exhibited fewer pathologic alterations in the lung and higher survival indexes, when compared to the placebo-treated group. Further, the authors showed that this lectin targets the viral invasion by binding to the S-protein, since the UDA protective effects were impaired in the presence of GlcNAc (Kumaki, Y., Wandersee, M.K., et al. 2011).

Other plant lectins have been shown to exhibit inhibitory action towards other coronaviruses. Examples include the following mannose-binding lectins: Concanavalin A (Con A), *Hippeastrum hybrid* agglutinin (HHA), *Galanthus nivalis agglutinin* (GNA or GNL) (Greig, A.S. and Bouillant, A.M. 1977, Hsieh, L.E., Lin, C.N., et al. 2010, Nguyen, T.D., Bottreau, E., et al. 1987, van der Meer, F.J., de Haan, C.A., et al. 2007a). One of these studies highlighted the importance of glycosylation in the sensibility of two types of coronaviruses (mouse hepatitis virus and feline infectious peritonitis virus) towards GNA, UDA and HHA. In this case, the inhibition of host mannosidases resulted in the improved antiviral activity of

the mannose- and GlcNAc-binding lectins (van der Meer, F.J., de Haan, C.A., et al. 2007b). GNA was also recently reported as an inhibitor of cell entry mechanism of influenza A H3N2 (Thompson, A.J., Cao, L., et al. 2020).

Non-plant derived agglutinins are also pointed as promising agents against coronaviruses, e.g. the mannose-binding-lectins cyanovirin-N (from cyanobacterium *Nostoc ellipsosporum*) and griffithsin (from red marine alga *Griffithsia* sp.) (O'Keefe, B.R., Giomarelli, B., et al. 2010, van der Meer, F.J., de Haan, C.A., et al. 2007a). However, only griffithsin (GRFT) has been evaluated against SARS-CoV and MERS-CoV. This protein binds to multiple sites of SARS-CoV and MERS-Cov glycoproteins with high affinity and inhibits viral entry (Millet, J.K., Seron, K., et al. 2016, O'Keefe, B.R., Giomarelli, B., et al. 2010). Additionally, this lectin also reduced the mortality and the severity of lethal pulmonary infection induced by SARS-CoV in mice. This effect is associated with the decrease of pro-inflammatory cytokines in infected lung tissue (O'Keefe, B.R., Giomarelli, B., et al. 2010).

Despite the potential antiviral activity of the aforementioned lectins, some limitations should be considered regarding their therapeutic utility. One practical issue is the rapid degradation of these macromolecules, imposing the need for multiple doses to maintain a therapeutic level. This obstacle could be addressed by the incorporation of the protein in formulations that promote controlled released and protection from denaturing agents (da Cunha, C.R., da Silva, L.C., et al. 2016, Tyo, K.M., Lasnik, A.B., et al. 2020, Yang, H., Li, J., et al. 2019).

Other relevant concern is the recognition of glycoconjugates present in host cells, since they could share same structural patterns observed in viral glycoproteins due their synthesis by host machinery (Cipollo, J.F. and Parsons, L.M. 2020). The action of lectins on these unwanted targets could induce hemagglutination, intravascular agglutination of cells, cell proliferation and impaired immune responses. Even the positive results from animal

models need to be examined with the appropriated caution, given the clear differences among the *in vivo* model species (most notably the mouse adapted strains) and the original viruses.

5. Strategies of protein engineering to improve the therapeutic application of lectins

The mitogenicity and pro-inflammatory properties of lectins (Carvalho, E., Oliveira, W.F., et al. 2018, Jandu, J.J.B., Moraes Neto, R.N., et al. 2017) raise several questions regarding their value to treat clinical conditions with severe inflammatory components, as seen in COVID-19 (Ye, Q., Wang, B., et al. 2020). Several attempts to overcome these issues have been performed, including protein engineering techniques. In the following sub-sections, we present the results obtained with Griffithsin (GRFT) and Cyanovirin-N that showed action against coronaviruses (O'Keefe, B.R., Giomarelli, B., et al. 2010, van der Meer, F.J., de Haan, C.A., et al. 2007a). The data obtained with the derivative agents from BanLec (from banana) and Microvirin (isolated from the cyanobacterium *Mycrocystis aeruginosa*) towards other enveloped viruses are also discussed (Shahid, M., Qadir, A., et al. 2020, Swanson, M.D., Boudreaux, D.M., et al. 2015). These studies are summarized in table II. The broad-spectrum activity of these agents and the techniques used in their design should be considered in the search for anti-infective compounds towards SARS-CoV-2.

5.1. Griffithsin derivatives

GRFT is a jacalin-related lectin found as domain-swapped dimers with six identical carbohydrate-binding sites (three in each monomer) (Lee, C. 2019, Ziolkowska, N.E., O'Keefe, B.R., et al. 2006). The dimerization promotes a multivalent interaction with mannose residues in the envelope glycoproteins and are essential for its antiviral action (Moulaei, T., Shenoy, S.R., et al. 2010). It has shown *in vitro* and *in vivo* action against

MERS-CoV, SARS-CoV and other coronaviruses, which make it a promising molecule for SARS-Cov-2 treatment (Lee, C. 2019, Mani, J.S., Johnson, J.B., et al. 2020).

