

Review Article

# Potent germline-like monoclonal antibodies: rapid identification of promising candidates for antibody-based antiviral therapy

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## Abstract

In recent years, fully human monoclonal antibodies (mAbs) are making up an increasing share of the pharmaceutical market. However, to improve affinity and efficacy of antibodies, many somatic hypermutations could be introduced during affinity maturation, which cause several issues including safety and efficacy and limit their application in clinic. Here, we propose a special class of human mAbs with limited level of somatic mutations, referred to as germline-like mAbs. Remarkably, germline-like mAbs could have high affinity and potent neutralizing activity *in vitro* and in various animal models, despite lacking of extensive affinity maturation. Furthermore, the germline nature of these mAbs implies that they exhibit lower immunogenicity and can be elicited relatively fast *in vivo* compared with highly somatically mutated antibodies. In this review, we summarize germline-like mAbs with strong therapeutic and protection activity against various viruses that caused large-scale outbreaks in the last decade, including influenza virus H7N9, Zika virus, Dengue virus, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus 2. We also illustrate underlying molecular mechanisms of these germline-like antibodies against viral infections from the structural and genetic perspective, thus providing insight into further development as therapeutic agents for the treatment of infectious diseases and implication for rational design of effective vaccines.

**STATEMENT OF SIGNIFICANCE:** A special class of antibodies with low level of somatic mutations, termed as germline-like antibodies, has been proved to have high binding affinity and neutralizing potency against emerging and re-emerging viruses. Their unique characteristics, such as lower immunogenicity and rapid identification, make them promising candidates in the treatment of infectious diseases.

**KEYWORDS:** monoclonal antibody; germline-like; somatic mutation; infectious disease

## INTRODUCTION

In the past decade, the emergence and reemergence of viral pathogens, such as influenza virus H7N9, Zika virus (ZIKV), Dengue virus (DENV), Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have posed significant threats to public health. The neutralizing monoclonal antibodies (mAbs) targeting these viruses have

been demonstrated effective in preventing and treating viral infection [1]. However, in contrast to the clinical and commercial success enjoyed by antitumor antibodies in the pharmaceutical market [2], the application of mAbs in the infectious diseases treatment has been largely impeded by high production costs and limited commercial market [3]. Besides, mAbs are expected to target multiple viral strains so as to provide broadly protective efficacy due

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to the fact that many viral pathogens evolve to undergo mutations frequently to elude the attack of host immune system or to improve their pathogenicity and transmission ability [4]. More importantly, immunogenicity problem remains a significant concern for the wide use of mAbs in the treatment of infectious diseases, especially given the heterogeneity of immunity in population [5].

It is broadly accepted that immunogenic responses to antibody therapeutics can impact both their safety and pharmacokinetic properties [6]. Historically, great efforts have been made to avoid immunogenicity seen with rodent-source antibodies, such as the development of chimeric and humanized antibodies. One may expect that the notion of fully human antibodies implies that human would be tolerant to the complementary determining regions (CDRs), which is the most diverse region of antibody molecules, and the framework-overlapping regions of these antibodies, but this cannot be the case [7]. Because of the evolution of an antibody response, the CDRs can be changed away from their germline predecessors. As a consequence, the presence of strong CD4<sup>+</sup> T helper cell epitopes is found in the CDRs of variable regions within some fully human antibodies [7]. It is one of the key contributors to induce clinically relevant antidrug antibodies. Actually, clinical data suggest that fully human mAbs adalimumab (Humira) and golimumab (Simponi) can induce antidrug antibodies in 5–89% (dependent on the disease and the therapy) and 16% patients, respectively [8–10]. Therefore, the immunogenicity problem of fully human mAbs is still of great concern so as to minimize the potential safety and efficacy issues. Some novel strategies are currently under consideration to further de-immunization of these biologic proteins. For example, one strategy referred to as tolerization, introduces tolerogenic sequences or Treg epitopes into the protein to trigger the expansion of Treg cells and promote a tolerogenic immune response [11,12]. Besides, identifying and replacing CD4<sup>+</sup> T helper cell epitopes in CDR regions is an alternative approach to reduce the immunogenic potential, but the number of modified amino acids is often constrained in order to retain the bioactivity of the antibody molecules [7]. Despite these efforts, there is still no generally applicable technology guaranteed to render therapeutic antibodies antigenically silent.

Interestingly, we and others have recently identified some special human mAbs with limited somatic mutations from their germline predecessors [13–16] called germline-like antibodies. The germline nature implies that these mAbs possess a higher purity of human origin, so they can exhibit a lower level of immunogenicity when compared with other environmentally selected or artificially engineered antibodies for higher affinity. As an essential part of human adaptive immune system, B-lymphocytes are capable of specifically binding foreign antigens by expressing receptors on their surface (B-cell receptors, BCRs). When encountered antigen for the first time, BCRs undergo a process of affinity maturation in germinal center (GC), whereby cycles of rapid somatic mutation and selection lead to improved antigen binding together with elimination of auto-reactive products [17]. This is the case especially for antibodies against HIV-1 and some other possible chronic infections, which are highly divergent from their

