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Review Article

Antibody Therapy: Past, Present and Future

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Abstract

The emergence of pandemics like SARS-CoV-2 and a gradual increase in Multidrug Resistant (MDR) infections highlights the need of innovation in therapeutics. Antibodies are one of the potential solutions for long. Antibody therapy has come very long way from the fight against infectious diseases, bacterial toxins to hybridoma technology and monoclonal antibodies. Hybridoma cells receive a deserving attention due to their antigen-specificity. But, as they were murine in origin, Human Anti Murine Antibody (HAMA) emerged. To achieve this, phage display was introduced. The emergence of molecular cloning lead to the generation of genetically engineered recombinant antibodies such as Fab, Fc, Variable Fragment (Fv), Single Chain Variable Fragments (scFv), single domain antibodies, diabodies; like scFv fragments to different moieties, such as drugs toxins, radionuclides, liposomes or quantum dots etc. Minimized antibodies have several advantages like rapid blood clearance, reduced immunogenicity, low retention time in non-target tissues, access to cryptic epitopes facilitating tumor penetration, rapid growth facilitating higher yield and lower production cost. This paper gives an overview of the history of development of antibodies and its fragments as potential therapeutic agents for the treatment of infectious diseases, one of the biggest challenges of humanity.

Keywords: scFv; Recombinant antibody; Human anti murine antibody; Single domain antibodies; Fv fragments; Fab fragments; SARS-CoV-2

Introduction

Antibodies can be potent inhibitors of a number of viral infections which also include the present pandemic Coronavirus Disease 2019 COVID-19 [1-4]. The Coronavirus Disease 2019 (COVID-19) caused by a novel coronavirus Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) has started in Wuhan, China and then received a worldwide attention. Researchers all over the world are trying their best to find effective therapeutic agents against this virus but no such potent antiviral drug has been discovered yet. Convalescent plasma collected from recovered patients is supposed to contain viral neutralizing antibodies and thus can be potentially used for the treatment of infected individuals [2-4]. In several cases, this therapy has been applied with success [5-6]. On the other hand, the majority of death from the pandemic COVID-19 is taking place due to respiratory failure from Acute Respiratory Distress Syndrome (ARDS) [7-9]. In several patients with COVID-19, a state of hyper inflammation known as secondary haemophagocytic lymphohistiocytosis has been observed which develops a storm of cytokines after infection [10]. This hypercytokinemia is mainly responsible for acute lung injury in the infected individuals. This can be prevented by cytokine inhibitors which are mainly modulators of cytokine responses, thus playing a therapeutic role in the pathogenesis of COVID-19 [11]. Among the proinflammatory cytokines, Tumor Necrosis Factor-α (TNF-α) has been suspected as the major mediator of the immune-based lung injury following infection with SARS coronavirus [12]. Thus, if TNF-α can be inhibited, it can potentially reduce the lung damage which is actually the major cause of the mortality. On the other hand, multidrug resistant bacterial strains have emerged due to the misuse, use or overuse of antibiotics and this creates a global therapeutic challenge. On August 25, 2016, a strain of *Klebsiella pneumoniae* was found to be resistant to all 26 antibiotics which include aminoglycosides and polymyxins tested at an acute care hospital in the Washoe County Health District in Reno [13]. The emergence of multi drug resistant bacterial strains underlines the necessity of potent treatment for drug resistant bacteria. Thus, antibodies may represent as one of the potential and promising futuristic alternative to the commercial drug therapies for the treatment of challenging infectious diseases. Serum therapy was successfully developed and first used for the treatment of bacterial infections in human in the 1890s [14-16]. But serum therapy was partly abandoned with the emergence of antibody because of toxicities and difficulty in purification and production of antibodies to single determinants at that time [14]. But, gradually with the advancement of technology, high Specificity of Monoclonal Antibodies (mAbs) is responsible for remarkable developments in the field of therapeutics, diagnostics and research till date [17]. But, monoclonal antibodies like Adalimumab, infliximab, rituximab etc. have the major disadvantages of high cost, difficulties in production and low tissue penetration [18,19]. This has led to the shift in attention to the production of small fragments which are more specific. Due to the gradual improvement in recombinant DNA technology, genetic manipulation has been facilitated [20,21]. The cost effective alternative is therapeutics with antibody fragments which has led to the emergence of second and third generation of antibody therapy, offering innovative opportunities in biopharmacy and therapeutic world.

Classic Drug Development

Conventional drugs that are used for therapeutic purposes are synthesized by chemical reactions between different organic or inorganic compounds. These are small active molecules processed into ingestible tablets/capsules after dissolution of which in the

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gastrointestinal tract, the Active Product Ingredient (API) is absorbed in the bloodstream through the intestinal wall [22]. In Biologics, Biopharmaceuticals refer to the drugs based on therapeutic proteins that bind to specific cell receptors associated with the anomaly [23]. But in case of conventional drug therapy, drugs also attack body's own cells, besides the foreign substance [24]. Since these drugs are very small in size and many molecular components are present inside the cell, it is difficult for the new small drug to block only problematic processes leading to nonspecific interaction [24]. This results in serious side effects due to off target interactions. Not only that, it takes a lot of time for the development of traditional drugs [25]. Moreover, due to the emergence of multidrug resistant as well as novel pathogens, the antimicrobial conventional drugs have become less effective and it is difficult to implement the anti-infective therapy successfully. Again, it is also difficult to treat infections in immunocompromised patients [26].

Emergence of Antibody Therapy

Antibodies are the glycoproteins and antigen binding proteins present on the B cell membrane and secreted by plasma cells, that recognize the foreign invading molecules. Each of them can recognize and bind a target molecule with the help of its antigen-binding site, paratope (located on the two Fab segments) specific to a particular epitope on an antigen [27]. Thus antigens act as powerful defense system protecting the body against foreign non-self-agents [27]. Moreover, in contrast to conventional drugs, antibodies have low toxicity, high specificity and comparatively need short term timescale for development [28]. Thus, antibodies can act as futuristic alternative to the commercial drug therapies for the treatment of infectious diseases such as novel viruses and multidrug resistant bacteria. Over time, with the development of technology and science, recombinant technology has been explored through somatic recombination and hypermutagenesis of sets of variant genes to give rise to diversity [27,29]. In the early 1890s, the concept of serum therapy was first developed by Behring and Kitasato and passive antibodies were transferred to combat against bacterial toxins [16]. By 1930s, passive antibody administration was being widely used for the treatment of individuals infected with bacteria, fungi and viruses [16]. After the introduction of Hybridoma technology [30], monoclonal antibodies are produced in remarkable quality and enormous quantity in the laboratory that are preferable as they can specifically target a certain antigen. But initially, these were exclusively murine in origin, so after introducing to human, Human Anti Murine Antibody (HAMA) were created [31,32]. Moreover, production technology is laborious and time consuming [27]. Also, high affinity antibody response to particular antigen cannot be provided by mammals like mice [33]. Due to these limitations, several research groups have tried to investigate the use of phage display for production of recombinant antibodies [27]. The recombinant antibody technology was first utilized in 1984 [34,35] in the bacteria but improper folding and polypeptide aggregation in the cytoplasm of the bacteria were the major difficulties for production of recombinant antibodies [20,27,36]. To overcome this, Skerra and Pluckthun in 1988 [37] started a technology where only parts of antibody molecule (Fab or Fv fragments) were used for expression. Various types of vectors were introduced for *E. coli* expression of antibody fragments, which could directly secrete the proteins into periplasmic space [27,37,38]. This offers an advantage with reduced size retaining the intact antigen binding site [27].