The monomeric forms of GRFT (mGRFT) were engineered in tandem repetitions leading to obtention of new proteins with two, three or four copies, designed as mGRFT tandemers. Cell-based assays showed that 3mGRFT and 4mGRFT tandemers exhibited higher anti-viral activity against HIV when compared to 2mGRGTs and native GRFT. The linker between each unit of mGRFT allowed strong interaction of carbohydrate-binding domains with the oligosaccharides in the virion surfaces, without inducing viral aggregation (Moulaei, T., Alexandre, K.B., et al. 2015).

In another study, the antiviral action of oligomeric tandemers (2mGRFT and 3mGRFT) and native GRFT was assessed against Nipah virus (NiV; *Paramyxoviridae*), which is an emerging etiologic agent of respiratory diseases and fatal human encephalitis. The authors also evaluated the action a recombinant form with improved resistance to oxidation designated as Q-GRFT (Met78 → Gln). The 3mGRFT showed the highest anti-NiV effects among the tested GRFT versions, with more efficacy in preventing syncytia formation. Interestingly, the prophylactic intranasal administration of Q-GRFT induced more protection than 3mGRFT in a lethal model of intranasal infection using golden Syrian hamsters (Lo, M.K., Spengler, J.R., et al. 2020).

5.2. Cyanovirin-N derivatives

Cyanovirin-N is one of the lectins with activity against coronaviruses and others enveloped viruses (Barrientos, L.G., Matei, E., et al. 2006, Hu, B., Du, T., et al. 2015, Kachko, A., Loesgen, S., et al. 2013, Smee, D.F., Bailey, K.W., et al. 2008, van der Meer, F.J., de Haan, C.A., et al. 2007a). This protein has two domains and can be found as monomers in solution (with two sugar-binding sites) or domain-swapped dimers when

crystalized (with four sugar-binding sites). The dimerization was shown to restore the viral inhibition of mutants (Matei, E., Zheng, A., et al. 2010).

The antiviral activity of the designed cyanovirin-N oligomers (dimers, trimers and tetramers) was evaluated against HIV. The dimers showed higher anti-HIV efficacy than the parental protein and other forms. Importantly, the dimers exhibited neutralizing activity at comparable or even superior levels than antibodies (Keeffe, J.R., Gnanapragasam, P.N., et al. 2011). A similar approach was later reported based in the construction of "Nested" dimers of cyanovirin-N by rational design resulting in enhanced anti-HIV activity in comparison to the wild type protein (Woodrum, B.W., Maxwell, J., et al. 2016).

Other strategy was based on the addition of poly(ethylene glycol) (PEGylate) to reduce the immunogenicity and cytotoxicity of cyanovirin-N as well as to enhance its half-time in plasm (Chen, J., Huang, D., et al. 2014, Wu, C., Chen, W., et al. 2015, Zappe, H., Snell, M.E., et al. 2008). For instance, a recombinant form of cyanovirin-N with a flexible and hydrophilic linker (Gly4Ser)3 at the N-terminus (denominated LCVN) was produced and then PEGylated in its N-terminal α-amine resulting in the 10 K PEG-aldehyde (ALD)-LCVN. Both cyanovirin-N derivatives exhibited anti-HIV-1 activity with reduced cytotoxicity, however, the mono-PEGylated version still showed the best results (Chen, J., Huang, D., et al. 2014).

Later, a new form of mono-PEGylated cyanovirin-N (PEG20k-LCVN) was described as a potent anti-influenza A inhibitor. PEG20k-LCVN showed higher action than Ribavirin. The usefulness of PEG20k-LCVN was shown in infection models using mice and chicken embryos infected by influenza H3N2. The infected mice treated with PEG20k-LCVN showed improved life span associated with reduction of viral genome expression and attenuation of lung damage (Wu, C., Chen, W., et al. 2015).

The derivative PEG10k-LCV-N also inhibited normal and acyclovir-resistant strains of HSV-1, even though with less action than its precursors (wild type and linked-cyanovirin-

N). Despite of this, the reduction in cytotoxicity, antigenicity and mitogenicity observed for the pegylated version are important aspects that advocate for its therapeutic value (Lei, Y., Chen, W., et al. 2019). Overall, these results with mono-PEGylated derivatives represent alternative models to increase the usefulness of lectins in antiviral therapy.

5.3. Recombinant BanLec (H84T)

BanLec is a mannose-binding protein from the group of lectin related jacalin with tetrameric structure (two binding sites in each monomer) and remarkable anti-infective activity towards enveloped viruses (de Camargo, L.J., Picoli, T., et al. 2020, Hopper, J.T.S., Ambrose, S., et al. 2017, Meagher, J.L., Winter, H.C., et al. 2005, Swanson, M.D., Winter, H.C., et al. 2010). A single amino acid replacement at position 84 (histidine replacement by threonine) impaired the mitogenic action without altering its ability to bind to mannose residues present at viral glycoproteins (Swanson, M.D., Boudreaux, D.M., et al. 2015).

These positive effects were attributed to a reduction in the multivalent lectin-glycans interactions that are associated with the proliferative induction (Swanson, M.D., Boudreaux, D.M., et al. 2015). The engineered BanLec (H84T) has shown *in vitro* and *in vivo* antiviral activity against HIV, hepatitis C virus (HCV), Influenza and Ebola (Coves-Datson, E.M., Dyall, J., et al. 2019, Coves-Datson, E.M., King, S.R., et al. 2020, Swanson, M.D., Boudreaux, D.M., et al. 2015).

