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# Leveraging coronavirus binding to gangliosides for innovative vaccine and therapeutic strategies against COVID-19

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Abbreviations: ACE-2, angiotensin converting enzyme-2; COVID-19, coronavirus disease 19; NTD, N-terminal domain; RBD, receptor binding domain; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, SARS-coronavirus-2.

## abstract

Covid-19 is an infectious respiratory disease due to a coronavirus named SARS-CoV-2. A critical step of the infection cycle is the binding of the virus spike S protein to the cellular ACE-2 receptor. This interaction involves a receptor binding domain (RBD) located at the center of the S trimer, whereas the lateral N-terminal domain (NTD) displays a flat ganglioside binding site that enables the virus to bind to lipid rafts of the plasma membrane, where the ACE-2 receptor resides. S protein binding to lipid rafts can be blocked by hydroxychloroquine, which binds to gangliosides, and by azithromycin, which binds to the NTD. Based on these data, we identified the NTD of SARS-CoV-2 as a promising target for both therapeutic and vaccine strategies, a notion later supported by the discovery, in convalescent Covid-19 patients, of a neutralizing antibody (4A8) that selectively binds to the NTD. The 4A8 epitope overlaps the ganglioside binding domain, denying any access of the virus to lipid rafts when the antibody is bound to the S protein. Thus, our data explain why antibody binding to the tip of the NTD results in SARS-CoV-2 neutralization. The high level of conservation of the ganglioside binding domain of SARS-CoV-2 (100% identity in 584 of 600 isolates analyzed worldwide) offers unique opportunities for innovative vaccine/therapeutic strategies.

## 1. Introduction

Covid-19 is a threatening infectious pandemic disease due to the novel coronavirus SARS-Cov-2 [1]. Considerable research efforts are currently engaged to find both prophylactic and therapeutic solutions to fight the virus. As other members of the coronavirus family, SARS-Cov-2 infects host target cells via a specific interaction with angiotensin converting enzyme 2 (ACE-2), a membrane bound protein with an ubiquitous expression in various tissues including the lung, heart, vessels, gut, kidney, and brain [2]. ACE-2 is recognized by the trimeric spike (S) viral protein which belongs to the viral envelope and protrudes outwards with a typical corona-like appearance [3,4]. The receptor binding domain (RBD), which is located at the center of the spike trimer interacts with a single ACE-2 receptor. This domain has received most attention from researchers and laboratories worldwide for therapeutic and vaccine strategies [3,5-7].

Despite the lack of a clear-cut consensual therapy for Covid-19 patients, several repositioned drugs, especially anti-malarial hydroxychloroquine and antibiotics (azithromycin), are currently used in several countries [8,9]. Retrospective studies cumulating thousands of Covid-19 patients in Belgium, France, Italy, and the USA gave remarkably converging evidences that hydroxychloroquine (often used in combination with azithromycin), significantly reduced mortality rates, especially when administered at early infection stages [10-13]. In vitro, hydroxychloroquine and azithromycin act synergistically to reduce SARS-Cov-2 infection [14], giving a rationale for the combination therapy used in Covid-19 patients. Since none of these drugs is primarily an antiviral compound, the mechanistic explanation of such synergy is puzzling. Recently, we used in silico methods to study the mode of action of hydroxychloroquine and azithromycin at the molecular level. We first identified a ganglioside binding domain at the tip of the N-terminal domain (NTD) of the spike protein, which may allow a functional interaction of the virus with lipid rafts of the plasma membrane independently of the RBD [15]. Then we showed that hydroxychloroquine has a remarkable affinity for sialic acids which are key structural determinants of glycoproteins and gangliosides [15]. Therefore, hydroxychloroquine can bind to lipid raft gangliosides and neutralize virus binding and infection [15]. In a subsequent study we demonstrated that the ganglioside binding domain is also recognized by azithromycin [16]. Overall, our data showed that hydroxychloroquine and azithromycin could block SARS-CoV-2 binding to lipid raft gangliosides through two distinct synergistic mechanisms: hydroxychloroquine binds to gangliosides, azithromycin binds to the ganglioside binding domain of the NTD. Based on these data we suggested to focus on the NTD -rather than the RBD- to define innovative therapies and vaccine strategies against Covid-19 [16]. The importance of lipid rafts in SARS-CoV-2 infection [17-19] is consistent with the established role of these microdomains in the infection cycle of related coronaviruses [20,21]. It may also explain how SARS-CoV-2 infects ganglioside-rich cell types such as epithelial intestinal [22] and brain cells [17].