corresponding germline predecessors to obtain breadth and potency [18–20]. The prevalent thought is that antibodies experiencing extensive and complex maturation pathways would have better properties. However, for some acute infections, the process of affinity maturation may not be necessary for antibodies to have prominent characteristics, or even redundant under some circumstances [21]. Indeed, by using B cells from the blood of healthy individuals, our group constructed a very large naïve antibody library so as to enhance the selection of high-affinity antibodies with minimal divergence from their germline predecessors, and successfully isolated potent germline-like antibodies against viruses including MERS-CoV [13,22], ZIKV [14], DENV [23] and H7N9 [15]. Meanwhile, some potent antiviral human mAbs with germline-like nature have also been identified by other laboratories including antibodies against respiratory syncytial virus [24], hepatitis C virus [25], Ebola virus [26,27] and SARS-CoV-2 [16,28,29]. Notably, because of the lower number of mutations, germline-like antibodies can be elicited *in vivo* relatively fast to guide the design of effective candidate vaccine immunogens [30]. Here, we focus on potent germline-like mAbs against viruses that caused large-scale outbreaks in the last decade such as H7N9, DENV, ZIKV, MERS-CoV and SARS-CoV-2, highlighting their characteristics and potential as prophylactic or therapeutic candidates (Table 1). We also provide illustration of known mechanisms of these germline-like mAbs from the structural and genetic perspective as well as insight into their successful application in the treatment of human viral diseases.

## H7N9

Since the first case of avian influenza A virus subtype H7N9 infection reported in China in 2013 [31], there have been five seasonal epidemics of H7N9, with a fatality rate of approximately 40%. During the latest wave between 2016 and 2017, a highly pathogenic H7N9 strain emerged and caused the greatest number of human infections and widest geographical distribution of human cases than ever [32]. Meanwhile, the H7N9 virus continues to accumulate mutations and its affinity for human respiratory epithelial sialic acid receptor is increasing [33]. Moreover, there have been laboratory-scale studies indicating drug resistance to neuraminidase inhibitors, such as oseltamivir and zanamivir that are currently used as first-line antiviral drugs [34,35]. These issues emphasize the need to develop novel safe and effective strategies to protect against H7N9 infection. As a principal target of neutralizing antibodies, the viral surface envelope protein hemagglutinin (HA) is composed of a conserved membrane-proximal stem region and a globular head region (HA1) with a shallow receptor-binding pocket. From four individuals with acute H7N9 infections in 2013–2014, several H7-specific neutralizing antibodies were isolated albeit with few somatic mutations in variable domain sequences [36]. L4A-14 binds to the conserved epitopes among H7N9 isolates and has therapeutic activity in murine infection model infected by both 2013 and 2017 H7N9 viruses. Structural analysis of the L4A-14/H7 complex revealed that the antibody recognizes the region

