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THE ROLE OF ARTIFICIAL INTELLIGENCE IN DESIGNING ANTIBODY-BASED THERAPY FOR COVID-19

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ABSTRACT

For several decades ago, passive immunization has already proven its ability to treat some diseases, especially pandemic diseases. On the contrary, after antibiotics discovery, the usage of passive immunization becomes neglected. Nowadays, we face a pandemic situation, COVID-19, which needs the possible treatment to save patients lives while medicines and vaccines are under development. By learning from history, passive immunization seems to be the best choice to save patient lives. As a kind of passive immunization, antibody-based therapy successfully treats diseases, including infectious diseases. Several antibody-based therapies are developed, as vast as the technology development, especially after the genetic codes announced. This article highlighted the influence of genomics tools, which helps researchers develop various platforms in developing monoclonal antibodies with high safety and efficiency in production and application.

Keywords: artificial intelligence, immunoinformatics, monoclonal antibody, antibody therapy

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INTRODUCTION

Disease prevention and treatment efforts, especially treatment, have developed so rapidly that various treatment models based on herbs, synthetic compounds, vaccines, and immunotherapy. Immunotherapy is conducted by providing an agent that eliminates pathogens without activating the body's immune system. In general, immunotherapy is carried out by delivering immune system components into the body of individuals[1,2]One of the immunotherapy is antibody-based therapy or passive immunization. Passive immunization is an immunotherapy method performed by providing antibody neutralization into the body of the individual[3]

At the time of an outbreak of the disease, passive immunization becomes the most viable treatment method to be applied. At present, when FDA approved one of passive immunization, convalescent plasma, to treat COVID-19 patients with severe symptoms. The approach used to surpress the increasing number of mortality rate caused by the infection of SARS-CoV-2 (FDA)[4]. Treatment with convalescent plasma, which introduced in 1890s by Kitasato and von Behring could eliminate pathogen by neutralizing antibodies provides by convalescent plasma. However, there are several limitations for convalescence plasma, such as difficulties in finding the people who proper to become a donor, the volume of antibodies delivered to the recipient, and antibodies purification to separate antibodies from blood cloths[5].

To overcome the weakness of convalescent plasma, monoclonal antibodies (mAbs) become an option to save patients' lives. At the past, mAbs developed by fusing immortal cells with splenocytes from immunized mice. Nowadays, the big data of sequence information available on the DNA libraries provide a new wave on antibody-based therapy. By using machine learning, antibody therapeutics are developed as humanization and synthetic monoclonal antibody which called engineered antibody[6]. This review will discuss the kinds of

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antibody therapies developed and the role of artificial intelligence in developing antibodies for treatment.

METHODS

This article used systematic review according to guidance provided on PRISMA 2009.

Objectives

To find out the role of artificial intelligence in the development of antibody-based therapy for COVID-19.

The Strategy of Literature Searching and Selection

Literatures were searched on January 20th 2021 by using two kinds of electronic database, PubMed and SCOPUS, with keywords "artificial intelligence" AND "antibody", "antibody-based therapy", "immunoinformatics" AND “antibody”, "engineered antibody" and “COVID-19” AND “monoclonal antibodies”. We limit the articles which only articles written in English and published in 2019 until 2021. For collecting the articles, we did not restrict to specific type of articles. We sorted the articles to find out the redundancy possibility by filtering the title. We put several specifications which differ as inclusion and exclusion criteria. All articles for this review were research articles which develop monoclonal antibody using artificial intelligence as part of methods to treat COVID-19. We excluded research articles which related with vaccine development for COVID-19 and avalaible antibody-based therapy on hospitalized patients.

