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► **To cite this version:**

Jordan D. Dimitrov. Harnessing the Therapeutic Potential of ‘Rogue’ Antibodies. Trends in Pharmacological Sciences, 2020, 41, pp.409 - 417. <10.1016/j.tips.2020.03.005>. <hal-03490641>

HAL Id: hal-03490641

<https://hal.science/hal-03490641v1>

Submitted on 22 Aug 2022

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Harnessing the therapeutic potential of “rogue” antibodies

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Abstract

Therapeutic antibodies have revolutionized the modern medicine. At present antibodies are successfully used for treatment of diverse human diseases, ranging from cancer to viral infections. All clinically approved antibodies rely on highly specific recognition of their target antigen. The antigen binding promiscuity, binding to autantigens and propensity for self-binding (homophilic interaction) are highly undesirable characteristics of antibody drug candidates. Nevertheless, immune system of all healthy individuals constantly produces and uses large quantities of antibodies that can be classified as inappropriate for development as drugs. Here I provide arguments that antibodies with “aberrant” properties have therapeutic potential. They could be useful in certain complex pathological conditions, thus enriching our armamentarium for treatment of human diseases.

Keywords: therapeutic antibodies; polyreactive antibodies; homophilic antibodies; autoantibodies; systemic inflammation; cancer, HIV-1.

Therapeutic antibodies are highly specific for their target

The advent of therapeutic antibodies has exerted transformative impact on medicine. Presently, Food and Drug Administration and European Medicines Agency have approved almost 90 antibodies for therapy of cancer, autoimmunity, infectious- and inflammatory diseases [1]. Hundreds of other antibodies are in clinical trials or under development. The concept behind this class of drugs reflects one of the fundamental characteristics of the immune system i.e. the potential for high specificity recognition of any target antigen. Following antigen recognition antibodies use different mechanisms to exert their therapeutic effects. The binding of the antibody might result in disruption of receptor-ligand interaction (e.g. interaction of a cytokine with its receptor). Other antibodies induce cell cytotoxicity via elicitation of innate immune reactions through their constant immunoglobulin portion (e.g. killing of cancer cell via **complement-dependent cytotoxicity** or **antibody-dependent cellular cytotoxicity**, see Glossary) [2]. Another class of therapeutic antibodies, that are still undergoing clinical trials, induce transmission of signal via membrane receptor (e.g. agonistic antibodies), thus modulating cellular functions [3]. Regardless of their actual mechanism, all currently approved therapeutic antibodies have been selected on basis of their high-specificity recognition of disease-associated target. In contrast, antibodies that show binding promiscuity (**polyreactivity**) or off-target activity are considered “risky” for entry into drug development process [4, 5, 6, 7]. Likewise, highly undesirable characteristics of candidate therapeutic antibody are the tendency for self-binding (**homophilic antibodies**) and reactivity towards autoantigens (autoreactivity) [4, 5, 6, 7].

Contrary to the biotechnology enterprise, immune system of healthy subjects constantly produces and uses substantial quantities of antibodies that manifest binding promiscuity and the other characteristics considered inappropriate for drug development. Accumulating evidence suggests that these antibodies play important functions in normal functioning of the immune system, contributing both to the immune defence and to the immune homeostasis. Here I argue that this obscured part of the immunoglobulin repertoires conceals yet underexploited source of ideas and inspirations for development of novel types of antibody therapeutics.

Therapeutic potential of polyreactive antibodies

One of the fundamental principles of the pharmaceutical design is that a potential drug should possess an exquisite specificity for its target. Indeed, a candidate molecule that demonstrates binding promiscuity, or off-target reactivity, almost certainly would be denounced as inappropriate for further development. The fondness for high degree of specificity is not solely valid for the low molecular weight drugs but it has been integrated into the concepts guiding the development of therapeutic antibodies as well. Indeed, antibodies that demonstrate antigen-binding promiscuity have poor chances to enter the clinical practice. It is recommended these antibodies to be rejected at early stages of the development processes [5, 6, 8, 9, 10]. Motives for excluding the promiscuous antibodies are related to their pharmacokinetic profiles and the possibility for eliciting detrimental immune reactions in patients, due to their reactivity with autoantigens. Accordingly, pre-clinical studies demonstrated that the polyreactive antibodies have considerably shorter circulatory half-life, owing to their promiscuous binding to different endogenous molecules [5, 11, 12]. Hence, when the purpose of the antibody therapeutic is to target a specific molecule with direct relevance to the pathology and to sustain a long-lasting therapeutic effect, the binding promiscuity of antibodies is indeed a detrimental trait.