**Table 1.** Germline-like antiviral mAbs

Virus	Binding region	mAb name	Screening method	VH germline	VL germline	Binding affinity (K <sub>D</sub> , nM)	Neutralizing activity (IC <sub>50</sub> , µg/mL)	References
H7N9	H7 head domain of HA	L4A-14 (IgG1)	Single plasmablast cell (IgM <sup>-</sup> ) sorting of H7N9 infected patients (acute phase)	IGHV3-30*04 (5 aa mutations)	IGLV6-57*01 (2 aa mutations)	16.6	0.02 ~ 0.2*	[36]
	H7 head domain of HA	H7.167 (IgG1)	EBV-mediated B cell immortalization of H7N9 vaccinated candidates	IGHV3-48*03(98% identity)	IGKV4-1*01	N/A	0.016	[37]
	H7 HA	m826 (IgG1)	Biopanning from a phage-displayed Fab library	IGHV1-69*01 (100% identity)	IGKV1-39*01 (99.6% identity)	0.8 (pH = 5.0), 9.4 (pH = 7.4)	No neutralization activity	[15]
	H7 head domain of HA	3L11 (IgG1)	Memory B cell (IgM <sup>-</sup> IgA <sup>-</sup> IgD <sup>-</sup> ) sorting of a H7N9 recovered patient	IGHV1-8 (91.13% identity)	IGLV2-13 (94.45% identity)	5.32	24.63	[39]
DENV	Envelope domain III	m366.6 (IgG1)	Biopanning from a yeast-displayed scFv library	IGHV3-21*01 (95.2% identity)	IGLV3-21*01 (95.2% identity)	DENV1-4: 0.8, 0.3, 0.3, 1.9	DENV1-4: 22, 2.4, 0.85, 0.36	[23]
ZIKV	Envelope domain III	m301 (IgG1)	Biopanning from a phage-displayed Fab library	IGHV3-30*03 (97.57% identity)	IGKV4-1*01 (97.64% identity)	0.2	N/A	[14]
	A unique non-E epitope	PIF12 (IgG1)	Single plasmablast cell sorting of a ZIKV infected patient (12 days post symptoms)	IGHV3-7*01 (100% identity)	IGKV1-8*01 (100% identity)	N/A	N/A	[53]
	Envelope domain III	ZK2B10 (IgG1)	Memory B cell (IgD <sup>-</sup> IgM <sup>-</sup> ) and plasmablast cell (IgD <sup>-</sup> IgM <sup>-</sup> ) sorting of a ZIKV infected patient (longitudinal analysis)	IGHV1-8 (92.3% identity)	IGLV1-47 (96.9% identity)	1.06	0.017	[47,48]
MERS-CoV	Envelope domain III	1C11 (IgG1)	Antigen-specific memory B cell (IgG <sup>+</sup> ) sorting of a ZIKV infected patient (64 days post symptoms)	IGHV3-23*04 (96.53% identity)	IGKV1-5*03 (91.67% identity)	N/A	0.083	[49]
	RBD of S protein	m336 (IgG1)	Biopanning from a phage-displayed Fab library	IGHV1-69*06 (1 aa mutation)	IGKV1-17*01 (5 aa mutations)	0.099	0.07	[13,22,54–56]
	RBD of S protein	MERS-4 (IgG1)	Biopanning from a yeast-displayed scFv library	IGHV3-30*01 (94.5% identity)	IGLV1-47*01 (98% identity)	0.978	0.5	[57,58]
	RBD of S protein	MERS-27 (IgG1)	Biopanning from a yeast-displayed scFv library	IGHV3-11*03 (93.9% identity)	IGKV1D-33*01 (93% identity)	71.2	2.0	[57,58]
	S protein	MERS-GD27(IgG)	Antigen-specific peripheral B cell screening of a MERS-CoV infected patient (23 days post diagnosis)	IGHV1-69*09 (97.92% identity)	IGLV1-40*01 (98.96% identity)	0.775	0.001	[59,60]
SARS-CoV-2	S protein	MERS-GD33 (IgG)	Antigen-specific peripheral B cell screening of a MERS-CoV infected patient (23 days post diagnosis)	IGHV1-69*09 (99.65% identity)	IGLV1-40*02 (97.22% identity)	0.575	0.001	[59]
	RBD of S protein	S309 (IgG1)	Memory B cell (IgG <sup>+</sup> ) sorting of SARS-CoV infected patients	IGHV1-18 (97.22% identity)	IGKV3-20 (97.52% identity)	0.0428	0.079	[16]
	RBD of S protein	CB6 (IgG1)	Antigen-specific memory B cell (IgG <sup>+</sup> ) sorting of a SARS-CoV-2 convalescent patient	IGHV3-66*01 (99.0% identity)	IGKV1-39*01 (99.6% identity)	2.49 ± 1.65	0.036 ± 0.007	[61]
	RBD of S protein	B38 (IgG1)	Antigen-specific memory B cell sorting of a SARS-CoV-2 convalescent patient	IGHV3-53*04 (99% identity)	IGKV1-9*01 (99.7% identity)	70.1	0.177	[62]
	RBD of S protein	H4 (IgG1)	Antigen-specific memory B cell sorting of a SARS-CoV-2 convalescent patient	IGHV1-2*06 (99.7% identity)	IGKV2-40*01 (98.3% identity)	4.48	0.896	[62]
	RBD of S protein	COVA1-18 (IgG1)	Antigen-specific B cell sorting from convalescent serum	IGHV3-53*01 (100% identity)	IGKV7-43*01 (97.9% identity)	N/A	0.007	[63]
	RBD of S protein	COVA2-15 (IgG1)	Antigen-specific B cell sorting from convalescent serum	IGHV3-23 (98.6% identity)	IGKV2-30*01 (98.3% identity)	N/A	0.009	[63]
	RBD of S protein	CC12.1 (IgG1)	Antigen-specific memory B cell (IgG <sup>+</sup> ) sorting of SARS-CoV-2 convalescent patients	IGHV3-53*01 (98.95% identity)	IGKV1-9*01 (99.64% identity)	17	0.12	[29]
	RBD of S protein	CV30 (IgG1)	Antigen-specific IgG <sup>+</sup> B cell sorting of a SARS-CoV-2 infected patient (21 days post symptoms)	IGHV3-53*01 (2 aa mutations)	IGKV3-20*01 (100% identity)	3.63	0.03*	[64]
	NTD of S protein	4A8 (IgG1)	Memory B cell (IgG <sup>+</sup> ) and plasmablast cell (CD38 <sup>+</sup> ) sorting of 10 SARS-CoV-2 convalescent patients	IGHV1-24*01 (97.92% identity)	IGKV2-24*01 (98.3% identity)	92.7	0.61	[28]
RBD of S protein	ab1 (IgG1)	Biopanning from a phage-displayed library	N/A	N/A	0.16	0.2	[66]	

N/A, not available. \*Pseudovirus.

surrounding the receptor-binding site (RBS) in the head region of the H7 protein. Natalie *et al.* reported the development of five germline-like antibodies which possessed neutralizing and HA-inhibiting activities from 75 recipients injected monovalent inactivated A/Shanghai/02/2013 H7N9 vaccine [37]. Epitope-mapping studies suggested that these mAbs bind near the receptor-binding pocket within HA region. The most potent mAb, H7.167, was tested as a prophylactic treatment in mice challenged by lethal dose of viruses intranasally, and the result showed that systemic administration of the mAb markedly reduced lung viral titers.