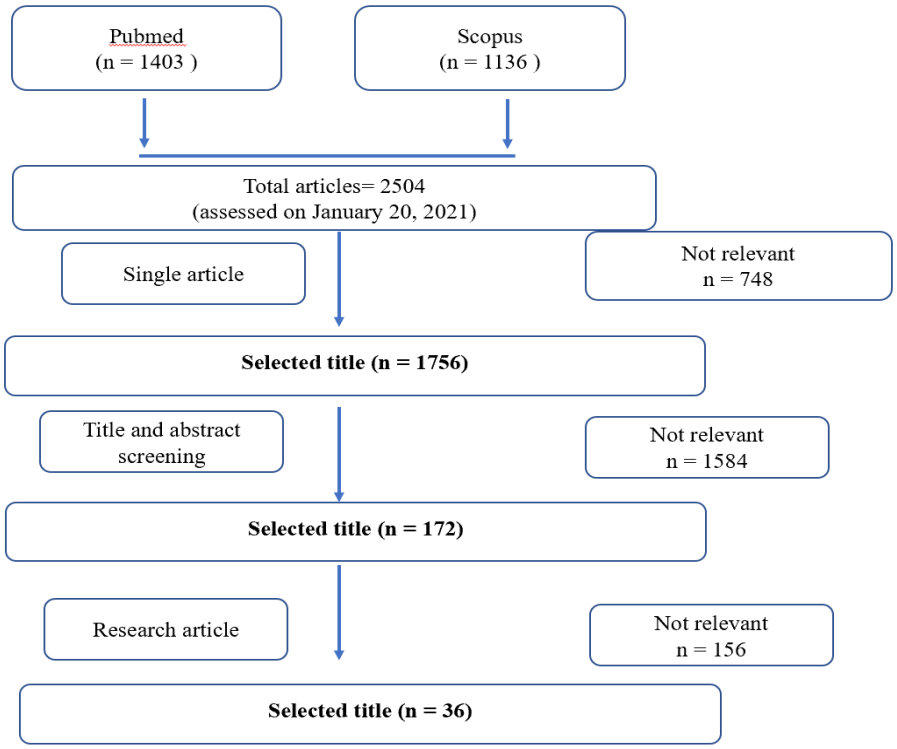

Figure 1. Process of literature searching and selection according PRISMA

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RESULTS

A total of 2504 articles obtained from two journal repositories, but only 70% were single articles. After screening the title and abstract only 172 articles related to artificial intelligence in the development of therapeutic antibodies for COVID-19, only 36 articles are research articles. Among the selected articles, only 1 study used only experiments in silico and 17 articles using in vivo experiments to determine the effectiveness of antibodies developed (Table 1). The majority of studies report using cloning strategies and protein expression by mammalian cells, though just two studies use a plant as their expression system, Nicotiana benthamina, to produce antibodies (Table 2).

Table 1. Development humanized antibody for therapy

Ref	Experiment			Animal model for in vivo experiment	Antigen Target	Potential Antibody
	in silico	in vitro	in vivo			
Alsoussi[7]	Y	Y	Y	C57BL/6J mice (for mAb from hybridoma); BALB/cJmice (for SARS-CoV-2 challenge)	RBD	2B04
Hansen[8]	Y	Y	Y	VelocImmune® (VI) mice	RBD	Antibody cocktail (REGN10933+REGN10987)
Brett[9]	Y	Y	N	N	Spike	
Chi[10]	Y	Y	N	N	Extracellular domain of S protein	4A8
Custódio[11]	Y	Y	N	N	RBD	Sb23
Dong[12]	Y	Y	N	N	RBD	9 VHH-Fc
Ejemel[13]	Y	Y	N	N	RBD	mAb362
Fagre[14]	Y	Y	Y	Syrian Hamster	RBD	AvGn-B
Hassan[15]	Y	Y	Y	BALB/c mice; C57BL/6J; DBA2/J	S protein	1B07 and 2F05
Huo[16]	Y	Y	N	N	RBD	CR3022
Kim[17]	Y	Y	Y	Ferret, golden Syrian hamster, and rhesus monkey	RBD	CT-P59
Kreye[18]	Y	Y	Y	C57BL/6J mice; hamster	RBD	CV07-209
Li[19]	Y	Y	Y	BALB/c mice; C3B6 mice	RBD	Ab1
Liu[20]	Y	Y	Y	Hamster	RBD	2-15 mAb
Lv[21]	Y	Y	Y	a humanized hACE2 C57BL/6 mice	RBD	H014 Fab fragment
Miao[22]	Y	Y	N	N	RBD	89C8-ACE2
Noy-Porat[23]	Y	Y	N	N	RBD	MD62 and MD65
Parzych[24]	Y	Y	N	N	RBD; IL-6R	anti-CR3022 dmAb; Anti-IL-6R dmAb
Piccoli[25]	Y	Y	N	N	RBD	None
Pinto[26]	Y	Y	N	N	spike	S309