5.4. Microvirin derivatives

Microvirin (MVN) is a monomeric lectin that exhibits two domains (A and B) with 35% of sequence similarity and able to neutralize some viruses (HIV, HCV) due the binding to high high-mannose type N-glycan present in envelope proteins. Some *in vitro* data suggest that MVN induces lower toxicity and cell proliferation than cyanovirin-N (Huskens, D., Ferir,

G., et al. 2010, Kachko, A., Loesgen, S., et al. 2013, Min, Y.Q., Duan, X.C., et al. 2017, Shahzad-ul-Hussan, S., Gustchina, E., et al. 2011).

In order to improve the inhibitory action of MVN, some oligomeric versions of were designed and tested *in vitro* against HCV. The results showed that tri- and tetramers have higher ability to block the HCV invasion than dimers and monomers (Min, Y.Q., Duan, X.C., et al. 2017). Recently, a version of microvirin (LUMS1) was engineered to have two identical domains and, therefore, two carbohydrate-binding sites. Although LUMS1 showed lower sugar affinity than wild type, it was able to block HIV and HCV infectivity. Undoubtedly, the most advantageous characteristics of LUMS1 are its lower cytotoxicity and immunogenicity. These properties should be associated to the structural homogeneity conferred by the two identical domains (Shahid, M., Qadir, A., et al. 2020).

5.5. Chimeras derived from antiviral lectins

The production of chimeras using the cyanobacterium-derived lectins (cyanovirin-N or recombinant microvirin) is another interesting strategy that has been largely exploited for HIV therapy (Contarino, M., Bastian, A.R., et al. 2013, McFadden, K., Cocklin, S., et al. 2007, Parajuli, B., Acharya, K., et al. 2018, Parajuli, B., Acharya, K., et al. 2016). A chimera (L5) was formulated using cyanovirin-N and a linear peptide (12p1) aiming to target the envelope gp120 glycoprotein with synergistic action. For this purpose, 12p1 was fused to the C-terminal domain of Cyanovirin-N. The chimera L5 significantly inhibited the binding of HIV to the host cell receptor protein (McFadden, K., Cocklin, S., et al. 2007).

Other chimeras were constructed with the fusion of cyanobacterial lectins (Cyanovirin-N or microvirin) and peptides derived from the membrane-proximal external region (MPER) of HIV-1 gp41 glycoprotein (Ang, C.G., Hossain, M.A., et al. 2020,

Contarino, M., Bastian, A.R., et al. 2013, Parajuli, B., Acharya, K., et al. 2018, Parajuli, B., Acharya, K., et al. 2016).

The chimeras were intended to inhibit viral binding to its receptor (through lectin portion) and to induce viral inactivation and were called as DAVEI (dual-acting virucidal entry inhibitor) (Contarino, M., Bastian, A.R., et al. 2013). The experimental data showed that the chimeras promoted simultaneous engagement of gp120 and gp41 domains and lead to the irreversibly lytic inactivation of HIV. However, the mechanism involved in the DAVEI action is not completely understood (Parajuli, B., Acharya, K., et al. 2018, Parajuli, B., Acharya, K., et al. 2016).

The microvirin-based DAVEI is considered a second generation chimera due its lower mitogenicity in comparison to Cyanovirin-N. The authors further improved this characteristic by designing a version of microvirin (MVN*) with two mutations (Q81K and M83R), which resulted in higher affinity to mannose residues (Huskens, D., Ferir, G., et al. 2010, Parajuli, B., Acharya, K., et al. 2018). MVN*-DAVEI was able to induce disruption of any membrane containing the HIV Env glycoprotein (Ang, C.G., Hossain, M.A., et al. 2020).

5.6. Lectibodies

Active lectins could be also fused with broadly neutralizing antibodies (bNAbs) to develop potent bispecific antiviral agents. The idea of ‘lectibody’ was experimentally applied for anti-HIV therapy using the Avaren recombinant protein. Avarin is derived from an antiviral lectin isolated from the actinomycete *Longispora albida* K97-0003T known as Actinohivin (Hamorsky, K.T., Kouokam, J.C., et al. 2019, Hoorelbeke, B., Huskens, D., et al. 2010, Seber Kasinger, L.E., Dent, M.W., et al. 2019). Avaren is expressed in the plant *Nicotiana benthamiana* and it was designed to have improved solubility and pharmacological activity than its parental lectin. These enhanced features were achieved by modifying 17

amino acid residues and by introducing a disulfide bond in the sugar-binding sites of domains 1 and 3 (the disulfide bond is found in the domain 2 of actinohivin) (Hamorsky, K.T., Kouokam, J.C., et al. 2019).

In the first report, Avaren was fused to the antigen-binding fragment (Fab) of the CD4 binding site-specific VRC01 bNAb, which resulted in the $VRC01_{Fab}$-Avaren lectibody. In this case, $VRC01_{Fab}$-Avaren was designed to: i. target the mannose residues present in the HIV-1 gp120 glycoprotein; and ii. the binding site of gp120 on the receptor of TCD4 lymphocytes (through Avaren and VRC01, respectively). Using cell-based assays, the authors proved the bispecific neutralization of HIV by $VRC01_{Fab}$-Avaren and showed that the lectibody had stronger activity than the isolated agents (Seber Kasinger, L.E., Dent, M.W., et al. 2019).

