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MONOCLONAL ANTIBODIES WITH ADCC AND CDC ENHANCEMENT FOR THERAPY

KOLLA BRAMHENDRA CHOUDARY*

Research and Development Department, Inbiopro Solutions Pvt. Ltd,
A Strides Enterprise, Bangalore-560 058, India.

ABSTRACT

Monoclonal antibodies are highly specific molecular-targeted therapeutic agents and represent fastest growing areas of the biopharmaceutical industry. Therapeutic efficacy of antibodies are mediated by variety of mechanisms including binding to antigen on target cells and recruiting effector functions by Fc region such as Antibody Dependent Cellular Cytotoxicity (ADCC) or Complement Dependent Cytotoxicity (CDC). Antibody engineering, glycovariation, engineering of expression host and selection of antibody isotype are powerful approaches for the generation of antibodies with fully human sequence and improved efficacy of ADCC, CDC. Various monoclonal antibodies (mabs) have been developed and approved as immunotherapeutic agents for various human diseases and improvement in efficacy of antibodies continues to be big challenge. Extensive reports have been made to increase the effector functions, here in this review we discuss some approaches like engineering of amino acid sequence in Fc region and glyco-modifications of an antibody for enhancing the binding to FcγR and C1q. These could be great value for the development of next generation of antibody therapeutics with promise clinical efficacy and safety.

KEYWORDS: **Monoclonal antibodies; Effector functions; Fc engineering; Glycomodification**

KOLLA BRAMHENDRA CHOUDARY
Research and Development Department, Inbiopro Solutions Pvt. Ltd,
A Strides Enterprise, Bangalore-560 058, India.

Corresponding author

INTRODUCTION

Recombinant monoclonal antibodies have become an increasingly important class of therapeutic agents for numerous indications including cancer, inflammatory diseases, and viral infections. The development of "Hybridoma technology" for monoclonal antibodies by Kohler and Milstein provided the capable of highly specific interaction of antibodies with their target antigens[1]. Immunogenicity of murine antibodies in humans leads to rapid development of Human Anti Murine Antibody (HAMA) response in the patients resulting in the rapid clearance of antibodies from the body[2]. With the advancement of molecular biology, it has become possible to produce recombinant antibodies with less immunogenic and increasing biological activities including enhancement of effector functions by chimeric and humanization approaches[3, 4]. Fully human antibodies have become a reality with new technologies such as phage display antibody library and transgenic mouse with human immunoglobulin genes[5, 6]. Antibody efficacy results from specificity for the target antigen by Fab and biological activities through Fc region. The Fab contains the variable region with three hyper variable Complementarity Determining Regions (CDRs) that form the antigen binding site of the antibody. The effector functions are activated through the interactions of the Fc with either FcγRs or complements which lead to ADCC and CDC [7]. Structural studies indicate that presence of specific amino acid residue in Fc region and particular N-glycoform determine the structural conformation of the IgG which in turn may impact on antibody effector functions. Fc region also associated with serum half-life of antibody by binding to FcRn receptor on the endothelial cells and protects from degradation. The most notable feature of this binding is pH-dependent with good binding at slightly acidic pH such as 6.0 which exists in endosomes where the FcRn protects the pinocytosed and endocytosed IgG1 by binding from degradation. On recycling, the IgG is released into the extracellular regions because there is almost no binding at pH 7.4 of the extracellular space [8]. Due to their large molecular sizes (~150 kDa), therapeutic antibodies have some limitations in their delivery like inability to target intracellular antigens, less efficient tissue penetration, and poor bioavailability when given orally. The major mechanisms underlying the therapeutic effect of antibodies are Antibody-Dependent Cellular Cytotoxicity (ADCC) or Complement-Dependent Cytotoxicity (CDC), directly inducing programmed cell death (Apoptosis) and blocking essential cellular growth factors or receptors. Most of the existing antibodies that have been developed & licensed as therapeutic agents are IgG1 subclass (Table-1) and induce strong effector functions when compared to other isotypes. Lot of reports have been shown that several amino acid residues in the Fc region and glycan linked to the Asn 297 of the CH2 domain of the antibody plays an essential role in binding to FcγRs or C1q complement. Therefore, it is important to enhance the interaction with FcγRs on immune effector cells or C1q for increased clinical efficacy of mabs[9]. Currently the most promising approaches are towards Fc engineering, glyco-modifications to improve ADCC, CDC and FcRn mediated antibody half-life for potential therapeutic mabs. The Fc engineering approach involves the identification of critical residues in the Fc region that interact with FcγR or complement through alanine scanning of surface-exposed residues or protein structure design algorithm[10]. One example is Medimmune has been developed proprietary technology involves the introduction of 3 mutations in the Fc domain of an IgG1: M252Y, S254T, T256E also referred to as 'YTE' results in higher affinity to the neonatal Fc receptor (FcRn). Introduction of YTE residues resulted in increases 3-4 fold half-life but drastic reduction in the ADCC activity[11]. Additionally, optimizing culture conditions and genetic engineering of mammalian expression host are alternative strategies involves the enrichment of particular glycoform for enhancing the ADCC and CDC. It is well known that Fc glyco sylation is important for the interaction with FcγR or complement. These interactions are known to be sensitive to changes in the oligosaccharide structures of the Fc region. Multiple strategies have been developed and glycoengineering is one of the most promising technology to improve the efficacy of