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Ref	Experiment			Animal model for in vivo experiment	Antigen Target	Potential Antibody
	in silico	in vitro	in vivo			
Premkumar[27]	Y	Y	N	N	RBD	
Rattanapisit[28]	Y	Y	N	N	RBD	CR3022
Rogers[29]	Y	Y	Y	Syrian Hamster	Spike	CC12.1 mAb
Schafer[30]	Y	Y	Y	mouse adapted	ACE2; RBD region both surface and within the region	C104 shows Fc effector function
Seydoux[31]	Y	Y	N	N	RBD	CV30
Shah[32]	Y	N	N	N	RBD	in silico study for mutation on RBD influence mAb interaction
Shanmugaraj[33]	N	Y	N	N	RBD	B38; H4
Shi[34]	Y	Y	Y	Macaques	RBD; ACE2	CB6
Sun[35]	Y	Y	N	N	RBD region	2 VH-Fc (ab6 and m397) could bind to RBD
Tai[36]	Y	Y	N	N	RBD	None
Wan[37]	Y	Y	N	N	RBD	11 mAb
Wang[38]	Y	Y	Y	Rhesus Monkeys	RBD	MW05
Wu[39]	Y	Y	Y	hACE2 transgenic mouse	RBD	B38
Zhang[40]	Y	Y	Y	Balb/c	RBD	2H2/3C1
Zost[41]	Y	Y	Y	Balb/c, Macaques	Spike	cov2-2196; cov2-2130
Zost[42]	Y	Y	N	N	RBD	COV2-2130
Zylbermann[43]	Y	N	Y	Horse	RBD	F(ab')2 pAb

Table 2. Utilization on Artificial Intelligence (AI)

Ref	Source of Antibody	AI Utilization		Expression System		Organism for Cloning
		Antigen Design	Antibody Design	Ag	Ab	
Alsoussi[7]	murine lymph node	Y	Y	pFM1.2	pABVec6W Ab expression vector	mammalian cells
Hansen[8]	humanized mice; PBMC from human	Y	N	T7 promoter and helper plasmid expressing VSV and T7 RNA polymerase	expression vectors containing human heavy constant region and light constant region	HEK293T cells (for Ag); CHO cells (for mAb)
Brett[9]	Synthetic antibody	Y	Y	VSV N, P, L and G expression plasmids	pCAGGS	HEK293T cells

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Ref	Source of Antibody	AI Utilization		Expression System		Organism for Cloning
		Antigen Design	Antibody Design	Ag	Ab	
Chi[10]	PBMC from human	Y	Y	pCAG vector	pcDNA3.4	HEK 293F (for Ag); Expi293F cells (for mAb)
Custódio[11]	synthetic antibodies	Y	Y	pαH	pCMVExt-Fc	HEK293-F (for Ag); mammalian cells (for Sybody)
Dong[12]	PBMC from Llama	NM	Y	NM	NM	NM
Ejemel[13]	Humanized mice	Y	Y	pcDNA 3.1 Myc/His	Immunoglobulin G1 (IgG) expression vector	Expi293 cells
Fagre[14]	PBMC from human	N	Y	N	various expression vector carrying the constant regions of human IgG1 heavy chain and the kappa chain	Expi293 cells
Hassan[15]	C57BL/6J mouse	N	Y	adenovirus vector	pABVec6W vectors	HEK293T cells (for Ag); Expi293F cells (For mAB)
Huo[16]	Synthetic human antibody	N	Y		pOPING-ET	ExpiCHO cells
Kim[17]	PBMC from human	N	Y		Fc fusion vector	CHO cells
Kreye[18]	PBMC from human	Y	Y	pFastBac	Human antibody expression vector	Sf9 cells (for Ag); ExpiCHO cells (for mAb)
Li[19]	PBMC from human	N	Y		pDR12 vector	Expi293 cells
Liu[20]	PBMC from human	Y	Y	pCAGGS	gWiz or pcDNA3.4	Expi293 cells
Lv[21]	spleen mRNA of mice immunized	Y	Y	pCAGGS	a phage-display scFv	HEK Expi 293F cells
Miao[22]	PBMC from human	N	Y		pFabVk vector and yeast gap repair	Expi293 cells
Noy-Porat[23]	PBMC from human	N	Y		pcDNA3.1+	ExpiCHO