Antibody Fragments-Minimized Antibodies

IgG can be cleaved, by papain into two identical Fab fragments of 45KDa, or by pepsin to form a $F(ab')_2$ with 100KDa [39] (Figure 1). Later with the advancement in technology, mAbs are further reduced to scFvs, monovalent Fabs, diabodies, minibodies [40,41]. There are several advantages of minimized antibodies- rapid blood clearance, reduced immunogenicity, lower retention time in non-target tissue, access to cryptic epitopes facilitating tumor penetration [27,42,43], large scale production using microbial expression system, rapid growth facilitating higher yield and lower production cost [43,44]. The advancement of the latest technology and molecular cloning lead to the generation of genetically engineered recombinant antibodies [45-47] such as fragment antigen binding (Fab) [48], the Fragment Crystallizable (Fc) domain, Fragment Variable (Fv) in which V domains are connected by non-covalent forces [37], the single chain variable fragment in which V_H and V_L genes are joined together with short peptide linker [49,50], bi-scFv where V_H and V_L are region joined through disulphide bridge by the introduction of cysteine residues [51], single domain antibodies etc. Peptide linkers of bigger than 12 residues provide sufficient spatial flexibility for V_H and V_L domains to associate as an independent scFv monomer. Two separate scFv molecules are required for complementary V_H/V_L pairs to associate and form a bivalent dimer, termed as a diabody [52,53]. Increase in avidity takes place when Fab or scFv molecules are complexed to form diabodies or triabodies [40]. There have been many attempts to conjugate Fab or scFv molecules into diabodies and triabodies to produce high avidity, which are capable of rapid tissue penetration without fast renal clearance [41]. In clinical trials, 30 percent of Biopharmaceuticals are recombinant antibodies [54]. Gradually with the emergence of new technologies, antibodies are reduced in size, valency is increased for higher affinity to form bivalent [55] and multivalent molecules [56,57], and also antibody fragments with different specificities are linked to form bispecific [40], multi-

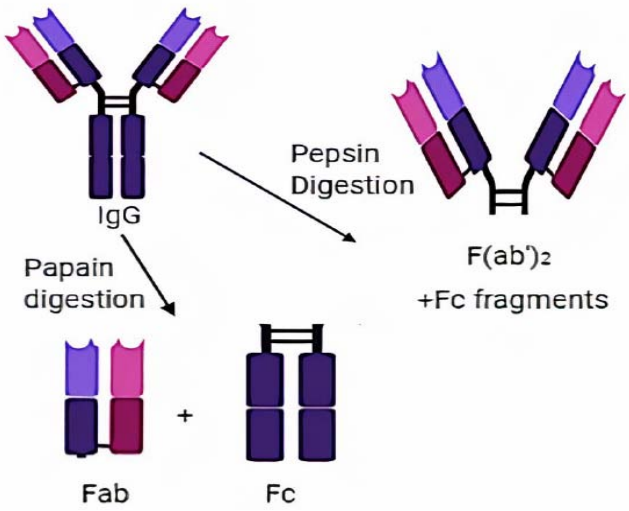
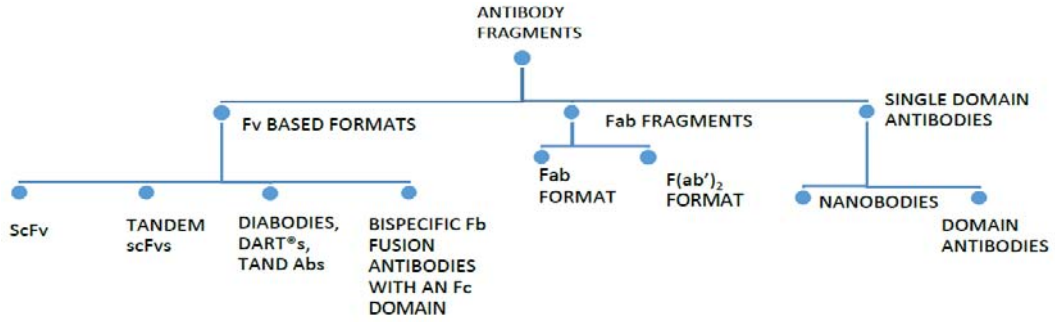


Figure 1: Generation of Fab, $F(ab')_2$ and Fc fragments [39].
IgG on papain digestion gives rise to Fab and Fc fragments (left side down). By pepsin digestion of IgG, $F(ab')_2$ fragment is generated (right side).
Fab: Fragment Antigen Binding; Fc: Fragment Crystallizable; $F(ab')_2$: Fragment Affinity-Purified Secondary Antibody.



Scheme 1: Different antibody fragments and their classification [43].
Antibody fragments are broadly classified into Fv based fragments, Fab fragments and single domain antibodies. Fv based fragments are further classified into scFv, tandem scFv, diabodies, DARTs, TAND Abs and bispecifics antibodies fused with Fc domain. Fab fragments can be of Fab format or $F(ab')_2$ format. Single Domain Antibodies are further classified into nanobodies and domain antibodies.
Fv: Fragment Variable; Fab: Fragment Antigen Binding; $F(ab')_2$: Fragment Affinity-Purified Secondary Antibody; scFv: Single Chain Fragment Variable; DARTs: Dual Affinity Retargeting Proteins; TAND Ab: Tetravalent Bispecific Antibody.

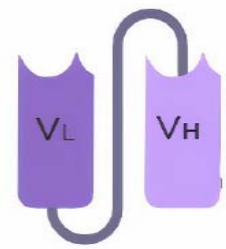


Figure 2: The single chain fragment variable format [43].
V_H and V_L are connected together by a glycine and serine rich flexible peptide linker.
V_H: Variable region of the heavy chain of an antibody; V_L: Variable region of the light chain of an antibody.

specific, multimeric, or multifunctional molecules [43,58]. Different antibody fragments and their classification is depicted in Scheme 1.

Single chain variable fragment (scFv)

ScFv is the most common recombinant antibody format. It is the smallest functional unit of an antibody containing the complete antigen binding domains of an intact antibody. The heavy chain variable domain and the light chain variable domain are connected together by a flexible peptide linker. The connection can be either in the V_H - linker - V_L or V_L - linker -V_H orientation, the former being more common (Figure 2). The expression of scFv of *Pichia pastoris* system is V_H linker V_L orientation [59]. This can be easily expressed in functional form in *E. coli* and it helps to improve the properties of scFv such as increase of affinity, avidity and structural stability. There are few drawbacks of using scFv like low retention on the target and rapid clearance as they are monovalent. It has short *in vivo* half-life for which the target molecule cannot be exposed to the scFv for a long time [51,60]. So scFvs are modified to multivalent and multifunctional multimers that have higher affidity with tissue penetration abilities not being compromised. In fact diabody is considered as the optimal tumor targeting fragment [61]. Moreover, through linking of scFv fragments to different moieties, such as drugs toxins, radionuclides, liposomes or quantum dots and also through fusion with albumin or Poly-Ethylene Glycol (PEG), further engineering of scFv fragments can be done [43].