Nevertheless, it has been suggested that binding promiscuity of some low molecular weight drugs can be advantageous in polygenic diseases such as psychiatric disorders and cancer [13, 14, 15]. Alike the therapeutic benefit of the promiscuity of the low molecular weight drugs, the promiscuous antibodies may offer unique therapeutic potential in certain complex pathological conditions characterized by a systemic derangement of homeostasis and simultaneous presence of many pathology-related targets.

In contrast to the current doctrine governing the development of antibody therapeutics, the immune system of healthy humans is not relying exclusively on antibodies with high fidelity in terms of antigen recognition. Actually, a substantial fraction (ca. 20 %) of antibodies and B cell receptors in healthy individuals display considerable degree of antigen binding promiscuity [16, 17, 18, 19, 20]. Although, the functions of the polyreactive antibodies in immunity have not yet been completely deciphered, there are plenty of clues that these antibodies represent a functionally important

constituent of the immune repertoires. Thus, it was demonstrated that natural polyreactive antibodies serve as a first line of defence against pathogens [21, 22] and might contribute to priming of specific adaptive immune responses against viruses and bacteria [23, 24].

The polyreactive antibodies also have immune-regulatory functions. Thus, they take part in the scavenging and the clearance of oxidised plasma lipoproteins (such as low density lipoprotein), apoptotic and senescent cells or damaged macromolecules [25, 26]. Moreover, it was hypothesized that the repertoire of promiscuous antibodies can act as a buffer that scavenge, and therefore prevent, any abrupt changes in the levels of endogenous molecules with physiological activity, including cytokines, anaphylatoxins, hormones, or **danger-associated molecular patterns** (DAMPs) [26, 27, 28, 29]. The significance of immune regulatory potential of polyreactive antibodies is supported by results from pre-clinical studies. Thus, immunoglobulin preparations with enhanced polyreactivity exerted a potent protective effect when applied in different models of septic shock – a severe condition characterized by systemic disbalance of the immune system and uncontrolled release of endogenous DAMPs, pro-inflammatory cytokines and anaphylatoxins [30]. In addition to their beneficial effect in systemic inflammation, the human IgG preparations deliberately enriched in promiscuous antibodies, demonstrated considerable therapeutic efficacy in pre-clinical models of different immune-mediate pathologies e.g. autoimmune diabetes, rheumatoid arthritis and multiple sclerosis [31].

Apart from its systemic repercussions, the antigen-binding promiscuity can provide an individual antibody molecule with a better adaptability and tolerance to the sequence variability in the target antigen. This fact may explain the findings that many of the broadly neutralizing antibodies specific for highly mutable pathogens, such as HIV-1, display a high degree of antigen-binding promiscuity [32, 33, 34, 35]. Accordingly, it was recently demonstrated that mutations that introduce or augment the promiscuity and autoreactivity of different broadly neutralizing HIV-1 antibodies, concomitantly increase their virus-neutralization potency and breadth [36]. The works on broadly neutralizing HIV-1 antibodies have also highlighted that the binding promiscuity of an antibody does not exclude high affinity antigen recognition. Indeed, most of

identified polyreactive HIV-1 specific antibodies recognize their epitopes on envelope proteins gp120 or gp41 with high binding affinity (<https://www.hiv.lanl.gov> and [35]). It is noteworthy that patients able to mount broadly neutralizing HIV-1 immune responses have aberrations in their B cell repertoires associated with higher prevalence of autoreactive and polyreactive traits [37]. Additionally, a large fraction (>70 %) of the antibodies specific for HIV-1 envelope proteins, isolated from such patients, manifests antigen binding polyreactivity [38]. Collectively all these findings highlight the importance of the autoreactivity and promiscuity for successful control of infection with a pathogen with high genetic variability.