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Ref	Source of Antibody	AI Utilization		Expression System		Organism for Cloning
		Antigen Design	Antibody Design	Ag	Ab	
Parzych[24]	Nucleic acid	Y	N	NM	NM	NM
Piccoli[25]	PBMC from human	Y	Y	pcDNA3.1	human Igγ1, Igκ and Igλ expression vectors (for mAb)[26]	Expi293 cells (for Ag and mAb)
Pinto[27]	PBMC from human	Y	Y	phCMV1	human Igγ1, Igκ and Igλ expression vectors	Expi-CHO cells
Premkumar[28]	PBMC from human	Y	N	pαH		Expi293 cells
Rattanapisit[29]	N	Y	Y	pBY2e	pBY2e	Nicotiana benthamiana
Rogers[30]	PBMC from human	y	Y	phCMV3	expression vectors encoding the human IgG1, Ig kappa or Ig lambda constant domains	FreeStyle293F cells (Ag); DH5 alpha (mAB)
Schafer[31]	N	Y	Y	pNL4-3-nanoluc and lentiviral	Ig heavy and light chain expression vectors	FreeStyle293-6E cells (mAb)
Seydoux[32]	PBMC from human	Y	Y	pαH	pTT3 or pT4-341 HC	FreeStyle293-6E cells (Ag)
Shah[33]	N	Y	Y	N	N	n
Shanmugaraj[34]	N	N	Y	N	pBYR2eK2Md	Nicotiana benthamina
Shi[35]	PBMC from human	Y	N	pFasbac1 (ACE2) pCAGGS (RBD);	N	Hi5 cells
Sun[36]	N	Y	N	pFUSE1-Fc2	pFUSE1-Fc2 (Ag)	293T cells
Tai[37]	PBMC from human	N	Y	N	pComb3x vector	Expi293 cell
Wan[38]	PBMC from human	Y	Y	N	pcDNA3.4 mAb)	HEK293E
Wang[39]	PBMC from human	Y	Y	pKN293E	pKN293E	HEK293
Wu[40]	PBMC from human	y	y	pEt21 n pFastBac1, pEGFP-N1	pCAGGS	E coli, Baculovirus (Ag); HEK293T cells (mAb)
Zhang[41]	PBMC from mice	Y	Y	N	pcDNA3.4	ExpiCHO cells

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Ref	Source of Antibody	AI Utilization		Expression System		Organism for Cloning
		Antigen Design	Antibody Design	Ag	Ab	
Zost[42]	PBMC from human	Y	Y	pαH	pTwist-mCis	ExpiCHO cells
Zost[43]	PBMC from human	y	y	pαH	pTwist-mCis	ExpiCHO cells
Zylbermann[43]	Serum from Horse	Y	N	pCAGGS	N	HEK-293T cells

DISCUSSION

The discovery of antibody utilization for Kitasato and von Behring treatment in 1870 elicits people to employ antibodies to treat disease. The employment of human plasma as the source of antibodies to treat patients was started in 1907. Nowadays, it is known as convalescence plasma therapy[5,44]. The use of convalescent plasma later turned into intravenous immunoglobulin (IvIg) after Cohn's antibody separation method and the team in the 1940s (figure 1).

Due to several requirements must be fulfilled by potential donors and also the number of donors to obtain enough antibodies become the obstacles to apply convalescence plasma as a routine therapy. On the other side, there is a demand for antibodies to treat diseases due to their ability to identify specific pathogens. The discovery of hybridoma technology in the production of antibodies by Kohler and Millstein makes the production of antibodies easier and more efficient [5].

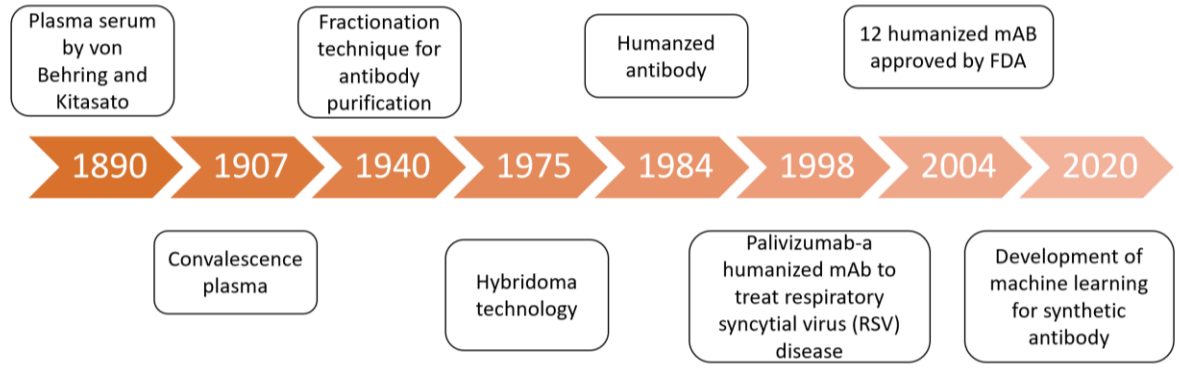

Figure 2. Timeline of antibody-based therapy

The hybridoma technology produces monoclonal antibodies from immortal cells known as hybridoma cells. However, another problem arises due to the patient's immune reaction to the antibodies given, namely the rejection of antibodies which occurs by the formation of HAMA (Human anti-mouse antibody. Two antibody production methods are developed to overcome the problem[45–48]. The first method uses hybridoma technology using human B lymphocyte cells and other methods using engineered antibodies (figure 2).