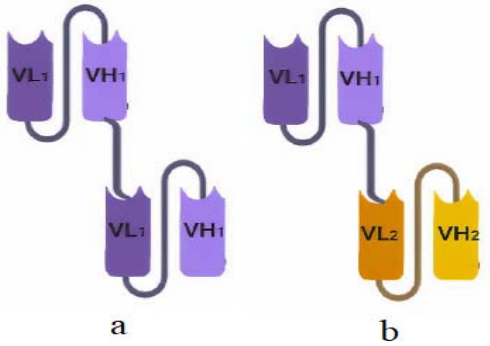


Figure 3: Tandem scFv format [43]. a) Two identical scFvs are joined together through helical peptide linker in the orientation NH_2-VL_1-VH_1-(linker-VL_1-VH_1)$_n$-COOH to form monospecific bivalent tandem scFv. b) Two different scFvs are joined together through helical peptide linker in the orientation NH_2-VL_1-VH_1-(linker-VL_2-VH_2)$_n$-COOH to form bispecific bivalent tandem scFv.
VL_1: Variable fragment of the light chain of the first antibody; VH_1: Variable fragment of the heavy chain of the first antibody; VL_2: Variable fragment of the light chain of the second antibody; VH_2: Variable fragment of the heavy chain of the second antibody.

Tandem scFvs

Two scFvs can be connected through a linker to form bivalent antibodies known as tandem scFv molecules (taFv, $scFv_2$) [62] (Figure 3). The first tandem scFv molecule was generated 20 years back when fusion of two scFvs was done by a 27 amino acid helical linker [63-65]. Due to the presence of the molecular interaction only between the V_H-V_L interface with no covalent linkage, scFv molecules are not stable individually. This stability can be improved in case of tandem scFvs through the introduction of an interdomain disulphide bond between the V_H and V_L domain which generates disulphide stabilized scFv (ds Fv, ds scFv) [66].These bivalent tandem scFvs can be monospecific when two identical scFvs are joined by a helical linker or bispecific when two different scFvs are joined by a helical linker.

Diabodies, DART®s, TAND Abs

Diabodies consist of two different chains each composed of one VL and VH domain from different antibodies arranged in a head to tail manner [61] (Figure 4). A pentameric glycine-rich linker (G4S) separates the two domains within a chain. Diabodies are bivalent and

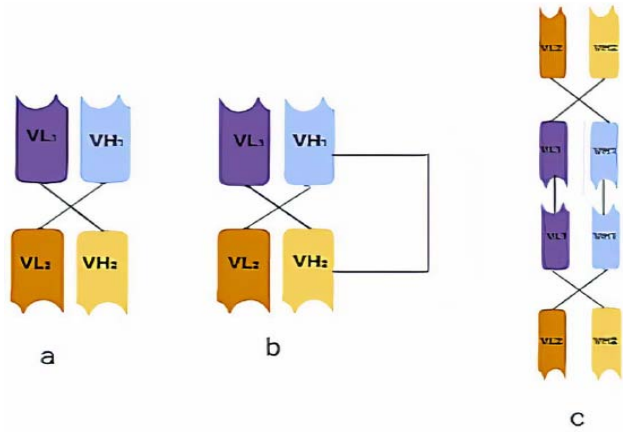


Figure 4: Bispecific diabody, DART®, and TandAb fragments [43]. a) Bispecific Diabody with two different chains each composed of one V_L and V_H domain from different antibodies arranged in a head to tail manner. b) Dual Affinity Re-Targeting (DART®) protein with two distinct polypeptide chains linked by a non-covalent interaction and disulphide bond. c) Tetravalent Bispecific Molecule (TandAb) formed by fusion of two diabodies in a linear way.
V_{L1}: Variable fragment of the light chain of the first antibody; V_{H1}: Variable fragment of the heavy chain of the first antibody; V_{L2}: Variable fragment of the light chain of the second antibody; V_{H2}: Variable fragment of the heavy chain of the second antibody.

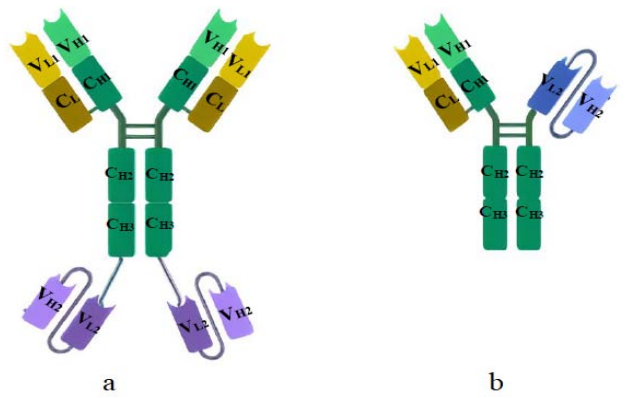


Figure 5: Bispecific Fv Fusion Antibody formats [43]. a) IgG-scFv where scFv of one antibody is attached with another extra antigen or epitope. b) Fab-scFv-Fc-IgG where one arm of IgG is exchanged for a scFv of another IgG. V_{H1}: Variable region of heavy chain of the first antibody; V_{L1}: Variable region of light chain of the first antibody; V_{H2}: Variable region of heavy chain of the second antibody; V_{L2}: Variable region of heavy chain of the second antibody; C_{H1}, C_{H2} and C_{H3}: Constant regions of heavy chain of the first antibody.

bispecific in nature, playing a major role in cancer immunotherapy, e.g., Bispecific T-Cell Engagers (BiTE®s) [52,67]. Dual Affinity Retargeting Proteins (DART®s) are modified diabodies with improved stability where two distinct polypeptide chains are linked by a non-covalent interaction and disulphide bond [67] (Figure 4). These are bispecific in nature. Three or four variable domains can be linked together in a single chain to produce modified tri and tetravalent molecules with increased stability and similar structure to diabodies. When two diabodies are fused in a linear way, they lead to the formation Of Tetravalent Bispecific molecules (TandAbs) (Figure 4).

Bispecific Fv fusion antibodies

Some examples of bispecific modified scFv formats are the IgG-scFv where scFv is attached with an extra antigen or epitope and Fab-scFv-Fc-IgG where one arm of IgG is exchanged for a scFv [62] (Figure 5). These formats are more flexible.

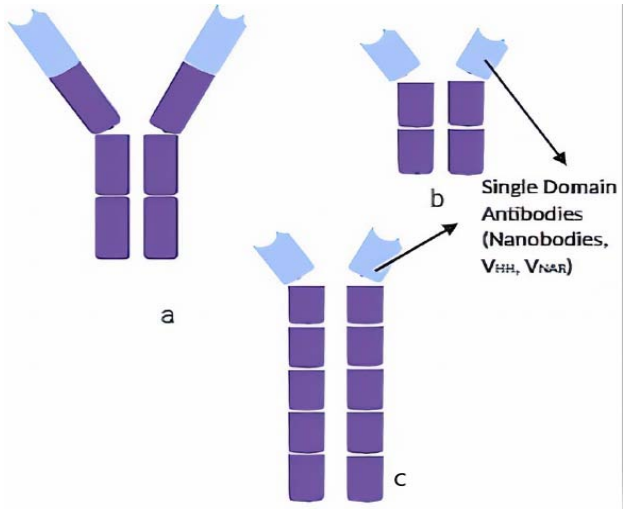


Figure 6: Single Domain Antibody format [71]. a) Structure of a full length regular antibody. b) Structure of a Heavy Chain Only Antibody (H_{CAb}) derived from camelid (V_{HH}). c) Structure of a Heavy Chain Only Antibody (H_{CAb}) derived from shark (V_{NAR}).