Evidences presented above imply that the polyreactive antibodies provide opportunities for development of novel types of therapeutics that may be applicable in distinct pathological conditions. Different sources of polyreactive therapeutic antibodies can be envisioned. Thus, candidate therapeutic polyreactive antibodies can be isolated from the B cells from healthy individuals or from patients with certain pathological conditions, e.g. HIV-1-infected individuals with broadly neutralizing antibody titers. Artificial antibody libraries might also serve as a source of therapeutic polyreactive antibodies. Moreover, the antigen binding promiscuity might also be rationally introduced by engineering of antigen binding sites of antibodies with stringent antigen-binding specificity [36]. Careful evaluation of the repertoires of targeted antigens, using proteomic approaches, as well as of the biological effects, using pre-clinical disease models, would result in categorizing of the therapeutic applicability of different polyreactive antibodies.

One may argue, however, that polyreactive antibodies would pose a risk for unwanted immune reactions. Actually, immunoglobulin preparations, which are functionally polyreactive, have been successfully used for therapy of immunodeficiencies and various autoimmune disorders for more than 40 years. Intravenous immunoglobulin (IVIg) represent pooled human IgG obtained from >3000 healthy blood donors, containing both antibodies specific for pathogen-associated antigens and for autoantigens [28]. These preparations, irrespectively of their polyreactive nature, are generally safe and efficient when administrated at very high doses (i.e. up to 2 g/kg). Besides therapeutic mechanisms related to **Fc fragment**, as saturation of **neonatal Fc receptor** resulting in accelerated clearance of pathogenic autoantibodies, IVIg also manifests **Fab**-dependent therapeutic activities. The fraction

of natural polyreactive antibodies in IVIg was demonstrated to be the main contributor to those therapeutic effects [28, 31].

In conclusion, the promiscuous antibodies despite their shorter half-life can offer therapeutic solution of conditions accompanied by systemic derangement of immune homeostasis such as septic shock and autoimmunity as well as systemic inflammation (cytokine storms) triggered by certain viral infections (Figure 1). These pathological situations are usually accompanied by high mortality and have acute course; hence the shorter half-life of the antibodies would not be an important issue. Application of polyreactive monoclonal antibodies that have capacity to bind and neutralize endogenous DAMPs, multiple cytokines or endogenously generated **anaphylatoxins** (such as C3a and C5a) can restore the perturbed immune homeostasis. Promiscuous antibodies may also provide therapeutic benefit in some chronic conditions accompanied by an impaired clearance of apoptotic or senescence cells as well as in cases of infection with viruses with high antigenic variability. The advantages of treatment of complex disorders with a single polyreactive antibody versus mixture of monoclonal antibodies with different specificities might be related to lower production costs and the potential of the former to interact and regulate not yet identified disease targets.

Therapeutic potential of homophilic antibodies

Some antibody molecules have tendency for self-association via interactions between their antigen-binding sites. These antibodies are referred to as homophilic or homotypic and were first described more than 30 years ago [39, 40, 41, 42]. Importantly, antigen-binding polyreactivity of antibodies correlates with a significantly higher propensity for homophilic interactions [9]. Therefore, many antibody clones can exhibit the two features considered as negative for therapeutics development. In particular, self-binding of monoclonal therapeutic antibodies is deemed as a detrimental property, because it might be responsible for aggregation and high viscosity of the biopharmaceuticals [6, 9, 10, 43]. Nevertheless, the fact that the immune system uses this phenomenon in certain situations should not be neglected. Thus, a recent work revealed that a group of highly protective antibodies against *Plasmodium falciparum* (the pathogen causing malaria) employ homophilic binding

as mechanism for achieving more efficient binding to the antigen PfCSP [44] (Figure 2A). Homophilic binding was also implicated in the mechanism of antibody R24 with anti-cancer activity, specific for carbohydrate epitope on the cell surface [45]. Notably, the target antigens of the both homophilic antibodies described above have repetitive nature.