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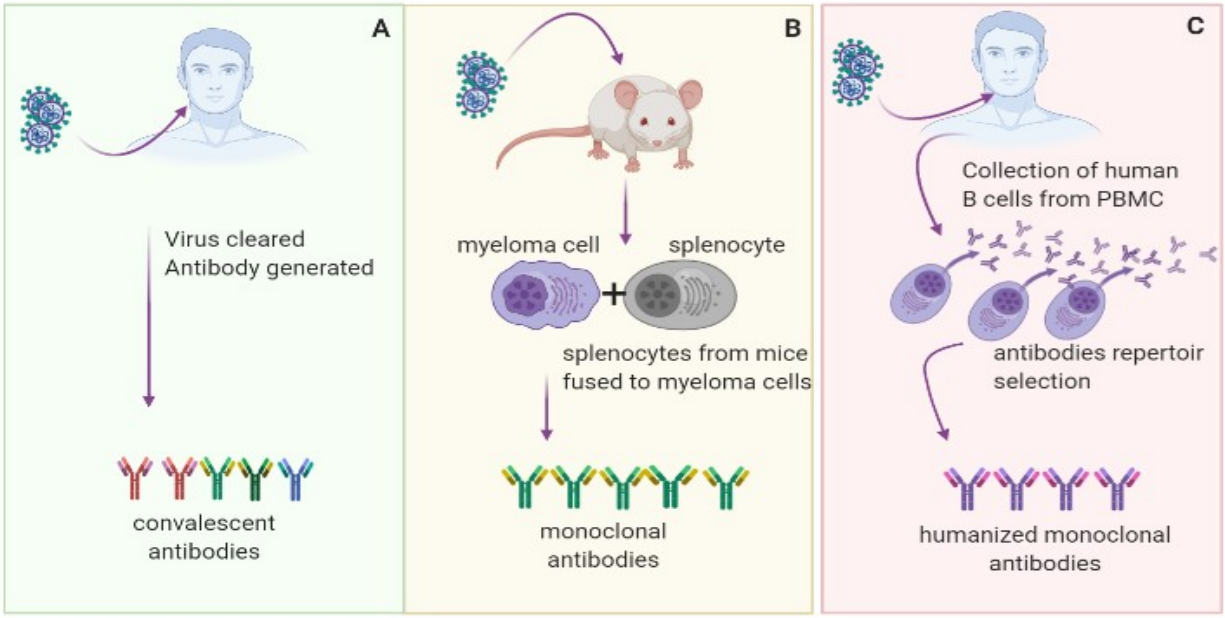

Figure 3. Development of therapy using antibodies. A. Production of antibodies with convalescent plasma method. B. Production of the antibody with the hybridoma method. C. Production of humanized antibody from peripheral blood mononuclear cells. Figure created by biorender.com.

Plasma Therapy for Covid-19

Convalescent plasma therapy has been used as one of medications for some outbreak diseases such as SARS (2003), avian influenza H5N1 (2005-2015), avian influenza H1N1 (2009-2010), and ebola (2013-2015). At present, the therapy becomes the potential therapy to cure COVID-19 patients, although the appliance must be done under supervision from EUA[49] Several studies have reported the effects of convalescent plasma administration in patients who have severe and critical symptoms.

Li et al. (2020) has reported that patients who get plasma therapy show some improvement. In the group of patients who received plasma therapy (n = 52), only 28.6% of patients who died were lower than the other group. Furthermore, the virus clearance is more effective by administering plasma therapy which is confirmed by PCR and shows that it is not detected the presence of viruses that cause COVID-19 from 24 hours to 72 hours after therapy with a 1x24-hour examination interval[50].

Shen (2020) reported his research results on 5 COVID-19 patients who were not smokers and received ventilators during treatment. The study reported that three patients did not use ventilators after plasma administration while the other two patients had been released from ecmo-type ventilators (extra-corporeal membrane oxygenation). In addition, the CT scores of the five patients also reached negative with a range of 1-12 days after convalescent plasma therapy. Similar result also reported by Duan et al who stated that plasma therapy improved the lung condition of patients indicated by the changing in the type of ventilator[51]

The positive effects of plasma administration in patients with severe and critical symptom criteria were also reported by Zeng et al and Duan et al[52,53]. Zeng et al. conducted the research by providing plasma therapy to 6 critical patients. The study reported that 5 out of 6 patients had negative CT scores but died, but the five patients' length of life was longer compared to patients in the group of patients who did not get plasma therapy. This study shows that plasma

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therapy can improve the patient's immune system. Research conducted by Duan et al. reported that the administration of plasma convalescence could improve the patient's lung condition, and there is an elimination of the virus characterized by an increase in CT value until it is indicated negatively on the third or sixth day after convalescence plasma administration[52,53].