Single domain antibodies

These are fully human variable domains (either variable heavy or light chain V_L or V_H) in which replacement of the hydrophobic residues at the V_H/V_L interface is done with the hydrophilic residues found in camelid V_{HH} through camelisation. [68,69] (Figure 6). It helps in preventing dimerization. These are small antigen binding fragments of molecular weight approx. 15 Da with high solubility, stability and short half-life. Yield of expression of this protein is high in both prokaryotes and eukaryotes [69]. Recently, natural Single Domain Antibody (sdAb) fragments are discovered in camelids (V_{HHs}), cartilaginous fish (VNARs) and lampreys (variable lymphocyte receptors VLRs) and they have remarkable features of identifying clefts and cavities on protein surfaces that are not found in conventional recombinant antibodies [70]. An overview of the history of development of antibody and antibody fragments as therapeutic agents are depicted in Table 1. Chronological timeline from 1975 to 2019 showing the successful scientific development of therapeutic antibodies is depicted in Figure 7. Gradual emergence of first, second and third generation of antibodies for therapeutic purpose is shown in Table 2. Few names of antibody fragments which are FDA/EMA approved or evaluated till clinical phase trials are shown in a chart in Scheme2.

Application of Antibody Therapy in Infectious Diseases: Potential Treatment for Pandemic COVID-19

Antibody therapy has been widely used for the treatment of infectious diseases. Synagis® (Palivizumab), Abthrax® (Raxibacumab) and Zinplava™ (Bezlotoxumab) are the three mAbs approved for the treatment of Respiratory Syncytial Virus (RSV) infection, anthrax and *C. difficile* infection respectively [97]. Among the antibody fragments, Afelimomab ($F(ab')_2$), Rivabazumab pegol (pegylated Fab) and ALX-

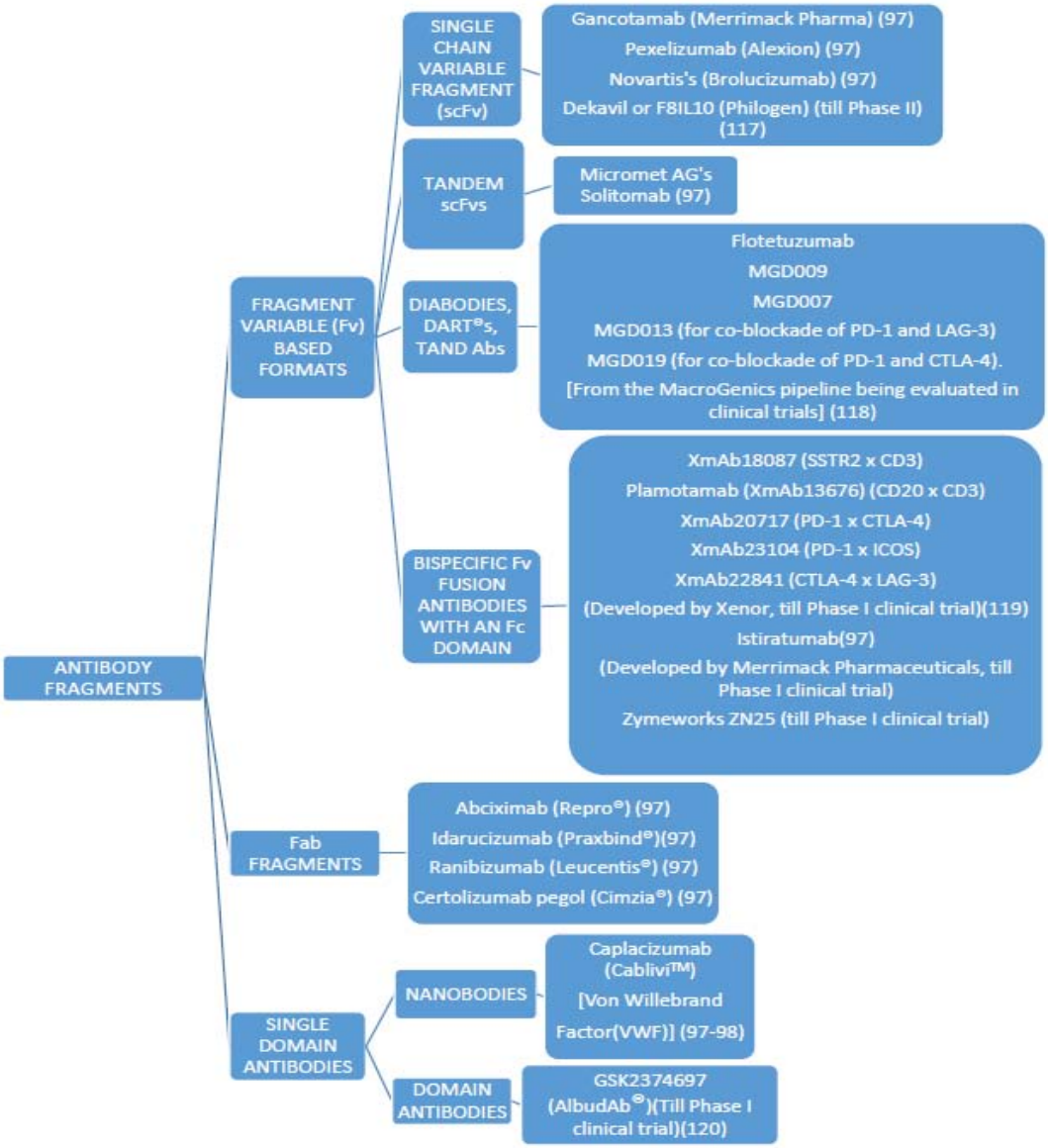
Table 1: Overview of History of Development of Therapeutic Antibodies (With the Mention of Year and Major Scientific Developments).

Year	Major Scientific Developments	Reference
1890	Serum Therapy by Behring And Kitasato.	[16]
1897	Magic Bullet Concept by Paul Ehlrich.	[72,73]
1960	First Mention of the Bispecific Antibody (bsAb) Concept.	[74,75]
1964	First Demonstration of the bsAb concept.	[75,76]
	First fragment based format.	
1975	Hybridoma technology by Kohler and Milstein.	[30]
1982	FDA approval of first recombinant protein product- HUMULIN.	[77]
1984	Chimerization of antibodies.	[78]
1985	Phage display technology.	[79]
1986	FDA approval of the first monoclonal antibody (mAb)-Muromonab CD3 (Orthoclone OKT3®).	[80-82]
1988	SKERRA et al. Suggested the use of only some parts of fragments from the whole antibody molecule.	[37]
	First strategy to link the variable light and heavy region via peptide linker.	[49,50]
1990	Introduction of cysteine residues to aid in formation of disulphide bridge between V_L and V_H region (biScFv).	[51]
1994	FDA approval of the first Fab Fragment-Abciximab (REOPRO®).	[80-83]
1997	FDA approval of first chimeric IgG-RITUXIMAB (RITUXAN®).	[80-82,84]
	FDA approval of the first humanized Monoclonal Antibody-Daclizumab (ZENAPAX®).	[80-82,85,86]
2000	FDA approval of the first Ab-DRUG conjugate - gemtuzumab ozogamicin (MYLOTARG®).	[80-82]
2002	FDA approval of the first fully human monoclonal antibody→ Adalimumab (HUMIRA®).	[80-82,87]
	Soluble expression of recombinant immunotoxin in insect cells	[88]
2005	FDA approval of first modified Fc Fusion Protein- Abatacept (ORENCIA®).	[81-82,89]
2006	Functional expression of a single chain antibody in plant system.	[90]
	Isolation of anti Fv by Fv display on human cells.	[91]
2007	FDA approval of first IgG with modified Fc-Ecolizumab (SOLIRIS®).	[80-82,92]
2008	FDA approval of first peptibody-Romiplostim (NPLATE®).	[93]
2009	EMA approval of first bi-specific monoclonal antibody- Catumaxomab (REMOVAB®).	[80-82,94]
	FDA approval of first human monoclonal antibodies from medarex mice-golimumab (SIMPONI®) and Ustekinumab (STELARA®).	[80-82]
2014	FDA approval of bispecific t-cell engager (BiTE®) –BLINATUMOMAB (BLINCYTO®).	[80-82,95]
2018	Phase I clinical trial of an ImmTAC® against different varieties of cancer.	[96]
	EMA approval of bivalent vhh targeting von Willebrand factor (vWF).	[97]
2019	Phase I clinical trial of imc-c101c against melanoma associated antigen-4.	[96]
	FDA approval of bivalent VHH targeting VON Willebrand factor (vWF)-CAPLACIZUMAB (CABLIVI®).	[97,98]