The second lectibody was generated by fusion of Avaren and the crystallizable region fragment (Fc) of the human immunoglobulin G1 (Fc IgG1). It was called AvFc and showed higher affinity (approximately 10-folds) to gp120 than actinohivin. AvFc also showed higher *in vitro* activity towards several strains of HIV-1 and HIV-2 than its wild-type counterpart. Employing biochemical assays, the authors showed the bispecific function of AvFc: (i) the treatment with mannosidases impaired its antiviral activity; (ii) a mutant of AvFc with reduced affinity to Fcγ receptors also showed lower action than AvFc. This lectibody did not induce toxic effects to human and mice peripheral blood mononuclear cells (PBMC). Finally, AvFc was effective in an infection model of simian immunodeficiency virus (SIV) in rhesus macaques (Hamorsky, K.T., Kouokam, J.C., et al. 2019).

In general, the advances obtained in these studies with engineered lectins provide several lessons that could be applied in the development of therapeutic strategies against COVID-19. However, the possible adverse effects discussed above due to systemic administration of carbohydrate-binding agents need to be carefully assessed for each type of infection. In the context of COVID-19, the intranasal administration of these agents seems to

be an attractive option. However, some aspects are also important to be examined, including the tissue distribution of these macromolecules (associated with their size), their influence in tissue metabolism, engagement of cells receptors and induction of local inflammation (especially in the lower respiratory tract).

6. Lectins can be used as adjuvant for vaccines.

The implementation of global community immunity using vaccines is believed to be the best and safest strategy for management and eradication of SARS-CoV-2 (Corey, L., Mascola, J.R., et al. 2020). Since the beginning of this epidemic, several academic and industrial groups have made considerable efforts to design effective vaccines (Cohen, J. 2020). According to vaccine tracking data from the Milken Institute, as of 18 July 2020, a total of 198 projects are in development for COVID-19, of which 18 candidates are already in the clinical phase and the others are in pre-clinical or exploratory phases.

The vaccines are distributed in the following categories: (i) Live attenuated virus: 4; (ii) Inactivated virus: 13; (iii) RNA-based: 26; (iv) DNA-based: 16; (v) Protein subunit: 63; (vi) Non-replicating viral vector: 23; (vii) Replicating the viral vector: 18; (ix) virus-like particles: 14; (x) Replicating bacterial vector: 1; (xi) unknown category (those that not fail in the groups or the details are not described): 20. There are at least 3 vaccines candidates in phase III clinical trials: AZD1222 (formally known as ChAdOx1 nCoV-19), an adenovirus-vectored vaccine encoding the spike protein of SARS-CoV-2 which is developed by the University of Oxford in partnership with AstraZeneca (British pharmaceutical company); Coronavac, a vaccine using inactivated virus from Sinovac Biotech (Chinese biotechnology company); mRNA-1273, a mRNA-based vaccine encoding the spike protein of SARS-CoV-2 which is manufactured by Moderna Therapeutics (American Company) (Jackson, L.A., Anderson, E.J., et al. 2020, van Doremalen, N., Lambe, T., et al. 2020).

The majority of these initiatives are focused on the development of gene-based vaccines (including viral- or nucleic acid–vectors that encode protein antigens that are produced by host cells) and protein-based vaccines (using recombinant or synthetic antigenic proteins or subdomains, or viral proteins assembled as virus-like particles) (Gao, Q., Bao, L., et al. 2020, Peeples, L. 2020). The biggest challenge with these types of vaccines is the induction of long-lasting immunity, which is usually lower than the traditional approaches using live or inactivated organisms (Shi, S., Zhu, H., et al. 2019, Vetter, V., Denizer, G., et al. 2018).

In this scenario, the employment of powerful adjuvants is important to improve immunization efficacy. These compounds need to enhance the immune response induced by the vaccine, while keeping the equilibrium between humoral and cellular immune responses (Del Giudice, G., Rappuoli, R., et al. 2018, Shi, S., Zhu, H., et al. 2019). Noteworthy to mention that even the proper induction of Th1-biased response, which is important for protection against viral infections, still remains as a limitation for some adjuvants (Golos, A. and Lutynska, A. 2015).

Lectins are well-known to promote proliferation of lymphocytes and modulate the release of effector molecules (cytokines and nitric oxide) by immune cells. For instance, several lectins are reported as potent inducers of IL-12 and IFN-γ production, which are key cytokines in the establishment of Th1 axis (da Silva, L.C., Alves, N.M., et al. 2015, de Souza Feitosa Lima, I.M., Zagmignan, A., et al. 2019, Oliveira Brito, P.K.M., Goncalves, T.E., et al. 2017, Ruterbusch, M., Pruner, K.B., et al. 2020). Some lectins can also bind to Toll-like receptors and/or increase their expression levels, which may also modulate the release of pro-inflammatory cytokines and increase the receptor's ability to recognize the pathogens (Batista, J., Ralph, M.T., et al. 2017, da Silva, L.C. and Correia, M.T. 2014, Ricci-Azevedo, R., Roque-Barreira, M.C., et al. 2017).