Human antibodies for therapeutic purposes can be developed with two approaches: hybridoma technology and genetic engineering divided into four platforms: hybrid mouse, phage display, transgenic mouse single B cell (figure 4)[54]. Both approaches require the involvement of artificial intelligence that can process genetic data into a monoclonal antibody. This artificial intelligence is used to design antigens used in hybridoma technology and design antibody structures that will then be produced using recombinant technology.

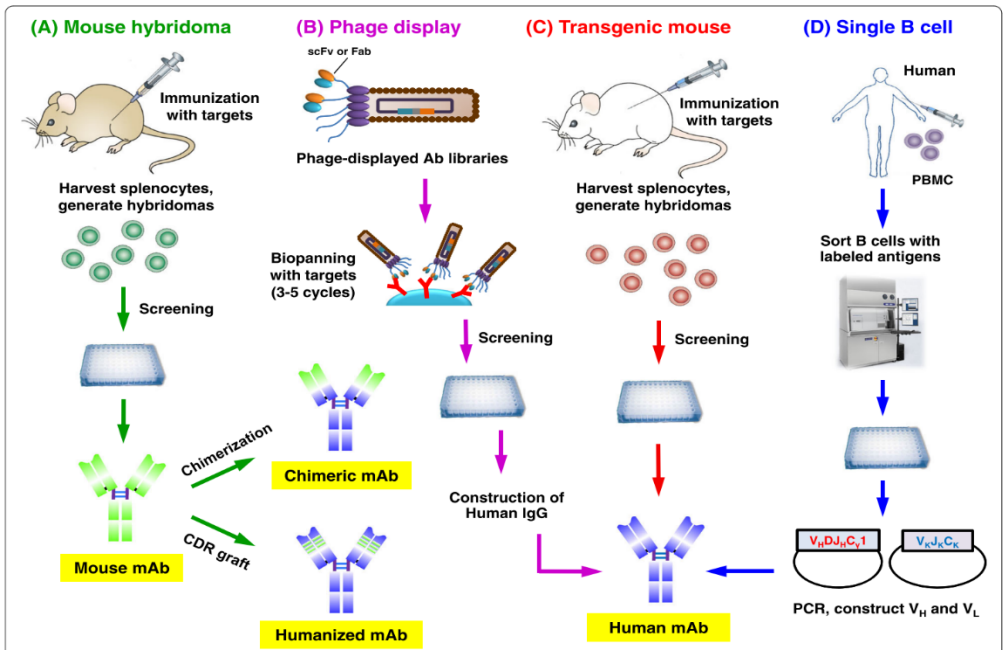

Figure 4. Development of antibody for therapy (cited from Lu et al.[54])

With hybridoma technology, specific antibodies can be obtained by stimulating the immune response to a particular antigen and a particular epitope. Meanwhile, with genetic engineering, antibodies can be produced using genetic information that develops antibodies' structure and is expressed by an expression system. Furthermore, the merging of mice and human antibodies are possible to be produced by engineered antibodies (recombinant antibodies). It is an urge to use bioinformatics tools to develop recombinant antibodies. The bioinformatics also applied to predict the promiscuous epitopes that can be conducted by immunoinformatics [55–57]. By applying bioinformatics, the structure of antibodies, antigen, and antigen-antibody interaction could be visualized by molecuar docking and molecular modelling.

Based on the selected literature, there are two strategies to develop antibodies for therapy in SARS-CoV-2 infection. The first strategy is designing antigens to stimulate the formation of specific antibodies, and the second is by designing human monoclonal antibodies (tables 1 and 2). The in vivo experiments have been conducted to trial the antibodies by using several animal models, namely Llama, horses, and mice[8].

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Antigen design for the development of human antibodies with hybridoma technology

Antigen with high functionality is vital in producing antibodies through hybridoma technology. Due to the formation of antibodies requires cooperation between APC cells and T lymphocyte cells to stimulate B cells to release antibodies. With machine learning, the antigen is designed to interact with T and B cells that predicted or determined by various software for mapping epitopes. A wide range of epitope mapping immunoinformatics software can be used either through web servers or installed software.

The development of SARS-CoV-2 antigens to produce specific proteins was carried out using genetically engineered technology to produce both S and RBD proteins from the SARS-CoV-2 virus. Spike and RBD proteins chose because those proteins have an important role in the mechanism of viral infection in the host cell 57. SARS-CoV-2 virus infects humans by binding to human angiotensin-converting enzyme 2 (ACE2) on the cell surface with RBD region on S protein. The binding of RBD to ACE-2 is the main key to the process of viral infection into the cells[58].