0171 (trivalent Nanobody® -- VHH_3) are the three molecules which are in clinical trials for the treatment of sepsis toxic shock, chronic Pseudomonas aeruginosa infection and RSV infection respectively [97]. T-cell mediated adaptive immunity involves destruction of virus infected cells via CD8+ T cells which reduces the viral load, thereby preventing viral replication. Moreover, Antibody Dependent Cellular Cytotoxicity (ADCC) mediated natural killer cells, when activated, not only helps in neutralizing the virus but also kills the infected cells which express viral proteins on their surface. AstraZeneca scientists have pressure tested neutralizing antibodies against influenza-A with DARPA P3 program [1]. In the same way neutralizing antibodies can also be used against the novel coronavirus of Coronavirus Disease 2019 (COVID-19). The Coronavirus Disease 2019 (COVID-19) caused by a novel coronavirus - Severe Acute Respiratory Syndrome- Coronavirus-2 (SARS-CoV-2) has become pandemic throughout the world. As of 17th May, 2020, there are 4,770,876 positive cases and 314,599 deaths due to SARS-CoV-2 all over the world (https://worldometers.info/coronavirus/) [121]. Currently there is unavailability of any cure for COVID-19. Researchers are trying their best to find any potential drug against the novel coronavirus and attempts have been made in implication of several retroviral medications such as Lopinavir/Ritonavir and Remdesivir [122], anti-malarial drug chloroquine [123] and combination of hydroxychloroquine and azithromycin [124]. Neutralization of the SARS-CoV-2 can be done by antibody based therapy and thus can be a potential treatment option for the infected individuals. It can provide immediate effect in the patient [1]. The spike protein on the SARS-CoV-2 virus can be targeted and this will help the antibody to neutralize the SARS-CoV-2 and prevent to enter the host cell and infect normal healthy cells (Figure 8). Once the antibody can bind the spike protein and helps neutralizing it, develop ability tests can be carried out further [1]. Thus, it is hoped that antibody based

Table 2: 1st generation, 2nd generation and 3rd generation antibodies for therapeutic purpose.

1st generation antibodies	2nd generation antibodies	3rd generation antibodies
• Chimeric Antibodies Mouse variable region and human constant region Since they have the original murine variable region, they will bind to the same epitope and as they have the human constant region, so less immunogenic and can interact with human receptors for improved therapeutic effects. Ex: Rituximab (used to treat non- Hodgkins lymphoma) • Humanized Monoclonal Antibodies (mAbs) Same as chimeric mAbs, but more exchange of human sequences for murine sequences within the variable region to further reduce the immunogenicity, with a chance of high efficiency [113]. Ex: Alemtuzumab. • Fully Human Monoclonal Antibodies Genetically engineered mice to have B- lymphocytes with totally human genes for all parts of Antibodies-manufactured with higher efficiency [114]. Ex: Adalimumab.	• Introduction of Antibody fragments (Fab and scFv). • Power of phage display technology in late 1990s [115,116]. • Humira is the first FDA approved fully human mAb obtained from a phage displayed human antibody library [80-82,87]. • Further development in antibody engineering.	• Additional features over conventional monoclonal antibodies and antibody fragments (Fab and scFv). • First VH-H based therapeutic drug-Caplacizum [98], a bivalent anti-Von Willebrand factor (vWF) antibody for treating rare blood clotting disorders approved by the EMA in October 2018 and the FDA in February 2019 [80-82].



Scheme 2: Antibody fragments which are FDA/EMA approved or evaluated till clinical phase trials [43].
As per the classification of antibody fragments, the names of few FDA/EMA approved antibodies from each type of antibody fragment are mentioned including their brand name. Several antibody fragments which are evaluated only till clinical trials are also mentioned [43].

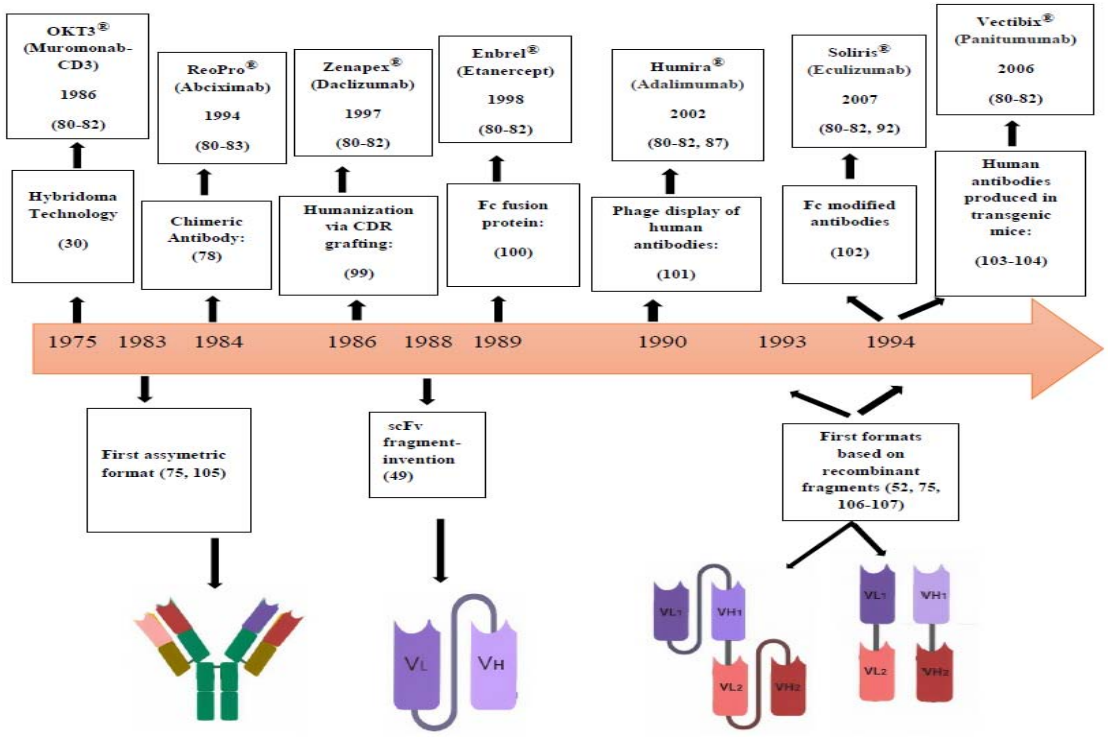


Figure 7a: Chronological timeline showing the successful scientific development of therapeutic antibodies [110,112]. Timeline from 1975-1994 showing successful developments in the field of antibody engineering starting from the discovery of hybridoma technology to the regulatory approval of antibody and antibody fragments.