An important step toward the design of an efficient Covid-19 vaccine is the demonstration that neutralizing anti-SARS-CoV-2 antibodies can be found in convalescent patients. Due to the prominent role of ACE-2, most scientists believed that the epitopes recognized by those neutralizing antibodies should be part of the RBD. This proved to be the case for some of these neutralizing antibodies, but not for all. Indeed, two recent studies have characterized monoclonal neutralizing antibodies (nAb) that recognize the tip of the NTD and do not interfere at all with the ACE-2-spike interaction [23,24]. This unexpected result led the authors of one of these studies to state: "it is unclear how binding to the tip of the NTD results in neutralization of SRAS-CoV-2" [24]. Our molecular dynamics studies shed, ahead of this publication, some light on this issue. Indeed, we concluded that "the NTD region of the SARS-CoV-2 spike protein, which is recognized by both gangliosides and azithromycin, should be considered as a target for neutralizing antibodies in vaccine strategies" [16].

## 2. Structural relationship between the 4A8 epitope and the ganglioside binding domain of SARS-CoV-2

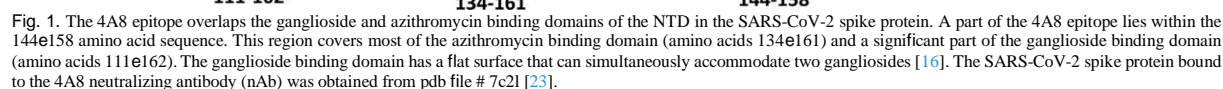
The ganglioside binding domain of the SARS-CoV-2 S protein consists in a flat electropositive surface at the tip of the NTD. This region encompasses amino acid residues 111 to 162 (Fig. 1). This domain includes a shorter motif (residues 134-161) that is recognized by azithromycin, a sugar-containing macrolide antibiotic which has some ganglioside mimicry properties [16]. The 4A8 antibody that has been characterized in convalescent Covid-19 patients recognizes a discontinuous epitope of the NTD [23] which includes a significant part of the ganglioside binding domain (amino acid residues 144-158). Thus, as illustrated in Fig. 1, the 4A8 antibody may prevent any access of the spike protein to lipid raft gangliosides through a competitive inhibition mechanism.

As previously reported, the ganglioside binding domain of the spike protein is very well conserved among SARS-CoV-2 isolates worldwide [15,16]. Nevertheless, the study of a larger sample of SARS-CoV-2 sequences performed in late September 2020 allowed to identify minor

### 3. Molecular mechanism of 4A8 neutralization: key role of lipid rafts

#### 4. Conclusions and perspectives

An important feature of the ganglioside binding motif is that it forms a flat platform at the tip of the NTD of the spike protein. Indeed, this large smooth surface forms a perfect landing pad for the spike trimer (Fig. 3), especially as its electronegative surface potential due to the negative charges of sialic acids fits with the electropositive potential of the NTD. Interestingly, it has been emphasized that such a flat glycan binding domain conflicts with the evolutionary host tropism/adaptation and survival pattern observed for all other coronaviruses, described by the “Canyon Hypothesis” [36]. According to this model, mutations near or at the glycan binding domain are hidden so that the virus escapes detection by host glycan binding immune receptors [37]. Hence it might not be the case for SARS-CoV-2, which, together with other sequence peculiarities, raises question concerning the proximal origin of this virus and its improved functional interaction with lipid rafts [36]. Although this puzzle warrants further discussion, we suggest using ganglioside-NTD interactions as molecular targets for therapeutic and vaccine strategies against Covid-19. In this context, a particular focus on the conserved QFN triad of the NTD [16] deserves careful consideration.







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