therapeutic antibodies. Thus, the importance of effector functions for the clinical efficacy of therapeutic antibodies is now widely recognized. ADCC and CDC enhancement technology is expected to play a key role in the development of next-generation therapeutic antibodies with improved clinical efficacy. This review will discuss on the current progress made in the development of Fc and glyco engineered antibodies.

Table-1
Therapeutic monoclonal antibodies approved by FDA

Generic Name (Trade Name)	Mab type	Isotype	Antigen	Indication	Approval year
Murine mabs					
Muromonab (Orthoclone-OKT3)	Murine	m IgG2a	CD3	Transplant rejection	1986
Irbitumomab (Zevalin)	Murine	m IgG1 (Antibody conjugate)	CD 20	Non-Hodgkin lymphoma	2002
Tositumomab (Bexxar)	Murine	m IgG2a (Antibody conjugate)	CD 20	Non-Hodgkin lymphoma	2003
Chimeric mabs					
Abciximab (Reopro)	Chimeric	h IgG1(Fab)	GpIIb/IIIa	Cardiovascular Disease	1994
Rituximab (Mabthera, Rituxane)	Chimeric	h IgG1	CD-20	Non-Hodgkin lymphoma	1997
Infliximab (Remicade)	Chimeric	h IgG1	TNFα	Inflammatory diseases	1998
Basiliximab (Simulect)	Chimeric	h IgG1	CD25	Transplant rejection	1998
Cetuximab (Erbitux)	Chimeric	h IgG1	EGFR-1	Colorectal cancer	2004
Brentuximab (Adcertis)	Chimeric	h IgG1 (Antibody conjugate)	CD 30	Hodgkin lymphoma	2011
Humanized mabs					
Dacilizumab (Zenapax)	Humanized	h IgG1	CD 25	Transplant rejection	1997
Trastuzumab (Herceptin)	Humanized	h IgG1	HER2	Brest cancer	1998
Palivizumab (Synagis)	Humanized	h IgG1	RSV F	Prevention of RSV	1998
Gemtuzumab (Mylotarg)	Humanized	h IgG4 (Antibody conjugate)	CD 33	Acute myeloid leukemia	2000
Alemtuzumab (Campath)	Humanized	h IgG1	CD 52	Chronic lymphocytic leukemia	2001
Tocilizumab (Actemra)	Humanized	h IgG1	IL-6R	Rheumatoid arthritis	2002
Efalizumab (Raptiva)	Humanized	h IgG1	CD 11a	Psoriasis	2003
Omalizumab (Xolair)	Humanized	h IgG1	Ig E	Asthma	2003
Bevacizumab (Avastin)	Humanized	h IgG1	VEGF	Colorectal cancer	2004
Natalizumab (Tysabri)	Humanized	h IgG4	Integrin-α4	Inflammatory diseases	2006
Ranibizumab (Lucentis)	Humanized	h IgG1(Fab)	VEGF	Macular degeneration	2006
Eculizumab (Soliris)	Humanized	h IgG2/4	CSP-C5	Paroxysmal noctumalhemoglobinuria	2007
Certolizumab (Cimzia)	Humanized	Fab(Pegylated)	TNFα	Crohns disease	2008
Pertuzumab (Perjeta)	Humanized	h IgG1	HER2	Brest cancer	2012
Trastuzumabemtansine(Kadcyla)	Humanized	h IgG1 (Antibody conjugate)	HER2	Brest cancer	2013
Human mabs					
Adalimumab (Humira)	Human	h IgG1	TNF α	Auto-immune disorders	2002
Panitumumab (Vectibix)	Human	h IgG2	EGFR	Colorectal cancer	2006
Canakinumab (Ilaris)	Human	h IgG1	IL-1β	Cryopyrin associated periodic syndrome	2009
Ofatumumab (Arzera)	Human	h IgG1	CD-20	Chronic lymphocytic leukemia	2009
Golimumab (Simponi)	Human	h IgG1	TNFα	RA, Psoriatic arthritis	2009
Denosumab (Prolia)	Human	h IgG2	RANK-L	Bone loss	2010
Belimumab (Benlysta)	Human	h IgG1	BLyS	Systemic Lupus erythematous	2011
Ipilimumab (Yervoy)	Human	h IgG1	CTLA-4	Metastatic melanoma	2011
Raxibacumab	Human	h IgG1	Anti-*B. anthrasis* PA	Anthrax infection	2012

ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY (ADCC)

ADCC is an important mechanism of action for several monoclonal antibodies used in cancer therapy. Most of the therapeutic antibodies are human Ig G1 isotype, which can induce strong ADCC and CDC when compared with other isotypes. Antibodies bind to antigens on tumour cells by Fab and Fc engage with receptors Fcγ RIII (CD16), Fcγ RII (32) and Fcγ RI (64) on surface of effector cells like Natural killer cells (NK cells), neutrophils, monocytes and macrophages. These cells become metabolically active as a result of release lytic components like lytic enzymes, TNFα, perporins mediate target cell destruction or phagocytosis of the target cell[12, 13]. These suggest that Fc receptor interaction in antitumor activity of mab be the important for efficacy of mabs in clinics. Several therapeutic mabs are capable of shown ADCC in in-vitro such as anti HER2 Trastuzumab, anti CD20 Rituximab and anti-tumour necrosis alpha (anti TNFα) Infliximab. In fact, current therapeutic mabs challenges the major issues of insufficient efficacy and relatively high cost of the antibodies. Therefore several strategies have been developed during the last decade like Fc, glycoengineering and cell culture process aimed to overcome critical issue of therapeutic efficacy with reduced doses for commercial success.

Fc engineering for enhancing ADCC

In general, the rank orders of potency are IgG1≥ IgG3≥ IgG4≥ IgG2 for ADCC. Human Fcγ receptors (FcγRs) are major mediators of ADCC through Fc region of IgG and the process of activation or inhibition of effector cells depending on which Fcγ receptors are engaged with antibody[14]. Activating FcγR are FcγRI, FcγRIIA/C, FcγRIIIA expressed on monocytes, macrophages and natural killer cells contains an immune receptor tyrosine-based activation motif (ITAM). Whereas inhibitory FcγR is only FcγRIIB presenting on B cells contains an immunoreceptor tyrosine-based inhibition motif (ITAM) which can interrupt intracellular signalling events triggered by activating FcγRs on same cells[15]. Activating and inhibitory FcγRs have high homology and the ratio of activating FcγR binding to inhibitory FcγRbinding (A/I ratio) is an important factor determining the therapeutic efficacy of antitumor antibody. Human FcγRIIIA has allotypes at position Val 158 showed significant higher binding to Rituximab than the allotype having Phe at that position (Phe may alter the interaction and reduce the binding) in non-hodgkin lymphoma[16]. Similarly combination of FcγRIIIA 158 V allotype with homozygous 131 histidine allele of FcγRIIA showed significantly higher trastuzumab mediated cytotoxicity than arginine 131 genotype in brest cancer patients[17]. Thus ADCC has been demonstrated that efficacy depends on polymorphisms of the effector cells and FcγR1 exhibits high binding for IgG than Fcγ RII and Fcγ RIII[18]. Optimization of the interaction of antibodies with FcγR by mutagenesis and glycoengineering has emerged as a promising technology to enhance the activity of therapeutic antibodies. In addition to enhancing the binding to activating FcγRs, minimizing the interaction with inhibitory