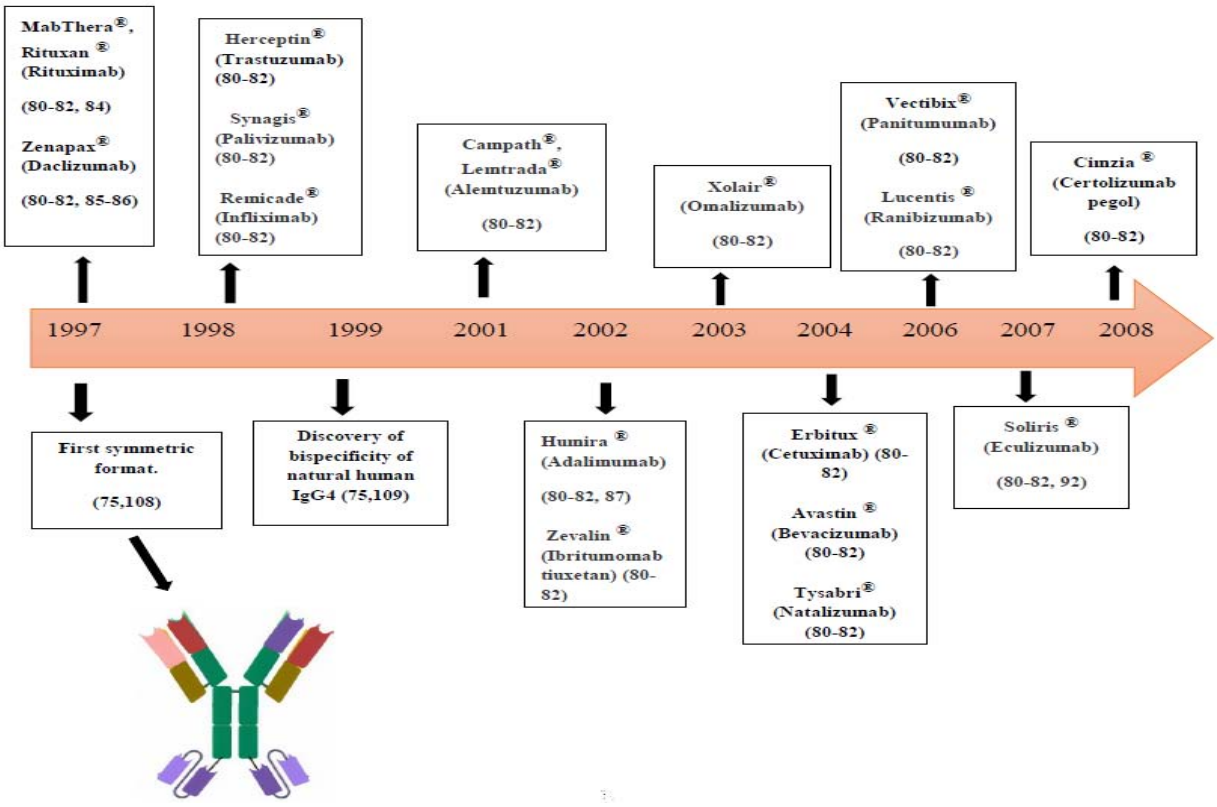


Figure 7b: Chronological timeline showing the successful scientific development of therapeutic antibodies [110,112]. Timeline from 1997 to 2008 including US FDA-approved monoclonal antibody on the market with the mention of their brand name and year of the first US FDA approval.

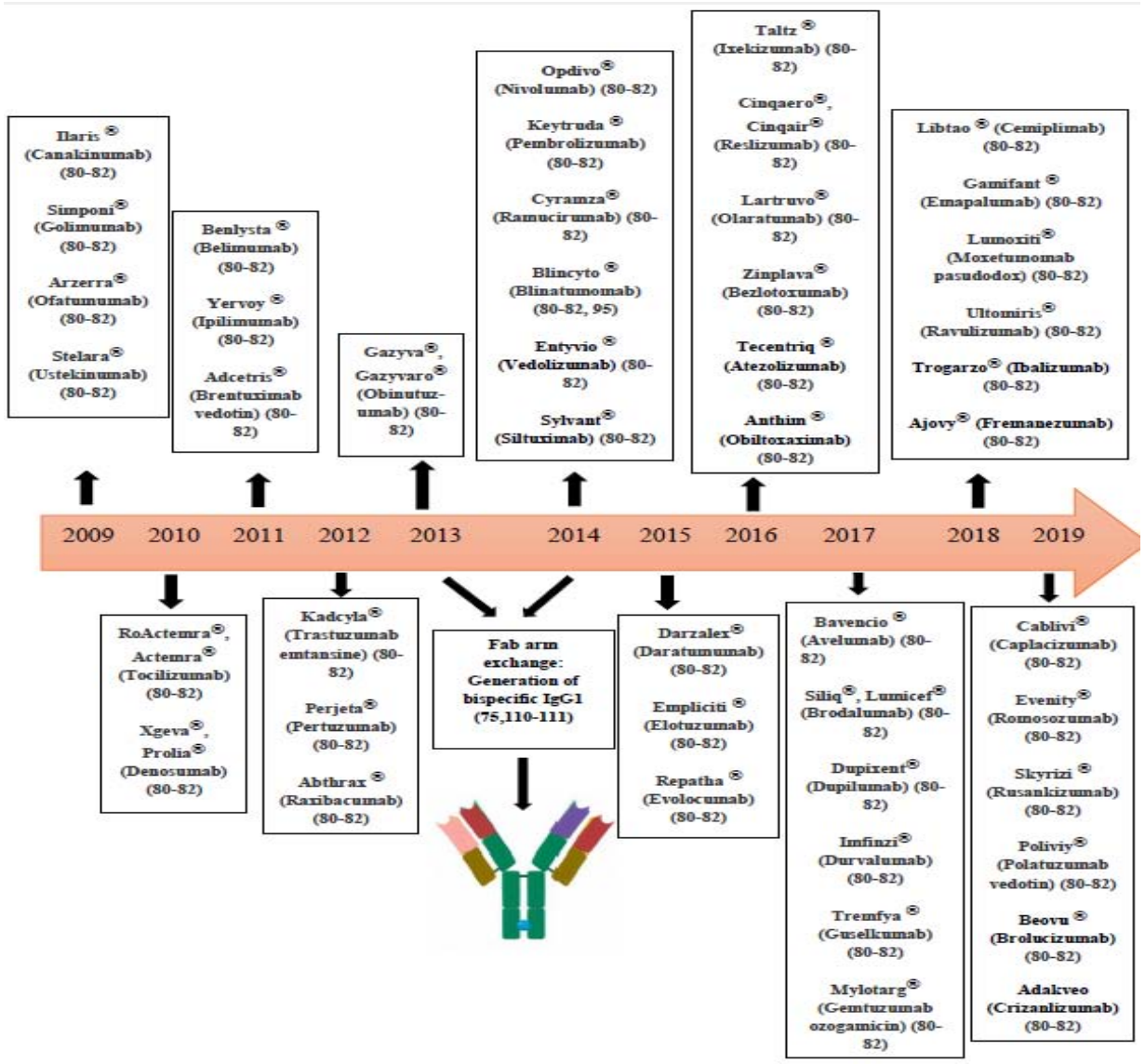


Figure 7c: Chronological timeline showing the successful scientific development of therapeutic antibodies [110,112]. Timeline from 2009 to 2019 showing major technical advances in the development of monoclonal antibodies as well as bispecific antibodies. A large number of antibodies received approval from the US FDA from 2018 to 2019.

therapy can be effectively used for treatment against coronavirus after scaling up and clinical trials. Antibodies can be captured and screened from different primary B cells through immune replica technology [1]. Hybridoma technology can also be used by fusing B cells with immortal myeloma cells to form hybrid cells for production of monoclonal antibodies [1]. The antibodies against the SARS coronavirus can be taken from various sources such as the patients recovered from COVID-19, SARS-CoV-2 spike protein immunized humanized mice and with phage display technique [1].