In addition to directly blocking viral attachment or membrane fusion, antibodies can exert antiviral effects through other mechanisms, such as Fc-effector functions, in particular antibody-dependent cellular cytotoxicity (ADCC). However, it has been recently proposed that anti-RBS antibodies that block the interactions between HA and sialic acid receptors on host cells would inhibit Fc $\gamma$ RIIIa activation and ADCC elicitation [38]. Therefore, mAbs targeting regions other than HA head domain are needed for alternative and adjunctive treatments for H7N9 infection. Indeed, m826, a germline-like human mAb selected by sequential panning of a large naïve antibody library using recombinant HA1 and HA proteins as antigens, could protect mice against lethal H7N9 challenge through mechanisms likely involving ADCC, despite the absence of neutralizing activity [15]. Interestingly, m826 binds to H7N9 HA in a pH-dependent way, with subnanomolar affinity at acidic pH but 10-fold lower affinity at neutral pH. Analysis of its crystal structure revealed a highly conserved epitope, which was buried in the trimeric structure and would undergo conformational changes for easier access by m826. Immunogenicity analysis indicates that m826 antibody is a germline-like antibody whose VH gene shared 100% identity with IGHV1-69\*01 germline, and VL gene shared 99.6% identity with IGKV1-39\*01 germline. Notably, m826-like sequences can be identified in not only H7N9-infected patients, but also healthy adults and newborn babies, suggesting the relatively easier elicitation in human immune response. Recently, another germline-like human antibody 3L11 with high binding affinity to HA was isolated from cytokine-stimulated switched memory B cells of a patient infected with H7N9 [39]. Similar to m826, 3L11 had poor neutralizing and hemagglutination inhibition activity but could promote killing of infected cells by ADCC, and protected mice against a lethal H7N9 virus challenge, in both pre- and postexposure administration regimens. Sequence analysis indicated that 3L11 used a rare lineage with IGHV1-8 and IGLV2-13 gene segments. Taken together, antibodies targeting cryptic epitopes may have low- or non-neutralizing activity, but can confer protection by other mechanisms such as ADCC.

## FLAVIVIRUSES

Flaviviruses are single-stranded RNA viruses that vectored mainly by arthropods and can cause a spectrum of diseases with diverse severity. So far, the global spread of flaviviruses remains a critical health issue, with up to

400 million cases of infection annually [40]. The envelope (E) protein of flaviviruses is a three-domain structure (referred to as E-DI, E-DII, E-DIII) mediating virus entry and membrane fusion, and is considered as a principal target for neutralizing antibodies. DENV is the largest pathogenic virus of vector-borne disease and has four antigenically distinct serotypes. Although primary infection induces lifelong immunity to the same serotype, when infected sequentially by other serotypes, cross-reactive but non- or poor-neutralizing antibodies elicited before can promote viral entry into Fc $\gamma$ R-expressing cells, resulting in increased virus production and symptom severity [41]. To avoid such effect known as antibody-dependent enhancement (ADE), developing neutralization antibodies against all the serotypes of DENV could be a promising strategy for anti-DENV agents. By sequential panning in a yeast-displayed antibody library derived from healthy donors using a competitive sorting strategy, Hu *et al.* [23] identified a germline-like antibody, m366.6, which can bind to all four DENV E-DIIIs with high affinity of 0.8 nM, 0.3 nM, 0.3 nM and 1.9 nM, respectively. Moreover, m366.6 could neutralize four serotypes DENV *in vitro* and protect mice against DENV infection *in vivo* without detectable ADE effect. Immunogenetic analysis indicated that m366.6 VH and V $\lambda$  gene shared 95.8% and 95.2% sequence identities with the IGHV3-21\*01 and IGLV3-21\*01 germlines, respectively, and m366.6-like V(D)J recombination can be found in IgM repertoires of both health adults and newborn babies. This significant degree of sequence resemblance between m366.6 and IgM repertoires showed the potential to elicit robust immune responses with m366.6-like germline antibodies by vaccination.

ZIKV is another important member of the Flaviviridae family which was previously regarded as a relatively mild pathogen that causes only sporadic infections in humans since its discovery in 1952 [42]. However, the large outbreak in 2007 and 2015–2016 triggered serious public health concerns due to increased geographic distribution, epidemic activity and some newly reported developmental and neurological complications, such as microcephaly and Guillain-Barré syndrome [43–45]. No specific therapy has been approved to date. Because of the high level of structural similarity between ZIKV and other flaviviruses, pre-existing of non- or poorly neutralizing antibodies may also result in ADE effect, which is of great concern when developing therapeutic antibodies or vaccines. Recently, an association between the epitope specificity of mAbs and neutralization potency and breadth has been observed [46]. These antibodies targeting E-DII are cross-reactive, weak or moderate neutralizing and have ADE effect, whereas antibodies that bind to E-DIII are type-specific and exhibit potently neutralizing activity. Using a large phage-display naïve antibody library, Wu *et al.* [14] identified a panel of germline-like mAbs that target ZIKV E-DIII specifically with high affinities and share 98–100% identity with their corresponding germline IGHV genes. Among them, a mAb, termed as m301, not only neutralized 5 representative ZIKV strains but also displayed a synergistic effect *in vitro* with another mAb, m302, as well as in an AG6 mouse model of ZIKV infection. To better understand