In developing antibodies for COVID-19 therapy, the artficial intelligence plays a role in antigen design from protein selection and predicting the interaction between epitopes with B cells and T cells with molecular dynamic and molecular docking 55 (figure 5). This method is commonly used in vaccine development, but it also used to design an antigen for developing antibody therapy[59].

The application of molecular dynamics and molecular docking as a machine learning provides a very beneficial effect for antigen design because it can provide an overview of the structure and model of the designed antigen. Both machine learning will provide good visualization by merging other methods such as electron microscope and small angle X-ray scattering (SAXS)[9]. In a study conducted by Brett et al (2020), the structure of an endogenous glycoprotein G found in a VSV particle can be compared in size with the SARS-CoV-2 virus S protein designed in the same VSV particle 9. Other researchers used machine learning to predict epitope position in RBD SARS-CoV-2 that can interact with ACE2[18].

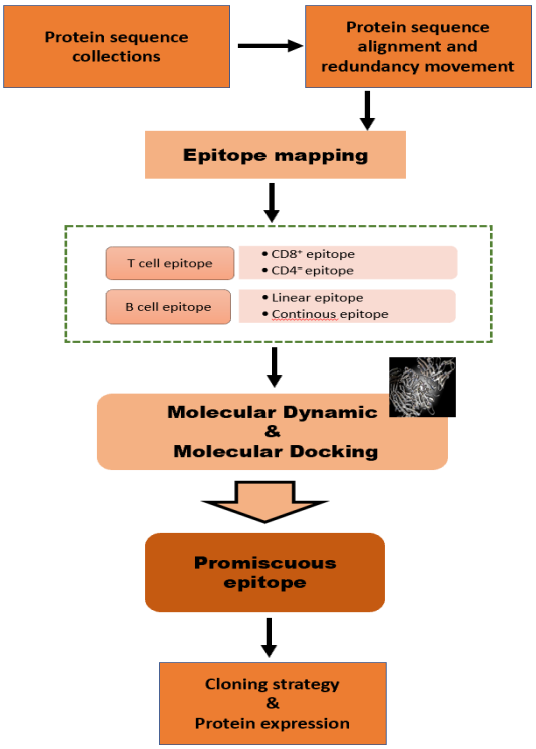

Figure 5. Schematic design of antigen by in silico experiment

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Artificial intelligence also provides another benefit to developing antibody therapy, such as designing the antigen and interaction prediction only with a computational-based study. Shah et al. (2020) conducted antigen and antibody design research just using in silico experiment. This study was conducted to determine the interaction of CR3022 and CR3014 antibodies with both the overall spike protein and RBD area with confirmation analysis and analysis of ligand interaction fingerprints (PLIF) proteins. The study also provides an overview of the 3-dimensional structure of spike proteins and RBD regions [32].

Human antibody design with recombinant technology

At the beginning of its development, there are three kinds of recombinant antibodies, namely chimeric antibody, humanized antibody and fully human antibody (Figure 4). Those three antibodies developed to minimize the rejection of patients who receive therapy Antibodies. On chimeric antibody, modifications are made on the constant domain of antibodies with maintains the territory of fragment antigen binding (Fab) derived from animals. Then chimeric antibody developed into humanized antibody conducted by inserting the animal's complementary-determining region (CDR) into the antibody sequence with CDR grafting technology and subsequently developed into a fully human antibody. Fully human antibody provides an auspicious opportunity for treatment with antibodies, but these antibodies can still cause rejection reactions from patients, so two antibody formats are developed, fragment antigen binding (Fab) and single chain fragment variable (ScFv) (Figure 5).[6,47]

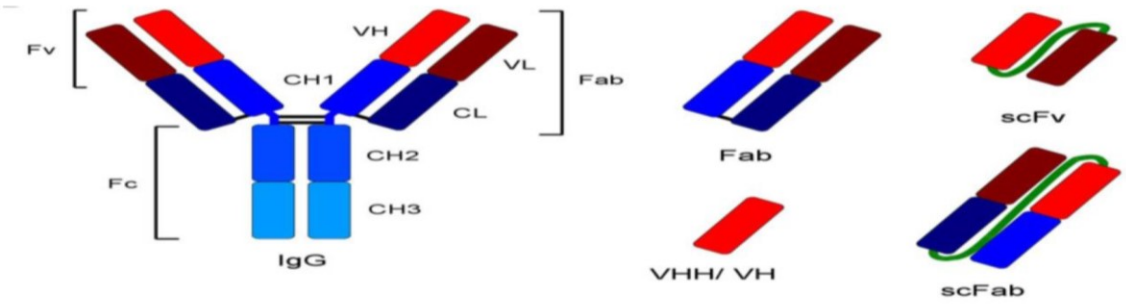

Figure 5. Types of recombinant antibodies (cited from Kuhn et al.[47])

The development of artificial intelligence has a very significant effect on the process of antibody development. In the first beginning, the antibody was employed using recombinant technology and followed by in vitro and in vivo experiments, which were performed by histology and statistics. Currently, the binding position of antibodies designed on antigen targets can be visualized using various machine learning (Figure 6). In developing antibody therapy for COVID-19, scientists not only used viral proteins as the antigen, but receptors (ACE-2) were also possible to be designed as antigens. Scientists used various mammalian cells to express ACE-2, such as CHO (Chinese Hamster Ovarian) and HEK (human embryonic kidney) cells (Table 2).