In fact, the enhancement of Th1-biased immune response is important for protective immunity against viruses and other intracellular pathogens, due to the activation of cytotoxic cells (natural killer cells and TCD8 lymphocytes) and production of neutralizing antibodies involved in immunological memory (Ruterbusch, M., Pruner, K.B., et al. 2020). In this sense, the immunomodulatory properties of lectins make them attractive candidates for vaccine adjuvants. This concept has been experimentally proven against different types of viral pathogens, including enveloped viruses such as hepatitis B virus, herpesvirus and influenza (Gao, W., Sun, Y., et al. 2013, Lavelle, E.C., Grant, G., et al. 2002, Reyna-Margarita, H.R., Irais, C.M., et al. 2019, Song, S.K., Moldoveanu, Z., et al. 2007) (Table III). In this section, some studies applying lectin as adjuvant for influenza vaccines are discussed.

The Korean Mistletoe Lectin C (KML-C) isolated from *Viscum album* Coloratum completely protected mice against this H1N1 influenza when the lectin was intranasally administrated along with inactivated virus. KML-C significantly increased the levels of anti-influenza antibodies (IgG and IgA), and the population of influenza-specific lymphocyte in spleens and in mediastinal lymph nodes. Moreover, the mice immunized with KML-C and inactivated H1N1 also exhibited partial protection towards H3N2 challenge (Song, S.K., Moldoveanu, Z., et al. 2007). Other interesting effect of KLM-C is the increase of perforin expression, an important cytotoxic protein secreted by natural killer cells and TCD8 lymphocytes (Kim, Y., Kim, I., et al. 2018).

The AAL molecule isolated from *Agrocybe aegerita* (mushroom) is another example of lectin with adjuvant properties for influenza immunization. In this study, mice were immunized by subcutaneous injection of inactivated H9N2 strain and AAL. The animals that received AAL and inactivated H9N2 exhibited higher levels of anti-influenza IgG1 and IgG2a than those treated with inactivated virus alone. By employing a recombinant-ALL with mutation in the carbohydrate-binding domain, the authors also showed that the adjuvant

properties of AAL were mediated by the recognition of glycoproteins in the viral surface (Ma, L.B., Xu, B.Y., et al. 2017). Transcriptome analysis revealed that the adjuvant effects of AAL were associated with increased expression of genes related to leukocyte migration and lymphocyte activation (Ma, L.B., Xu, B.Y., et al. 2018).

Although these studies show the lectins as promisor adjuvant candidates, the concerns regarding their administration listed before (agglutination of host cells, induction of undesired inflammation and proliferation) need to be considered. Specifically, long-term immunological and toxicological evaluations are required to prove the usefulness of an adjuvant compound (Shah, R.R., Hassett, K.J., et al. 2017). The potential induction of severe systemic inflammation by lectins is also a possibility to be evaluated in the early stage of the research. Regarding the application of lectins as adjuvants for viral vaccines (including those against SARS-CoV-2), one important challenge is to choose of a combination (vaccine/lectin) able to concomitantly promote an effective adaptive immune response with clinical safety.

7. Lectin-based devices for identification of glycosylation changes in viral and host proteins

The incorporation of glycans in envelope proteins has great influence in viral adhesion and recognition by the immune system, since epitopes of neutralizing antibodies are often protected by glycan molecules (Vankadari, N. and Wilce, J.A. 2020, Watanabe, Y., Bowden, T.A., et al. 2019). The differential patterns of glycosylation in a viral protein can be elucidated by lectin-based devices, e.g. lectin-microarrays and lectin-ELISA assays. This information is essential to expand our understanding about the role played by glycan shields in viral pathogenesis (Guo, Y., Yu, H., et al. 2018, Hiono, T., Matsuda, A., et al. 2019, Thompson, A.J., Cao, L., et al. 2020, Wagatsuma, T., Kuno, A., et al. 2018).

The comprehension of glycosylation of host proteins is other hot topic to be considered in the context of infectious diseases (Goncalves, B.S., Horta, M.A.P., et al. 2019, Irvine, E.B. and Alter, G. 2020, Kaplan, B.S. and Vincent, A.L. 2020). The dynamic of glycosylation has been evaluated for a plethora of viral diseases (Dalal, K., Dalal, B., et al. 2019, Major, M. and Law, M. 2019, Qin, X., Guo, Y., et al. 2017, Qin, Y., Zhong, Y., et al. 2016). In some cases, specific differential expression of these glycoconjugates is correlated with the severity degree of some conditions (Luna, D.M., Oliveira, M.D., et al. 2014, Yeh, M.L., Huang, C.F., et al. 2019). These experimental approaches are important to discovery and clinical validation of glycoconjugates as biomarkers for diagnosis.

The possible correlation among alterations in serum glycoproteins expression (and/or in their glycosylation patterns) and the clinical presentations of COVID-19 is another question that could be addressed by employing lectin-based tools such as lectin-affinity chromatography, lectin-microarrays and lectin-immunoassays (Lectin-ELISA). So far, some studies have associated changes in the serum constituents of patients with COVID-19 and disease severity. For example, serum levels of IL-6 and C-reactive protein had a significant correlation with the severity of the disease while the procalcitonin (a glycoprotein) may be a predictor of poor/good prognosis (Liu, F., Li, L., et al. 2020).

8. Lectin-based biosensors

The development of fast and sensitive methods for pathogens detection is essential to promote the effective treatment and to prevent their dissemination (Liang, K.H., Chang, T.J., et al. 2020, Qiu, G., Gai, Z., et al. 2020, Seo, G., Lee, G., et al. 2020). The techniques currently used for viral diagnosis comprise immunochromatography-based assays, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR and derivative assays). However, especially in the context of COVID-19, some serological tests do not

exhibit satisfactory accuracy; while the molecular assays require expensive reagents and extensive sample preparation, which may cause a delay in results (Acquah, C., Danquah, M.K., et al. 2016, Cesewski, E. and Johnson, B.N. 2020).