the process of antibody development in ZIKV-infected patients, Gao *et al.* applied next-generation sequencing (NGS) technology to probe the dynamic development of a potent and protective ZIKV E-DIII-specific antibody ZK2B10, which was isolated from a ZIKV convalescent individual [47,48]. Despite the dramatic changes in the usage of antibody variable region germline genes in the whole repertoire, lineage tracing of ZK2B10 revealed limited somatic hypermutation and transient expansion during the 12 months following the onset of symptoms. In addition, a significant increase in the population of germline-like sequences for both heavy and light chain repertoire was observed at a timepoint of Day 15. Both m301 and ZK2B10 could only neutralize ZIKV but showed no detectable cross-reactivity with DENV. Interestingly, from memory B cells of a ZIKV-infected patient that was seronegative for DENV infection, a germline-like mAb (1C11) targeting E-DIII was isolated and could neutralize both ZIKV and DENV1 [49]. The heavy and light chains of mAb 1C11 were derived from the IGHV3-23 and IGKV1-5, respectively, the pair of which has been reported in several mAbs that have unique capacity to neutralize both ZIKV and DENV1, regardless of whether they are germline-like or not [50–52]. Diogo *et al.* isolated another germline-like mAb without detectable somatic hypermutation, P1F12, from plasmablasts of an acutely ZIKV-infected patient, showing unusual feature of binding to an atypical non-E protein epitope and neutralizing the virus with IC<sub>50</sub> at ~2 µg/mL. Since the mAb was isolated only 12 days post onset of symptoms, it is hardly to undergo a GC-dependent development pathway, suggesting that affinity maturation is not necessary for the generation of isotype switched virus-neutralizing mAbs [53].

## MERS-COV

MERS-CoV was first reported to be isolated from a Saudi Arabian patient suffering from high fever, coughs and shortness of breath in 2012. As of September 2019, 2468 laboratory-confirmed cases have been reported by World Health Organization, with the highest mortality (~35%) among 7 known coronaviruses ([https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers#tab=tab\\_1](https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers#tab=tab_1)). Currently, there are no approved drugs or vaccines available for the prevention or treatment of MERS-CoV infection. Among the four major structural proteins encoded by MERS-CoV genome, the spike protein plays a key role in viral infection and pathogenesis. The receptor-binding domain (RBD) of the S protein, which binds to the cellular receptor, dipeptidyl peptidase 4 (DPP4), is a critical neutralizing domain and general target for the development of MERS vaccines and therapeutics.

M336 is a germline-like human mAb identified from a large naïve-antibody library targeting the RBD of MERS-CoV S protein with high (pM) avidity [13]. With its epitope almost completely overlapping the binding site of DPP4, m336 showed exceptional neutralizing potency against both pseudovirus and live MERS-CoV with 50% neutralization at 0.005 and 0.07 µg/mL, respectively. Notably, immunogenetic analysis indicated that m336 was

almost germline except for one- and five-point mutations in their VH and VL genes, respectively. Furthermore, structural and genetic analysis indicate that the naturally occurring Phe54 and Lys73-encoded allelic-specific amino acids coupled with specific junctional amino acid residues in VH gene play a critical role in achieving high affinity at such low levels of somatic hypermutation [22]. The mAb m336 exhibited potent protection in various animal models. Prophylaxis with m336 in rabbits infected with MERS-CoV resulted in 40–9000-fold reduction of pulmonary viral RNA titers [54]. The hDPP4-transgenic mice treated with m336 prior to or post lethal MERS-CoV challenge conferred full protection [55], and m336 treatment could also reduce signs of clinical disease in a non-human primate model [56]. Similar to m336, other two potent germline-like mAbs, MERS-4 and MERS-27, were also derived from a nonimmune human antibody library and inhibited infection of both pseudotyped and live MERS-CoV with IC<sub>50</sub> at nanomolar concentrations [57]. Remarkably, MERS-4 bound soluble RBD with ~45-fold higher affinity than DPP4 and showed inhibitory activity against syncytia formation between S glycoprotein and DPP4. Structural analysis suggested that MERS-4 adopted an unusual indirect-competing mechanism to interfere with DPP4 binding by approaching the RBD from the outside of the RBD-DPP4 binding interface [58]. This unique epitope specificity thereby enabled MERS-4 to synergize with other neutralizing antibodies, such as those against RBD (MERS-27, m336) or N-terminal domain (NTD, 5F9). Recently, a panel of 13 ultrapotent human mAbs was verified by another group from a naturally MERS-CoV infected individual [59]. Like m336, these mAbs also had IGHV1-69 derived VH genes with low somatic mutations, indicating that the IGHV1-69 gene is preferentially used in anti-MERS-CoV mAbs, whereas the VL genes were more diverse. Among these 13 mAbs, MERS-GD27 and MERS-GD33 showed the most potent neutralizing activity against pseudovirus and live MERS-CoV *in vitro*. Since they recognized distinct epitopes on the S protein and had a low level of competing activity, their combination demonstrated strong synergistic effect. Interestingly, MERS-GD27 and m336 had the largest overlap in the epitope with DPP4, correlating with the 10-fold lower pseudovirus IC<sub>50</sub> values for m336 (0.003 µg/mL) and MERS-GD27 (0.001 µg/mL) versus those for MERS-27 (63.96 nM), 4C2 (0.71 µg/mL), MCA1 (0.39 µg/mL) and D12 (0.013 µg/mL). MERS-GD27 could provide prophylactic and therapeutic efficacy before and after a lethal challenge with MERS-CoV in a hDPP4-transgenic mice model [60].