A recent study revealed a surprising finding that Rituximab – a therapeutic anti-**CD20** antibody that have been used for treatment of certain B cell malignancies and autoimmune disorders, utilizes homophilic binding in its mechanism for antigen recognition and cytotoxicity [46]. The study provides structural evidence that two Fab fragments from different rituximab molecules contact a single dimer of CD20 (Figure 2B, [46]). In addition to contacts with the antigen, the antigen binding sites of the antibody established substantial homophilic contacts via their heavy chain variable domains (Figure 2B). The homophilic contacts between two Rituximab molecules are contributed mainly by **CDR H3** regions and more specifically by Tyr93 residue, which is implicated in van der Waals contacts and aromatic interactions. Serine residue at position 31 in heavy immunoglobulin chain also plays a key role in the homophilic binding of Rituximab [46]. Site-directed mutagenesis analysis showed that self-binding of Rituximab during recognition of CD20 has a considerable impact for increase in the antigen binding affinity of the antibody. Moreover, the homophilic interaction of the therapeutic antibody and formation of dimer was found to be indispensable for induction of complement-mediated cellular cytotoxicity [46] – the main therapeutic mechanism of Rituximab. Even though the homophilic trait was not rationally designed in Rituximab, this study highlights that homophilic interactions have important repercussions for therapeutic activity of the antibody. It is noteworthy that engineering of the therapeutic antibody Trastuzumab, which recognizes Her2 receptor, to acquire homophilic potential resulted in a marked improvement of its therapeutic efficacy in pre-clinical models of human breast cancer [47, 48].

The self-binding of a given monoclonal antibody might have important functional consequences. Similarly, as Fc-mediated hexamerization of IgG [49], the homophilic interactions of IgG can lead to special clustering of many IgG molecules, followed by more efficient recruitment of the complement system [46]. Antibody self-association may also represent a common mechanism for avidity enhancement launched during local increase in the antibody concentration at surface of pathogens. Homophilic

antibodies might be especially efficient for induction of cytotoxic reaction by binding to surfaces with repetitive antigens (Figure 3). Taken together the presented results strongly suggest that in certain situations an apparently detrimental property for manufacturability of therapeutic antibodies may present with beneficial biological functionality. Finding an appropriate strategy for control of the propensity of antibodies to associate before administration to patients should allow harnessing the superior cytotoxic activity of some homophilic antibodies in clinical settings.

Therapeutic potential of autoantibodies

Immune repertoires of all healthy individuals contain antibodies that recognize various self-antigens with low affinity. These antibodies predominantly belong to IgM class, frequently show antigen-binding polyreactivity and are referred to as natural autoantibodies [26, 50]. In addition to low affinity natural autoantibodies, it was estimated that one fifth of the general population have detectable levels of typical disease-associated IgG autoantibodies, without any overt autoimmune disease [51, 52, 53]. There is substantial evidence that autoantibodies found in healthy individuals or in patients with autoimmune disorders might offer some therapeutic opportunities.

Autoantibodies in healthy individuals can exert important immune-regulatory activity. This activity may occur through interaction with cell-surface receptors. Thus, a recent work demonstrated that all healthy humans have substantial levels of IgG antibodies specific to different types of G protein-coupled receptors (GPCRs) [54]. GPCR are ubiquitous group of membrane receptors that trigger diverse physiological responses upon binding to their ligands, which include hormones, cytokines, neurotransmitters, and various low molecular weight substances. Importantly, GPCR-binding autoantibodies isolated from healthy subjects were shown to have agonistic effect when binding to the receptor, thus acting as chemokines for immune cells (neutrophils) [54]. These studies provide argument in favour of the role of autoantibodies for regulation of immune responses.

In some pathologies of the immune system appearance of autoantibodies has important disease ameliorating effect [55]. Thus, in **APS1/APECED** patients with congenital deficiency of the transcription factor AIRE, there is a systemic

perturbation of thymus tolerance. These patients have increased incidence of autoimmune diabetes. Interestingly, group of patients develop autoantibodies that recognize with extremely high affinity (K_D values in picomolar range) and specificity type I interferons [55]. Neutralization of interferons protects the patients from manifestation of autoimmunity [55]. These findings reveal the remarkable adaptability of the immune system. They also illustrate that particular autoantibodies can be applied as immunomodulatory therapeutics to compensate for inborn errors in the immune system.