Biosensors are analytical tools that have a bioreceptor element (such as a lectin), immobilized in a thin layer on the surface of the transducer. They are intended to provide specific and sensitive detection of an analyte in biological samples (de Oliveira, W.F., Dos Santos Silva, P.M., et al. 2019, Silva, M.L.S. 2018). A few biosensors have been proposed for SARS-CoV-2 diagnosis targeting S protein (Seo, G., Lee, G., et al. 2020) or nucleic acids (Qiu, G., Gai, Z., et al. 2020). In the following sub-sections, we discuss the construction of lectin-based biosensors targeting viral or host glycoproteins (Table IV).

8.1. Biosensors for viral glycoproteins

The intrinsic specificity of lectins towards glycans allows their use as an element of biorecognition of glycans present in viral proteins (Cesewski, E. and Johnson, B.N. 2020, Hong, S.A., Kwon, J., et al. 2015, Tung, Y.-T., Liang, J.-J., et al. 2019). In the case of SARS-CoV-2, its envelope glycoproteins could be also exploited for identification using lectin-based biosensors (as illustrated in Supplementary Figure 2C).

An electrochemical biosensor based on Con A-modified nanostructured gold electrodes was used to detect Norovirus (non-enveloped virus) in fecal samples using Cyclic voltammetry and impedance spectroscopy analyzes. This biosensor showed high specificity and did not exhibit cross-reaction with hepatitis A or E viruses (Hong, S.A., Kwon, J., et al. 2015). Another example of electrochemical biosensor was elaborated using cysteine, zinc oxide nanoparticles and Con A. The impedimetric response allowed the distinction of DENV-2, Zika virus, Chikungunya virus, and Yellow fever virus in serum samples (Simao, E.P., Silva, D.B.S., et al. 2020).

8.2. Biosensors for detection of glycan patterns in infected individuals

Some studies have suggested that glycoproteins are differentially expressed in the serum of subjects affected by acute and chronic viral infections (such as dengue and hepatitis), making the profile of serum glycoproteins an emerging target for diagnosis (Luna, D.M., Oliveira, M.D., et al. 2014, Yeh, M.L., Huang, C.F., et al. 2019). In this scenario, biosensors incorporated with lectins could provide a fast identification of people infected with SARS-CoV-2 based on the profile of glycoproteins in biological fluids. This approach could be extremely interesting if SARS-CoV-2-infected subjects exhibited a differential glycosylation profile that those individuals affected by other respiratory pathogens.

Two types of Con A-based biosensors have been proposed for Dengue diagnosis using serum glycoproteins. In the first, Con A was immobilized on gold electrode using polyvinyl butyral to construct a biosensor capable to identify different glycoprotein patterns in the sera of patients with dengue fever and dengue hemorrhagic fever. The distinction was based on changes in charge transfer resistance using electrochemical impedance spectroscopy (Oliveira, M.D., Correia, M.T., et al. 2009). The other system was designed using Con A and lipid membranes for differentiation of glycoproteins from serum of individuals infected with DENV-1, DENV-2 and DENV-3. The differentiation of the pattern of serum glycoproteins from subjects infected by each serotype was based in impedimetric analysis, with higher response to DENV-3 infection (Luna, D.M., Oliveira, M.D., et al. 2014).

Cramoll, a glucose/mannose-binding lectin extracted from *Cratylia mollis* seeds, is another lectin used in biosensors for Dengue diagnosis. It was used in two types of biosensors employing: (i) gold electrodes modified with Fe_3O_4 nanoparticles and polyvinyl butyral (Oliveira, M.D., Nogueira, M.L., et al. 2011); (ii) gold nanoparticles/polyaniline electrodes (Avelino, K., Andrade, C., et al. 2014). Similarly, the galactose-specific lectin isolated from

Bauhinia monandra leaves (BmoLL) was immobilized on gold nanoparticles-polyaniline hybrid composite (Andrade, C.A., Oliveira, M.D., et al. 2011). All these lectins-derived sensors were effective in correlating the glycosylation patterns of serum proteins with the type of infection.

These lectin-based biosensors could represent a more cost-effective alternative when compared to those that currently use antibodies and nucleic acids (Luna, D.M., Oliveira, M.D., et al. 2014). However, their sensitivity and specificity could be a limitation. In particular, the binding to glycans from other sources (host or other pathogens) could induce false results. Thus, these parameters need to be effectively optimized prior their use in clinical analysis.

9. Lectin affinity plasmapheresis for extracorporeal viral elimination

Plasmapheresis is the extracorporeal purification of pathogens and other products (toxins, cytokines) from blood. This could be performed in association with dialysis as a resource to reduce SARS-CoV-2 levels in the patient's blood (Turgutkaya, A., Yavasoglu, I., et al. 2020). Indeed, this technique has been used to remove SARS-CoV-2 from convalescent plasma transfusions (Li, L., Yang, R., et al. 2020) and has been considered as an alternative treatment considered for COVID-19 due the absence of vaccine or specific medication for SARS-CoV-2 (Brown, B.L. and McCullough, J. 2020, Duan, K., Liu, B., et al. 2020).