## SARS-COV-2

The outbreak of coronavirus disease 2019 (COVID-19) caused by a newly emerged coronavirus known as SARS-CoV-2 has taken over 2.4 million people's lives globally as of 21 February 2021 ([https://www.who.int/health-topics/coronavirus#tab=tab\\_1](https://www.who.int/health-topics/coronavirus#tab=tab_1)). Similar to SARS-CoV, viral entry of SARS-CoV-2 depends on the spike (S) glycoprotein and utilizes the angiotensin-converting enzyme 2

(ACE2) on host cells as receptors. Because of the homology of S protein between SARS-CoV and SARS-CoV-2, a cross-reactive mAb S309 was identified from a SARS-CoV survivor's memory B cells, which potently neutralizes SARS-CoV-2 with an  $IC_{50}$  of 79 ng/mL [16]. Cryo-electron microscopy of the complex containing S309 and trimeric S protein revealed that S309 recognized the epitope including a unique conserved N343-glycan and bound to RBD in an ACE2 non-competing way. Therefore, in combination with other neutralizing mAbs targeting different epitopes could improve neutralizing efficacy. Moreover, S309 can recruit efficient ADCC and antibody-dependent cellular phagocytosis via Fc fragment that binds to  $Fc\gamma$  receptors [16].

By isolating single B cells from COVID-19 convalescent patients, a panel of potent antibodies not only specifically binding to SARS-CoV-2 RBD, but also having a limited level of somatic mutations, was identified. For example, mAb CB6, identified from a COVID-19 survivor by recombinant RBD of SARS-CoV-2 S protein, shows an identity of  $\sim 99\%$  with its germline predecessors and exhibits strong neutralizing potency against live virus with an  $IC_{50}$  of  $\sim 0.036 \mu\text{g/mL}$  [61]. CB6 administration at a dose of 50 mg/kg showed strong inhibition of viral titer and pathological lung damage in rhesus macaques in both prophylactic and therapeutic settings. With a similar strategy, Wu *et al.* reported two ACE2-competing mAbs B38 and H4 (with an  $IC_{50}$  of 0.177  $\mu\text{g/mL}$  and 0.896  $\mu\text{g/mL}$ , respectively), which targeted different epitopes on the RBD and exhibited synergetic neutralizing ability [62]. *In vivo* study validated that the use of antibody combination can reduce virus titers in infected lungs of a mouse model, supporting its potential in clinical applications. Using a SARS-CoV-2 stabilized prefusion S protein, a group from the Netherlands isolated mAbs from three COVID-19 patients and these mAbs had low levels of somatic mutations [63]. Among them, two mAbs known as COVA1-18 and COVA2-15 showed extremely high potency against authentic SARS-CoV-2, with  $IC_{50}$  as low as 0.007 and 0.009  $\mu\text{g/mL}$ , respectively. Further structural analysis of Fab-S protein complex by cryo-electron microscope suggested that COVA2-15 bound RBD with a stoichiometry of three per trimer in both "up" and "down" conformations compared with one Fab per trimer in only "up" conformation for other less potent mAbs. By establishing a cohort of SARS-CoV-2 infected donors, Rogers *et al.* [29] developed a high-throughput antibody generation platform to screen more than 1800 antibodies and characterize potent neutralizing antibodies in 2 weeks. A mAb, CC12.1, with an  $IC_{50}$  of 0.019  $\mu\text{g/mL}$  against pseudovirus *in vitro*, provides protection against high-dose SARS-CoV-2 challenge in Syrian hamsters. Seydoux *et al.* [64] isolated 45 S-specific mAbs from a COVID-19-infected individual 21 days post the onset of clinical disease. These mAbs were minimally mutated and only three of them displayed neutralizing activity. The most potent one, CV30 ( $IC_{50} = 0.03 \mu\text{g/mL}$ ), bound the RBD in a manner that efficiently inhibited RBD-ACE2 interaction and displayed  $\sim 530$  times higher potency than two non-RBD binding mAbs, CV1 and CV35. Although RBD is the major target for mAbs against SARS-CoV-2,