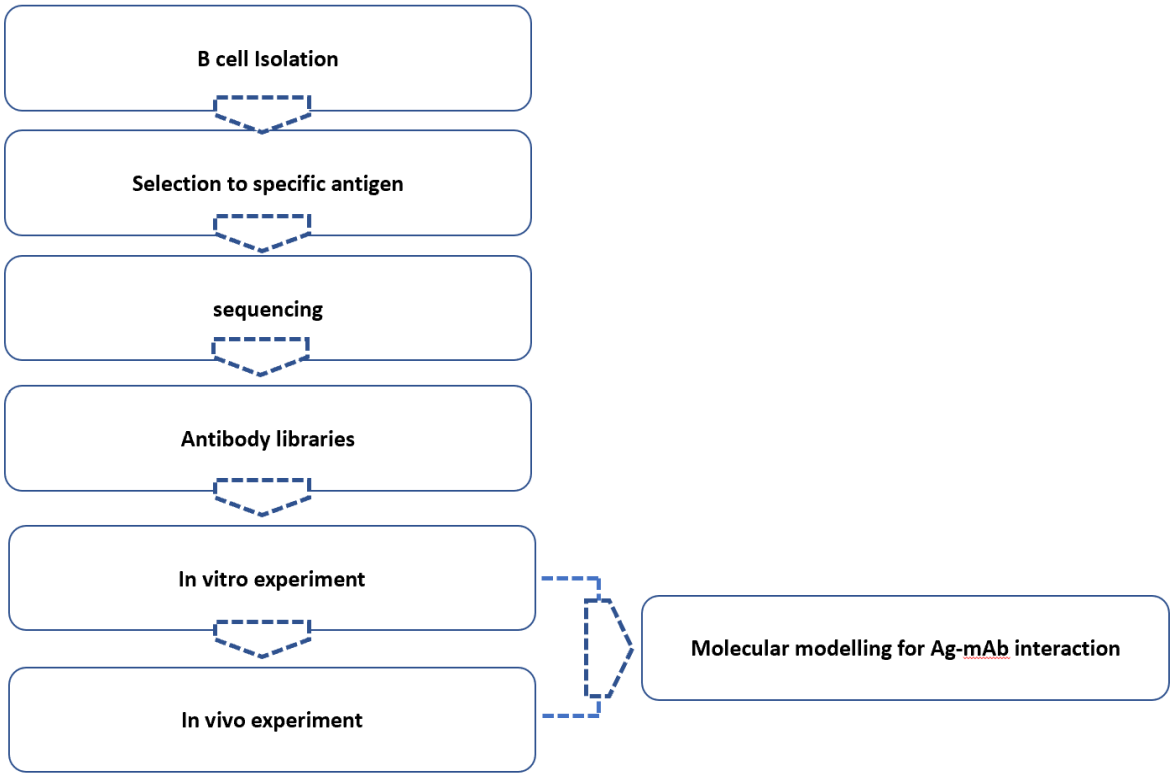

Figure 6. Schematic design of antibody development using artificial intelligence

The employment of machine learning has made it easier for researchers to get accurate information about antigens' interaction with antibodies designed. Besides, the information can be used by researchers to provide an explanation of its effects. Chi et al (2020) use cryo-EM to determine the interaction between the antibodies that have been designed (4A8) to the SARS-CoV-2 virus S-ECD antigen. The results of their study stated that the designed monoclonal antibodies have an excellent neutralization effect which is not caused by interruptions in the binding of RBD with ACE2. The molecullar modelling shows that neutralization is likely to occur as a result of inhibition of changes in confirmation of protein S[10].

CONCLUSION

Antibodies for therapeutic for COVID-19 have been utilized by artificial intelligence. It took parts in antigen and antibody design. Moreover, the visualization of antibody structures is one of the results from data processing using machine learning adopted to predict the neutralization effect by antibodies from research in vitro and in vivo. The utilization of artificial intelligence will enhance the research in developing antibodies as COVID-19 therapy.

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