Convalescent plasma therapy

Human convalescent plasma from the recovered patients could be used for treatment of SARS-CoV-2 infected patients [2-4]. The liquid component of blood known as convalescent plasma can be taken from the patients recovered from the coronavirus infection. This plasma will contain the neutralizing antibodies which will help in the neutralization of the spike protein of SARS-CoV-2 that attacks the human protein (Figure 9). The concept of treatment with convalescent plasma is not new. It was used for the treatment of measles and mumps till the availability of vaccines. It was also used against SARS and MERS epidemics [16]. Unfortunately, while using this donated plasma first time for the treatment of COVID-19 patients in Shenzhen, China, the impact of this treatment could not be understood clearly although few patients were recovered as of March 25, 2020 [5]. This was because antiviral medications were also given to the patients along with this treatment, and thus the cause of recovery could not be properly predicted [5]. U.S. Food and Drug Administration has recently authorized this therapy for emergency use and atleast 11 patients severely infected with COVID-19 in New York City and Houston received this experimental treatment

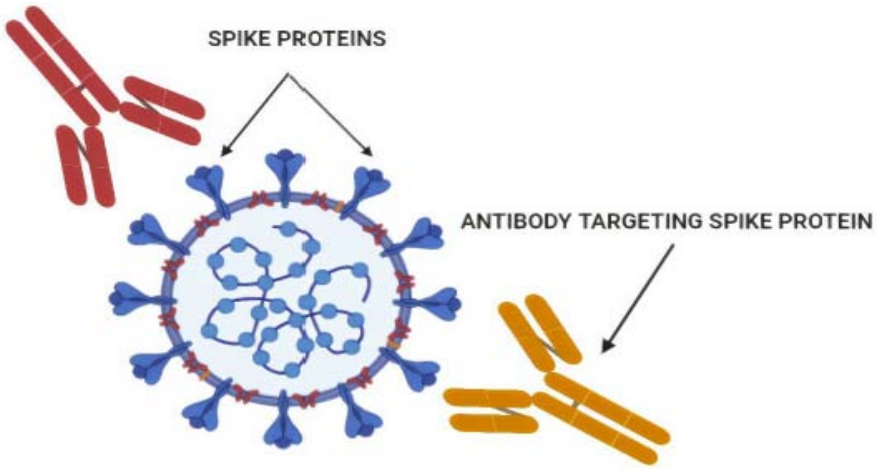


Figure 8: Neutralization of spike proteins of SARS-CoV-2 by antibodies [1].
The antibodies targeting the spike proteins can neutralize the spike proteins present in SARS-CoV-2 thus preventing its entry into the host cell and further infection in normal healthy cells [1].

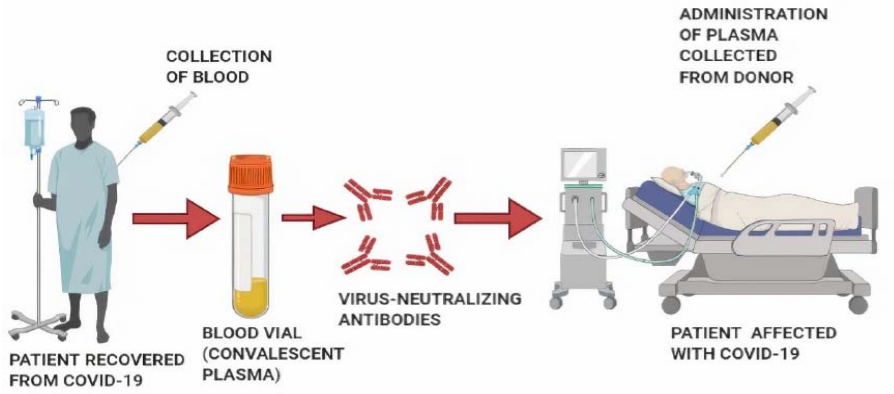


Figure 9: Convalescent plasma therapy for the treatment of COVID-19 patient [125].
Blood is collected from the patients recovered from COVID-19. The convalescent plasma collected contains the virus neutralizing antibodies which when administered into the affected individual, will help in fighting against the virus and thus in recovery of the patient as they help in neutralization of the spike protein of SARS CoV-2 that attacks the human protein [2-4].

[6]. Antibodies can be provided immediately through this passive immunity therapy. In India, the first patient who was given convalescent plasma therapy in Delhi has fully recovered as of 26th April 2020 [126]. The 49 year old male patient tested positive for COVID-19 on 4th April 2020 and on the night of April 14, he was administered plasma collected from the donor tested negative thrice for COVID-19 [126]. Progressive improvement has been observed and finally he is fully recovered on 26th April 2020 [126]. This passive antibody therapy using convalescent plasma needs more controlled clinical trials to check whether symptoms of COVID-19 can be improved or it can be prevented. The National COVID-19 Convalescent Plasma Project has been set up by Henderson, group of US researchers to find the effectiveness of convalescent plasma therapy [125]. The first trial will investigate whether the therapy will be effective in people in close contact with patients, the second trial will test whether convalescent plasma can help keep patient with moderate sickness from getting sicker and the last trial will be on critically affected patients [125].

Immunomodulators: Cytokine inhibitors

The COVID-19 is mainly associated with Acute Respiratory Distress Syndrome (ARDS) and cytokine storm where plasma cytokines are found to be elevated at the later stage of the disease [7-9]. This leads to the respiratory failure which is the major cause of the mortality [7-9]. The distinctive features of the infected lung pathology include inflamed endothelial cells, gap formation leading to capillary leakage of fluid and activation of inflammatory neutrophils, lymphocytes and macrophages [127] (Figure 10). These activated macrophages release increased levels of cytokines creating cytokine storm. The concentrations of Tumor Necrosis Factor-α (TNF-α), interleukine-2 (IL2), interleukine-2 (IL7), Granulocyte Colony-Stimulating Factor (G-CSF), granulocyte macrophage-colony stimulating factor, Interferon (IFN)-gamma, vascular endothelial growth factor, IP-10, Monocyte Chemoattractant Protein (MCP)-1, Macrophage Inflammatory Protein (MIP)-1alpha, Platelet-Derived Growth Factor (PDGF) in the initial plasma have been found to be higher in both the ICU and non ICU patients infected with 2019-novel