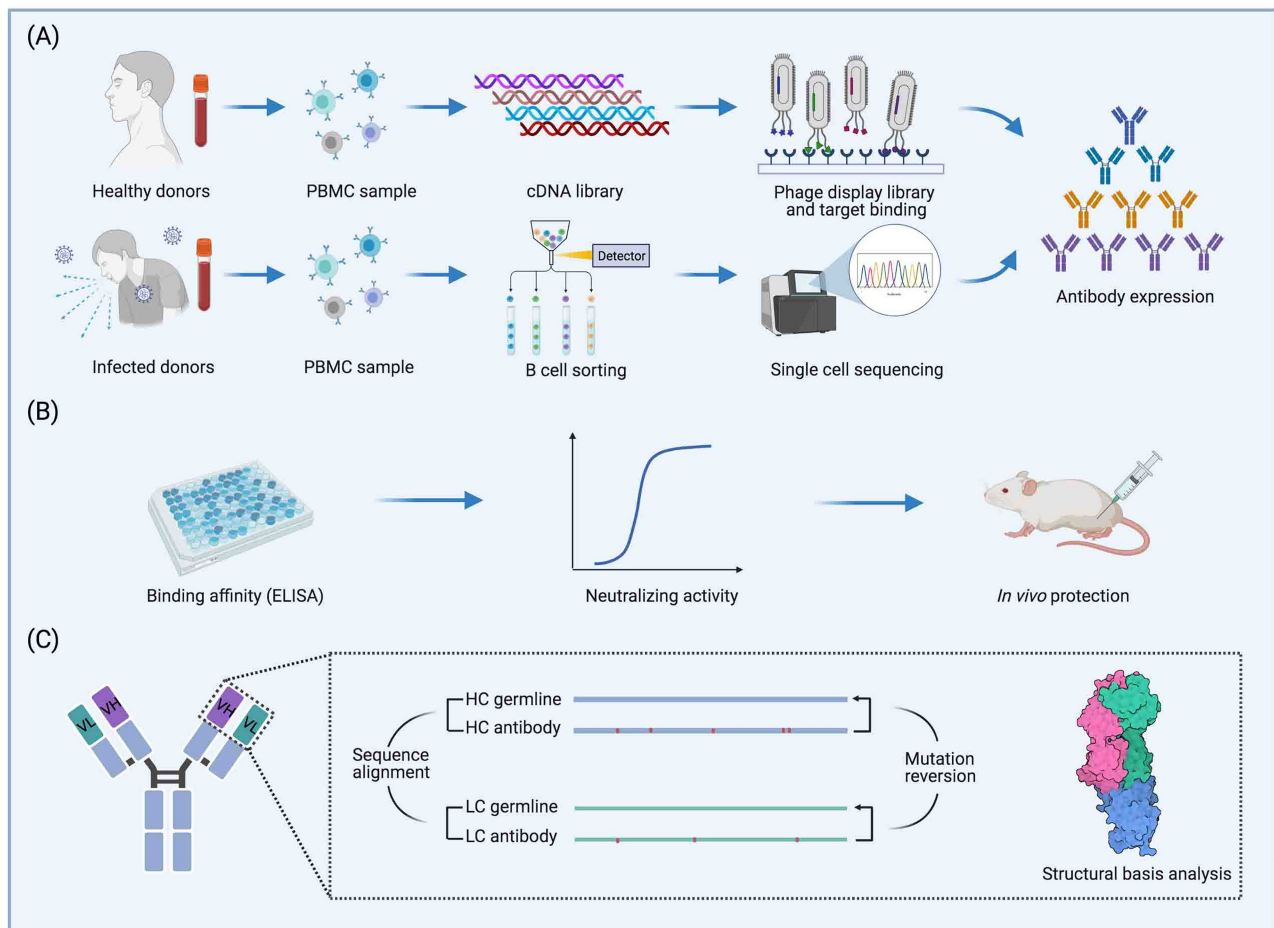
germline-like antibodies targeting NTD were also reported and may benefit the development of antibody cocktails to decrease potentials for virus escape. Antibody 4A8 was isolated by Chen's group from 10 convalescent patients and exhibited potent neutralizing activity against authentic SARS-CoV-2 with  $IC_{50}$  about 0.61  $\mu\text{g/mL}$  [28]. Structural characterization with cryo-electron microscopy suggested that 4A8 recognized a vulnerable epitope of the NTD on the S protein and the high neutralization capacity was possibly a result of blocking conformational changes from the pre- to post-fusion state of SARS-CoV-2 S protein.

Notably, four of the six germline-like mAbs mentioned above (B38, COVA1-18, CC12.1 and CV30) share the same germline of VH genes (IGHV3-53), despite the origins of their light chains are relatively diverse. Yuan *et al.* [65] analyzed 294 SARS-CoV-2 RBD-targeting antibodies and found that IGHV3-53 is the most frequently used IGHV gene. Further structural analysis revealed that two key motifs encoded in the IGHV3-53 germline gene enabled IGHV3-53 to target RBD, a NY motif at VH residues 32 and 33 in the CDR H1 and an SGG motif at VH residues 53–56 in the CDR H2, based on which mutations from affinity maturation are apparently not necessary. Another germline gene, IGHV3-66, highly close to IGHV3-53, also contains these two motifs. IGHV3-66 germline is also commonly used in neutralizing antibodies, such as SARS-CoV-2 specific mAb CB6. Moreover, the role of the two motifs in hydrophilic interactions between the CB6 heavy chain and SARS-CoV-2 RBD was confirmed by crystal structure of the CB6-RBD complex [61].

Screening human mAbs from large phage-displayed libraries is an alternative approach to develop antibodies against SARS-CoV-2. Li *et al.* built antibody libraries in Fab, scFv, or VH formats using peripheral blood mononuclear cells (PBMC) from 490 healthy donors before the SARS-CoV-2 outbreak. A human germline-like antibody was rapidly (within a week) identified, IgG1 ab1, which bound to RBD of S protein in an ACE2-competing manner. The antibody ab1 potently neutralized replication-competent SARS-CoV-2 and displayed high prophylactic and therapeutic efficacy against SARS-CoV-2 infection, as demonstrated by *in vitro* tissue culture assays, a mouse-ACE2-adapted SARS-CoV-2 in BALB/c mice, and native virus in hACE2-transgenic mice or hamster [66]. Additionally, IgG1 ab1 induced ADCC activity, which may contribute to the neutralizing activity and the control of viral infection *in vivo*. Moreover, IgG1 ab1 exhibited excellent properties including low aggregation and no binding activity to 5,300 human cell membrane-associated proteins. These results indicated that fully human germline-like antibodies have potential as therapeutic in the treatment of infectious diseases.