Preclinical studies revealed that typical autoantibodies as those found in **systemic lupus erythematosus** might also present with a therapeutic potential. One of these antibodies - 3E10, isolated from lupus prone MRL/lpr mice, was shown to penetrate cells and bind to nuclear DNA [56]. The binding of this antibody to nuclear DNA could increase the sensitivity of human cancer cells *in vitro* and *in vivo* to DNA-damaging agents i.e. ionizing radiation and certain chemotherapeutics [56]. Further studies showed that antibody 3E10 might directly affect the cancer cells with intrinsic defects in the mechanisms of single or double DNA-breaks repair (e.g. BRCA1/2 and PTEN deficient cancers) [57, 58, 59]. Notably this antibody did not perturb the viability of cells without defects in DNA repair machinery. Comprehensive studies and engineering of 3E10 and its scFv deciphered mechanism and therapeutic potential in certain forms of cancer, characterized with deficiency in DNA repair [57, 58, 59, 60]. In addition to 3E10, another cell penetrating DNA-binding IgG antibody – 5C6 showed enhanced tumorocidal activity by impairment of DNA repair pathways [61]. Besides by the impairment of DNA repair, the nucleus-penetrating DNA-binding autoantibodies can also be used for specific targeting of cytotoxic drugs to the cancer cells [62]. The mechanism by which anti-DNA antibodies penetrate cells and nucleus are not well understood. It was proposed that these antibodies use nucleoside transporters expressed on the cell membrane for entry into the cells following binding to traces of extracellular DNA [59].

Patients with lupus have overall higher incidence of cancer compared to general population but surprisingly significantly lower incidence of certain forms of cancer such as breast cancer, prostate cancer, ovarian cancer and endometrial cancer [59, 63]. The cancer types associated with lower incidence in lupus are frequently

associated with BRCA1 deficiency i.e. they have defects in DNA repair machinery and hence might be vulnerable to cell penetrating anti-DNA autoantibodies [59].

Taken together the presented findings suggests that autoantibodies found in healthy individuals or in patients with some immune disorders presents opportunities for development of innovative drugs that can be used for treatment of diverse disease conditions ranging from immune deregulation to cancer.

Concluding Remarks

Today all approved therapeutic antibodies are characterized with exquisite antigen binding specificity. Likewise, antibody development programmes are focused exclusively on such molecules. Indisputably, antibodies with precise target specificity have demonstrated, and will continue to demonstrate, their remarkable therapeutic potential. Literature on the development of therapeutic antibodies considers “polyreactivity”, “autoreactivity” and “self-binding” as negative features. Not surprisingly the ingenuity of researchers is predominantly focused on the canonical antibodies. We should endeavour to challenge the dogmas that only specific is reliable and try to embrace the whole picture of functionality of the adaptive immune system sustained by the 500M years of evolution. By overtly ignoring “risky” antibodies we may limit the scope of therapeutic possibilities that the diversity of immune repertoires offers. The history has witnessed that the stigmatization and ignorance may only negatively impact the progress in science. By better understanding the polyreactive, autoreactive and homophilic antibodies (see Outstanding Questions) we will certainly arrive with innovative strategies to harness these “rogue” antibodies for therapy of human disease.

Acknowledgments

The work was supported by INSERM, France and by “Fondation ARC pour la recherche sur le cancer”, France (PJA 20171206410).

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FIGURE LEGENDS

Figure 1. Comparison of therapeutic mechanisms of antibodies with stringent antigen specificity and polyreactive antibodies. An antibody with high specificity can discriminate among numerous targets and bind with high affinity to a single entity. Such “conventional” antibodies can be used for therapy of disease where there is well-identified and preferably invariable target, which is associated directly or indirectly with the disease. For example, binding to a protein associated with certain form of tumour or viral protein. Antibodies with high antigen promiscuity can bind to numerous targets. Beside disease associated targets they can interact with off-targets as well (red arrows pointing to empty spaces). These antibodies can exert a therapeutic effect through adaptation namely simultaneous recognition of set of targets or accommodation to the antigenic variability (typical for HIV-1 and certain forms of cancer). The promiscuous antibodies can be used for re-establishment of homeostasis in systemic disorders accompanied with massive release of different endogenous pathogenic molecules.

Figure 2. Structural models of homophilic antibodies. (A) Interaction of Fab fragments of human antibody 1210 with a NANP5 fragment of PfCSP antigen (depicted in red). The model was built by using PDB 6D01, published in [44] (B) Interaction of Fab fragments of Rituximab with human CD20 dimer. The protomers of CD20 are depicted in red and pink. The model was built by using PDB file 6VJA, published in [46]. The heavy immunoglobulin chains are depicted in blue; the light chains are depicted in red. Molecular graphics were performed with UCSF Chimera software, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.