The ability of GNA to bind mannose-containing glycans has been used to modify the plasmapheresis apparatus (Hemopurifier® cartridges) (Supplementary Figure 2D). The system is designed as a lectin affinity plasmapheresis (LAP) and has been employed in *in vitro* and clinical evaluations of viral disease. For instance, GNA-modified hemopurifier removed HCV from blood of HCV-infected patients. *In vivo* analysis showed that the association of this

system with dialysis was effective in reducing HCV load in the blood (Tullis, R.H., Duffin, R.P., et al. 2009).

Later, other study showed that the continuous blood purification using LAP resulted in undetectable viral load in HCV-positive patients. This therapy enhanced the effectiveness of standard drugs without plasma losses (Tullis, R.H., Duffin, R.P., et al. 2010). A GNA-based system was also successfully applied for extracorporeal removal of Ebola virus in a patient (Buttner, S., Koch, B., et al. 2014). In addition to these cases, *in vitro* analysis with LAP devices cleared MERS-CoV and Marburg virus as well as their respective soluble glycoproteins (Koch, B., Schult-Dietrich, P., et al. 2018).

Although the number of tested patients in these studies is rather limited, these results with LAP shall be considered for future applications of lectins in clinical settings (Table IV). Further evaluation of fluid composition after the procedures is performed needs to be conducted to rule out the possibility of removal of other important plasmatic proteins, in special neutralizing antibodies. The efficiency of these devices should be compared with other assays of pathogen inactivation.

10. Conclusions and perspectives

The presence of glycoproteins in the viral envelope opens a wide range of possibilities for application of lectins to solve different types of problems involved with viral infections such as COVID-19. In the therapeutic area, the lectins could be considered leading molecules for the development of new antiviral approaches due to their ability to inhibit viral entry in the host cell. The advances in protein design strategies are important to boost the clinical application of these agents considered for treatment of SARS-CoV-2 and other viral infections. The immunomodulatory action of some lectins can also be exploited to improve the effectiveness of immunization schemes for viral infections.

On the other hand, lectin-carbohydrates interactions can be used to design devices for diagnosis targeting viral glycoproteins or host glycoproteins alterations during viral infections. These apparatuses hold the promise to provide fast, sensitive and cost-effective identification of infected individuals and are of vital need during pandemic situations, as this imposed for COVID-19. Finally, the lectin affinity plasmapheresis is an interesting resource for blood purification and to help the implementation of convalescent plasm therapy.

The major limitation for these diverse applications is the possible binding of this lectin in unwanted glycosylated targets. For instance, the administration of lectins could result in agglutination and proliferation of cells, besides the exacerbation of immune response. Similarly, the lectin-based biosensors could have low specificity due the signals from other glycans. Several interesting strategies have been applied to overcome these issues.

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Legends to Tables

Table I: Antiviral activity of some lectins against SARS-CoV, MERS-CoV and other types of coronavirus.

Table II: Some examples of engineered proteins derived from antiviral lectins.

Table III: Examples of lectins used as adjuvant for antiviral vaccines.

Table IV: Examples of lectins used for development of biosensor and Lectin affinity plasmapheresis.

Tables

Table I

Lectin	Source	Sugar affinity	Anti-SARS-CoV-2 action	Anti-SARS-CoV action	Anti-MERS-CoV action	References
FRIL	*Lablab purpureus*	Man/Glu	*In vitro*	-	-	Liu and Shahed-Al-Mahmud et al. (2020)
APA	*Allium porrum*	Man	-	*In vitro*	-	Keyaerts and Vijgen et al. (2007)
Morniga M II	*Morus nigra*	Man	-	*In vitro*	-	Keyaerts and Vijgen et al. (2007)
EHA	*Epipactis helleborine*	Man	-	*In vitro*	-	Keyaerts and Vijgen et al. (2007)
UDA	*Urtica dioica*	GlcNAc	-	*In vivo* and *in vitro*	-	Keyaerts and Vijgen et al. (2007); Day and Baric et al. (2009); Kumaki, Y., Wandersee, M.K., et al. (2011)
NICTABA	*Nicotiana tabacum*	GlcNAc	-	*In vitro*	-	Keyaerts and Vijgen et al. (2007)
Con A	*Canavalia ensiformis*	Man/Glu	-	-	-	Greig and Bouillant (1977); Nguyen Bottreau et al. (1987)
HHA	*Hippeastrum hybrid*	Man	-	*In vitro*	-	van der Meer, de Haan et al. (2007a); van der Meer, de Haan et al. (2007b)
GNA (or GNL)	*Galanthus nivalis*	Man	-	*In vitro*	-	Hsieh, Lin et al. (2010)
Cyanovirin-N	*Nostoc ellipsosporum*	Man	-	-	-	van der Meer, de Haan et al. (2007a)

Griffithsin	*Griffithsia* sp.	Man	-	*In vivo* and *in vitro*	*In vitro*	Millet, Seron et al. (2016), O'Keefe, Giomarelli et al. (2010)

Man: mannose; GlcNAc: N-acetylglucosamine; Glu: glucose.