## CONCLUSION AND DISCUSSION

For millions of years, humankind is consistently faced with health threats from emerging or re-emerging viruses. To successfully eliminate these microbes that can cause serious infectious diseases, human immune system has evolved complex immunological repertoires for which there are



**Figure 1.** Overview of antiviral germline-like antibody discovery platform. (A) Two screening methods for identification of potent germline-like antibodies. In brief, very large phage-display libraries are constructed using PBMCs from healthy donors. After several rounds of panning using antigen as baits, a panel of antibodies is identified and converted to IgG format. Alternatively, PBMC samples from infected individuals are prepared for isolation of suitable B cells by single cell sorting. After sequencing these B cells, antibodies are cloned and expressed for further analysis. (B) Functional characterization of germline-like antibodies. The binding affinity of selected antibodies is determined by ELISA, followed by characterization of neutralizing activity using pseudoviruses or infectious viruses. Afterwards, *in vivo* efficacy is evaluated using suitable animal models. (C) Analysis of underlying molecular mechanism. Sequence of antibodies is aligned to the closet VH and VL germline predecessors using the IMGT tool and the mutation sites (red) can be reversed for analysis of their role in antibody efficacy. In parallel, the structure of antibody–antigen complex is analyzed to determine the interacting residues.

both historical and real-time components. The historical germline repertoire, which could be traced back to cartilaginous fish [67], is selected by evolutionary pressure for the ability to protect the host from infectious agents. In contrast, real-time evolution is unique to different antigens, where a process of somatic mutation ensues to select for B cell clones that bear antibodies with higher binding affinities to antigens [68]. However, real-time evolution does not play a dominant role in combating many viruses, especially those cause acute and highly pathogenic infections, because of time constraints. For example, when infected by Ebola virus, the survival of an individual depends on suppressing viruses within the first several days, a period too short to mutate of antibodies for high affinity and broad breadth. In this case, germline genes containing critical epitopes to inhibit infection would function, in forms of antibodies with limited mutations, as first responders to initiate defense against infection agents [69,70]. Indeed, Dr. Lerner [71] for the first time proposed that IGHV1-69 is the S.O.S.

component of the immunological repertoire, and sequent studies confirmed that IGHV1-69 is dominantly used in antibodies against acute infections caused by viruses, such as influenza [72], SARS-CoV [73] and MERS-CoV [13,59]. Typically, *VH1-69* gene encodes two hydrophobic residues (position 53 and position 54) in the CDR2 loop of the antibody heavy-chain that provides a structural basis for epitope recognition. Therefore, one unique feature of VH1-69-derived bnAbs is an unusually hydrophobic CDRH2 loop that recognizes conserved hydrophobic epitopes of viral envelope glycoproteins. Remarkably, these antibodies undergo a rapid lineage development and have only few mutations [74]. In parallel, as noted above, IGHV3-53 is the most frequently used IGHV gene among 294 SARS-CoV-2 RBD-targeting antibodies, among them many carry limited somatic mutations [65]. Herein, it is easy to understand that for some germline-like antibodies, allele-specific regions from their germline may play a dominant role in antigenic recognition, whereas the rest of the molecules

have diverse germline origin during recombination. In brief, we propose that potent germline-like antibodies are the result of historical evolution by selective pressure.

However, for a long time, antibodies with low levels of somatic mutations give an impression of immature in binding and neutralizing activity. As summarized in this review, actually a number of such germline-like antibodies, isolated from nonimmune library, plasmablasts or memory B cells, are capable of providing prophylactic and therapeutic benefits against lethal viral challenge in animal models, supporting their potential to serve as candidates for clinical application. In addition, germline-like antibodies have some advantages over somatically hypermutated antibodies. First, the germline nature of these antibodies implies that they exhibit low to no immunogenicity, a quality made them preferable when administrated in clinical use. Also, due to the skip of the affinity maturation process, germline-like mAbs can be elicited relatively fast in contrast to highly matured antibodies, like HIV-1 antibodies, which require extensive maturation pathways to obtain potency and breadth. Therefore, it would be efficient to use germline-like antibodies as templates for vaccine immunogen design. This is of special value to protect against viruses typically causing acute infections such like H7N9, MERS-CoV, SARS-CoV-2. Thirdly, given that potent germline-like antibodies against diverse viruses have been developed by us and other groups from universal libraries from healthy donors, such antibody discovery platforms (Fig. 1) can free researchers from limitations of infected patients' material access or animal immunization and thus allow rapid identification (can be as soon as 1 week) of potent mAbs when a novel virus infects human and leads to severe diseases [66]. Of note, the size and diversity of libraries are critical determinants for selection efficiency. Finally, in an era when antibody repertoire attracts unprecedented attention, the identification of potent germline-like antibodies against emerging microbes would provide unique insight into antibody development and evolution pathways by analyzing their immunogenetic and structural characteristics, thereby facilitating the engineering of more effective antibody therapeutics and vaccines to fight viral diseases.

#### DATA AVAILABILITY

The data presented in this study are available on request from the corresponding authors.

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#### CONFLICT OF INTEREST STATEMENT

None declared.

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