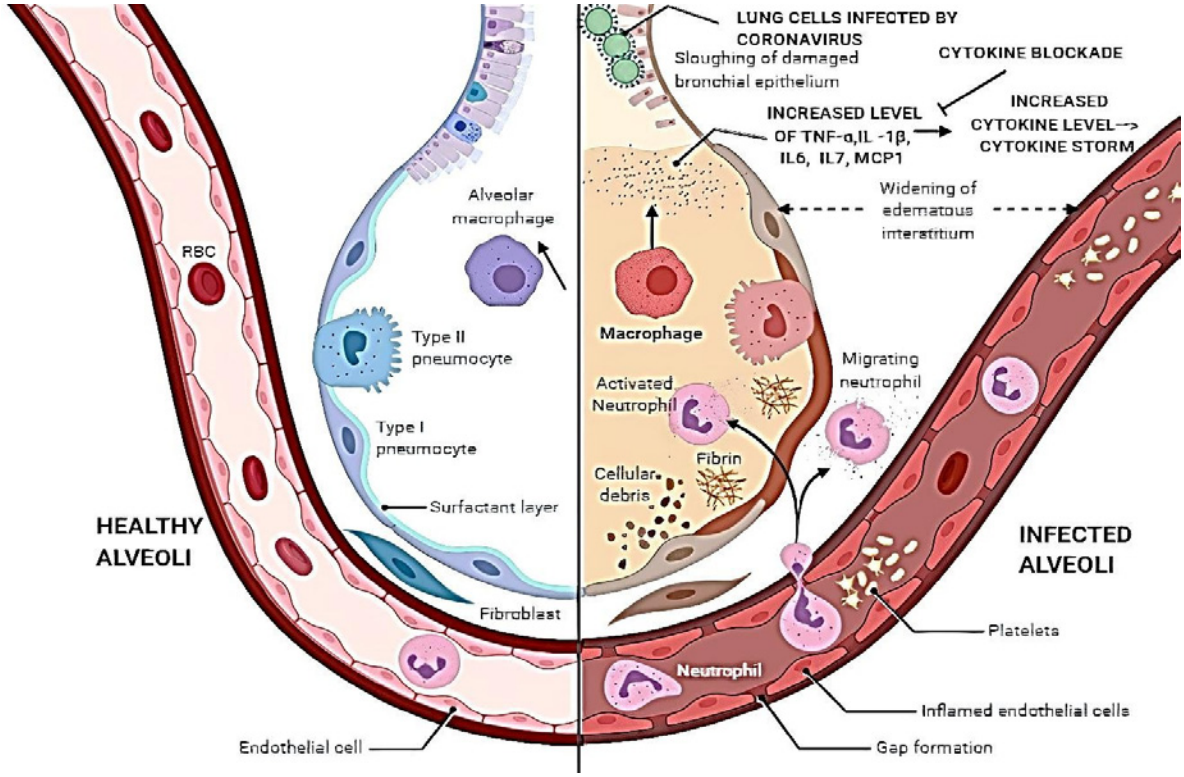


Figure 10: Lung pathology after coronavirus infection: Reduction of Cytokine storm by cytokine blockade [128,129].
When lung cells get infected with coronavirus, the pathology is characterized with inflamed endothelial cells, gap formation leading to capillary leakage of fluid, infiltration and activation of inflammatory neutrophils, lymphocytes and macrophages [127]. These activated macrophages release increased levels of cytokines creating cytokine storm. The concentrations of cytokines like Tumor Necrosis Factor- α (TNF-α), interleukine-2 (IL2), interleukine-2 (IL7), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor, Interferon (IFN)-gamma, vascular endothelial growth factor, IP-10, monocyte chemoattractant protein (MCP)-1, Macrophage Inflammatory Protein (MIP)-1alpha, Platelet-Derived Growth Factor (PDGF) get higher [130] after the infection which can be blocked with the help of cytokine blockade [9].

coronavirus when compared with the healthy patients admitted in the hospitals in Wuhan, China [130]. After detail comparison between ICU and non ICU patients, it has been further observed that patients requiring ICU admission have higher concentration of these cytokines than non ICU patients suggesting the co-relation of the cytokine with disease severity [130]. As the patients with pandemic COVID-19 have cytokine storm syndrome, identification and treatment of hyperinflammation using existing therapies have been recommended [9]. These include selective cytokine blockade as immunosuppression could improve mortality [9] (Figure 10). Among the proinflammatory cytokines, TNF-α released by virally stimulated macrophages in the alveoli plays an important role in the pathogenesis of coronavirus Severe Acute Respiratory Syndrome (SARS) [131] and it has been suspected as the mediator of the immune-based lung injury following infection with SARS coronavirus [12]. Though there are many proinflammatory cytokines, blockade of TNF alone plays a significant role in the pathogenesis of COVID-19 [132]. This is because of "TNF dependent cytokine cascade" [133,134] which implies that blocking of TNF also decreases the concentration of IL-6 and IL-1 [135]. If the inflammatory cascade takes place by TNF- α resulting in lung injury in SARS, inhibition of TNF-α can potentially lead to the reduction of lung damage [136]. One of the major remedies of such conditions aims at blocking TNF-α with anti-TNF antibodies. Thus, a single infusion of anti- TNF antibody will not only reduce TNF bound to it but may also help in reducing the levels of other inflammatory mediators during COVID-19 lung inflammation [132]. This leads to significant therapeutic implication anti- TNF-α-antibodies in the pathogenesis of SARS2 [137]. The role of TNF-α in the development of acute respiratory diseases induced by virus endotoxin has been studied in which etanercept has been reported to block porcine TNF-α activity *in vivo* in the lungs of pigs inoculated intratracheally with Porcine Respiratory Corona Virus (PRCV) followed by Lipopolysaccharide (LPS) [138]. As per the information that study was the first *in vivo* study showing direct blocking of TNF-α in the lungs of pigs by etanercept (Enbrel®) [138]. It has also been reported that inhibition of TNF-α by anti- TNF antibody helps in reducing the severity in virus specific lung immunopathology in mice, viral clearance being not interfered [139]. On the other hand, there has been no such cases indicating the harmfulness of TNF-α blockade to patients in the context of Corona Virus Disease of 2019 (COVID-19) [140].

Conclusion

With the progress of the COVID-19 disease, it is difficult to reverse the tissue damage caused by the virus as it cannot be done with something which is only targeted for the virus [1]. Moreover, the immunocompetence of the affected individual has an immense impact on the efficacy of the antibody therapeutics. The extent of

the effect of the antibody mediated immunity on viral clearance is still under investigation. But anyways, at primary stage the antibody based therapy may help to prevent the replication of the virus. In several cases, the recovery of COVID-19 infected patients treated with convalescent plasma therapy has been reported [5,126]. Investigation is going on to find the effectiveness of convalescent plasma against SARS-CoV-2 so that this treatment could be used against COVID-19 infected patients effectively. Again, in several cases, anti TNF-α antibodies have been found to block TNF-α in the lungs of pigs and mice infected with coronavirus [138,139]. But use of TNF-α inhibitors has not yet been recommended for treatment of COVID-19 patients due to poor evidence [141]. Moreover, a risk of increase in bacterial infection is also there with anti-TNF therapy [142]. On the other hand, development of second and third generations of antibodies are going on for the improvement of specificities of antibodies. The major problem that is being faced in this field is the requirement of antibody in bulk quantity, upscaling, maintaining the stability of antibodies in harsh environment and also recovery from inclusion bodies. Efficient purification is also a major challenge from the perspective of purity, product yield and specially cost. Purification of tagged protein has several shortcomings as the tags have been reported to alter the protein conformation, cause toxicity in the system [143,144] and also increase the cost of downstream processing required for the removal of tags [143]. This has to be taken in account and researchers are trying to investigate the purification in different cost effective methods such as using tag free protein and salt inducible system [145] instead of costly and toxic Isopropyl β-D-1-Thiogalactopyranoside (IPTG).

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