Figure 3. Therapeutic potential of homophilic antibodies. The self-binding of therapeutic antibodies is detrimental property. It prevents storage of the drug at high concentration. In such condition the antibody would start to polymerize, thus resulting in augmentation of the viscosity and precipitation (upper right panel). These outcomes make impossible the application of the therapeutic antibody in patients. The antibodies with self-binding capacity may have superior cytotoxic activity in vivo. Initiation of the self-association of the antibody at the cell surface as result to binding to repetitive antigen might result in an increase in the binding avidity (functional affinity) and aggregation of IgG molecules on the membrane surface, thus triggering

potent complement activation through recruitment of classical complement component C1q.

GLOSSARY

Anaphylatoxins – endogenous or exogenous molecules that can induce systemic inflammatory reaction (anaphylaxis).

Antibody-dependent cellular cytotoxicity (ADCC) – cytotoxic reactions triggered by antibodies, especially those belonging to IgG class, bound to cellular surface. Following, interaction of Fc-portion of IgG with Fc receptors expressed by NK cells, neutrophils or macrophages, the immune cell is activated and release cytotoxic molecules, thus killing the target cell.

APS1/APECED – autoimmune syndrome due to inherited deficiency of transcription factor AIRE. The disorder is characterized with abnormal functions of endocrine system, increased incidence of infections.

CD20 or cluster of differentiation 20 – transmembrane protein consisting of two identical protomers. It belongs to membrane-spanning 4A gene family. CD20 plays important role in B cell differentiation. It is used as specific marker for identification or targeting of B lymphocytes.

CDR – complementarity determining regions part of the immunoglobulin molecule characterized with highest variability in sequence. The most diverse are CDR3 regions of heavy immunoglobulin chains.

Complement-dependent cytotoxicity (CDC) – cytotoxic reaction that can be induced by constant portion (Fc) of antibodies bound to cell surface. The activation of a cascade of plasma proteins – complement system – results in ultimate lysis of the target cell.

Danger-associated molecular patterns (DAMP) – cellular constituents that if released in extracellular space can trigger strong inflammatory reactions. Examples of DAMPS include – histones, HMGB1, RNA, heme, etc.

Fab fragment – fragment antigen binding – a part of immunoglobulin molecule characterized by high sequence variability. In Fab is situated the antigen binding site of antibodies.

Fc fragment – fragment crystallizable or constant fragment a part of immunoglobulin molecules characterized with relatively low variability between different molecules. The Fc fragment is responsible for effector functions.

Homophilic antibody (also known as homotypic antibody) – an antibody molecule that has capacity to bind to itself, using its antigen-binding site.

Neonatal Fc receptor (FcRn) – cellular receptor that binds to Fc part of IgG. FcRn participate in maintenance of homeostasis and transfer of IgG through epithelial surfaces.

Polyreactive antibody – an immunoglobulin molecule that is able to recognize many structurally unrelated molecular targets (antigens).

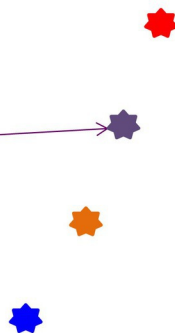
Systemic lupus erythematosus – a systemic autoimmune disorder characterized with production of autoantibodies binding to DNA and nuclear proteins. The formation of immune complexes of antigens-antibody results in severe inflammation and damage of kidneys.