Table II

Native Lectin	Source	Sugar affinity	Type(s) of derivative(s)	Action	References
Griffithsin	*Griffithsia* sp.	Man	Monomeric and (mGRFT) Oligomeric tandemer forms (2mGRFT, 3mGRFT, 4mGRFT)	3mGRFT and 4mGRFT exhibited improved *in vitro* anti-HIV action.	Moulaei, Alexandre et al. (2015)
				3mGRFT showed improved action towards NiV	Lo, Spengler et al. (2020)
			Q-GRFT	Q-GRFT has higher resistance to oxidation and *in vivo* anti-NiV action.	Lo, Spengler et al. (2020)
			Oligomers (dimers, trimers and tetramers)	The dimers showed highest anti-HIV efficacy.	Keeffe, Gnanapragasam et al. (2011); Woodrum, Maxwell et al. (2016)
Cyanovirin-N	*Nostoc ellipsosporum*	Man	PEGylated versions	10 K PEG-ALD-LCVN has improved anti-HIV and anti-HSV-1 actions.	Chen, Huang et al. (2014); Lei, Chen et al. (2019)
				PEG20k-LCVN has improved anti-influenza H3N2 action.	Wu, Chen et al. (2015)
			Chimera L5 (cyanovirin-N and 12p1)	*In vitro* inhibition of HIV.	McFadden, Cocklin et al. (2007)
			DAVEI: Chimeric Cyanovirin-MPER formulation	*In vitro* dual inhibition of HIV.	Contarino, Bastia et al. (2013); Parajuli, Acharya et al. (2018); Parajuli, Acharya et al. (2016).
BanLec	*Musa* sp.	Man	Recombinant BanLec	The mutation impaired the mitogenic	Coves-Datson, Dyall et al.

			(H84T)	activity. rBanLec showed antiviral action against HIV, HCV, Influenza and Ebola.	(2019); Coves-Datson, King, S.R. (2020), Swanson, Boudreaux et al. (2015).
			Oligomers (dimers, trimers and tetramers)	Trimers and tetramers exhibited improved *in vitro* anti-HCV action.	Min, Duan et al. (2017)
			LUMS1 with two identical structural domains.	Although LUMS1 has lower cytotoxicity and immunogenicity than native microvirin, it showed lower anti-HIV and anti-HCV actions.	Shahid, Qadir et al. (2020)
Microvirin	*Mycrocystis aeruginosa*	Man	MVN*	MVN* has two mutations (Q81K and M83R) that resulted in higher affinity to mannose residues and lower mitogenicity.	Ang, Hossain, et al. (2020)
			MVN*-DAVEI (second generation): fusion of MPER with MVN*	*In vitro* dual inhibition of HIV.	Parajuli, Acharya et al. (2018); Ang, Hossain et al. (2020)
			Avaren	It has improved solubility and pharmacological activity.	Hamorsky, Kouokam, et al. (2019).
Actinohivin	*Longispora albida*	Man	Lectibody: $VRC01_{Fab}$-Avaren	*In vitro* neutralization of HIV.	Seber Kasinger, Dent et al. (2019).
			Lectibody: AvFc	*In vitro* neutralization of HIV. Inhibition of SIV infection in rhesus macaques.	Hamorsky, Kouokam et al. (2019).

Man: mannose; NiV: Nipah virus; HIV: human immunodeficiency virus; HSV-1; herpes simplex virus; HCV: hepatitis C virus; SIV: simian immunodeficiency virus; $VRC01_{Fab}$: antigen-binding fragment (Fab) of the CD4 binding site-specific bNAb VRC01; AvFc: fusion of Avaren and human the fragment crystallizable region (Fc) of the human immunoglobulin G1 (Fc IgG1). MPER: membrane-proximal external region (MPER) of HIV-1 gp41.

Table III

Lectin	Source	Sugar affinity	Administration	Type of vaccine	References
KML-C	*Viscum album* Coloratum (Korean Mistletoe)	Gal and GalNAc	Intranasal route in mice	Inactivated vaccine for influenza H1N1	Kim, Kim et al. (2018); Song, Moldoveanu et al. (2007).
AAL	*Agrocybe aegerita*	GlcNAc	Subcutaneous route in mice	Inactivated vaccine for influenza H9N2	Ma, Xu et al. (2017).
POL	*Pleurotus ostreatus*	GalNAc	Intramuscular route in mice	DNA vaccine for HBV	Gao, Sun et al. (2013)
MLI, MLII, MLIII	*Viscum album L.* (Eupopean Mistletoe)	Gal and GalNAc	Intranasal route in mice	Subunit vaccine (Glycoprotein D2) for HSV	Lavelle, Grant et al. 2002

GlcNAc: N-acetylglucosamine; GalNAc: N-acetylgalactosamine; Gal: Galactose; Man: mannose.

Table IV

Lectin	Source	Sugar affinity	Type of application	References
Con A	*Canavalia ensiformis*	Man/Glu	Biosensor for viral detection	Hong, Kwon et al. (2015); Simao, Silva et al. (2020).
			Biosensor for detection of serum glycoproteins	Luna, Oliveira et al. (2014); Oliveira, Correia et al. (2009).
Cramoll	*Cratylia mollis*	Man/Glu	Biosensor for detection of serum glycoproteins	Oliveira, Nogueira et al. (2011); Avelino, Andrade et al. (2014)
BmoLL	Bauhinia monandra	Gal	Biosensor for detection of serum glycoproteins	Andrade, Oliveira et al. (2011)
GNA (or GNL)	*Galanthus nivalis*	Man	Lectin affinity plasmapheresis	Koch, B., Schult-Dietrich, P., et al. (2018); Tullis, R.H., Duffin, R.P., et al. (2010)

Gal: Galactose; Glu: glucose; Man: mannose.