Beneficial effects through discrimination

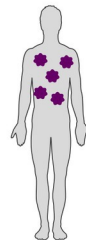
Monoreactive antibody



High affinity
High avidity



Single invariable
disease target

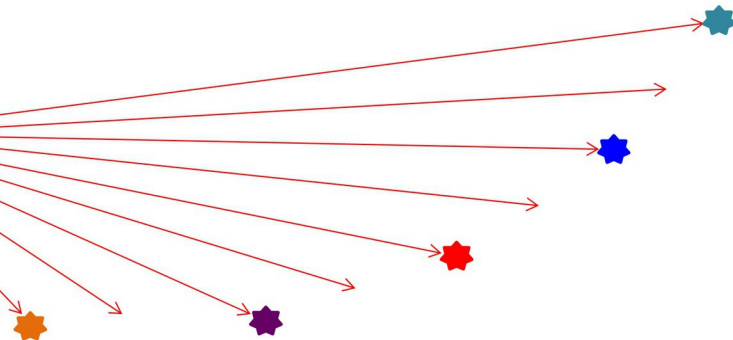


Beneficial effects through adaptation

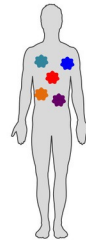
Polyreactive antibody

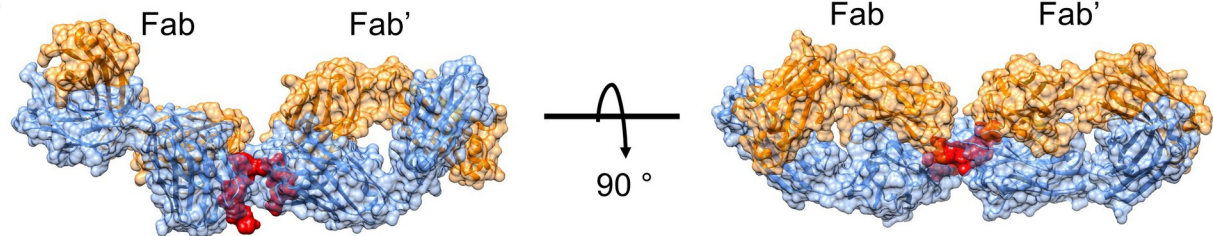
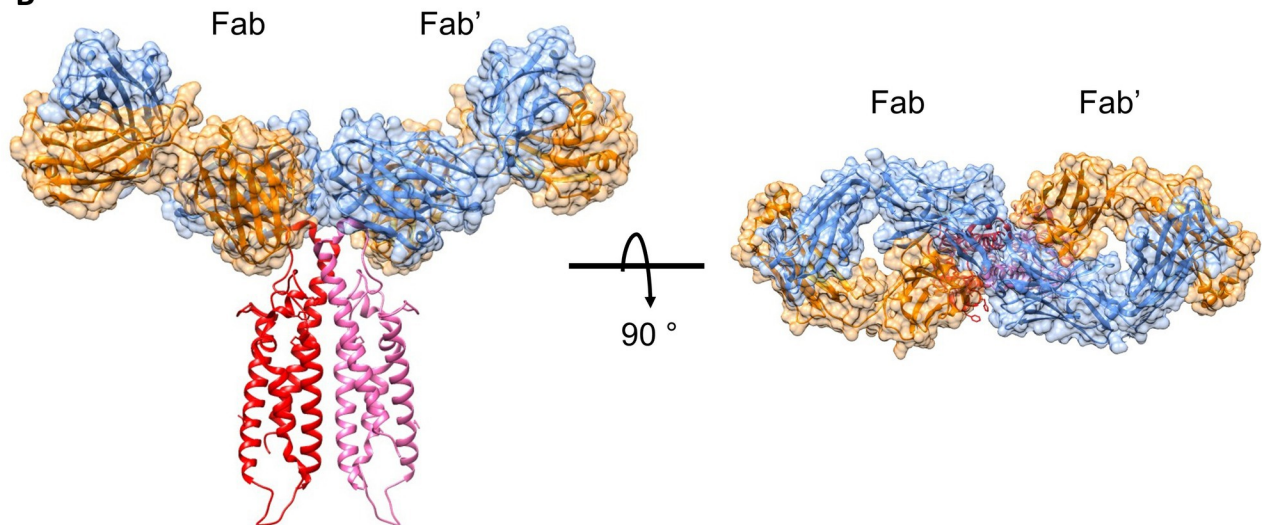


Modest affinity
High avidity



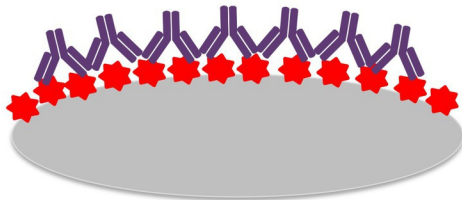
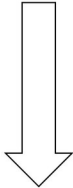
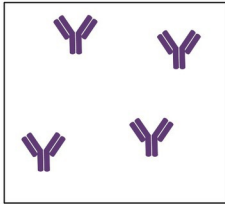
Multiple disease targets
Variable disease target



A**B**

Antibody storage

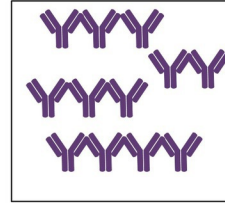
Homophilic antibody (low concentration)



Homophilic binding at cell surface

Increase in binding avidity / Higher recruitment of complement / Higher cytotoxic potential

Homophilic antibody (high concentration)



Homophilic binding in solution

Risk for aggregation/ High viscosity