FcγR is another strategy to enhance the potential of the antibody. Particularly FcγRIIIA dependent effector function may be one of the major critical mechanism responsible for clinical efficacy of mabs. Mapping of the binding site on human IgG1 for FcγR could provide the recruitment of effector cells for enhancing the efficacy of antibody therapeutics. Sondermann and Huber et al reported amino acid residues comprising in human Ig G1 includes Leu234–Ser239, Asp265–Glu269, Asn297–Thr299, and Ala327–Ile332 are involved in binding to FcγRIIIA receptors[19]. One example is anti-tumour activity of Herceptin was attenuated by a mutation (D265A) that impairs binding to FcγRIII and FcRγII. Revealing that, other studies proposed that specific positions L234, L235, A327 and P329 were important for FcγRI, FcγRIIIA binding whereas P238 crucial for all FcγRs in human IgG1. Engineering the antibody Fc region to enhance the FcγRIIIA binding and ADCC activity of therapeutic antibodies is currently an active area of investigation. Shields *et al*. have reported set of Fc-domain variants with a combination of three mutations E333A, K334A and A339T exhibited improved binding to FcγRIIIA. Other set of variant S298A, E333A, and K334A showed further enhanced ADCC by binding to FcγRIIIA than parental human IgG1[20]. Fc engineering with triple substitutions

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S239D/A330L/I332E shows a significant enhancement in binding affinity to human F/F and F/V genotypes of FcγRIIIA over wild type IgG antibody. However the variant also shows binding affinity to inhibitory FcγRIIB which is undesirable and may not be possible to achieve maximum ADCC activity considering the A/I ratio[21]. The latter approach has been revealed the substitution of several amino acids in Fc (F243L/ R292P/Y300L/V305I/P396L) showed greatest increase in ADCC through binding to FcγRIIIA of different IgG mabs but not increase the binding affinity against inhibitory FcγRIIB[22]. ADCC enhancement of antibodies produced by unique yeast display system is that Fc with five mutations (F243L, R292P, Y300L, V305I, and P396L) showed ~10-fold increased binding affinity to FcγRIIIA and improved ADCC activity[23]. Recently Chugai Pharmaceuticals introduces novel Fc engineering approach that introduces different substitutions in each Fc domain asymmetrically. Asymmetric mab with L234Y/L235Q/G236W/S239M/H268D/D270E/S 298A substitutions in one Fc domain and D270E/K326D/A330M/K334E substitutions in the other Fc region showed highest binding affinity for both FcγRIIIA allotypes without increasing affinity for FcγRIIB. This novel asymmetric engineering provides Fc variants with superior ADCC activity and also higher stability than previously reported symmetrically engineered Fc variants[24]. Genentech group has discovered single substitution of His229 with Tyr in the upper hinge region showed increased binding to FcγRIII and improved ADCC activity by 2-fold over the wild type IgG1. In addition to that, His/Tyr substitution improves the stability, production yield and could be first report that single substitution in mab engineering to improve multiple properties[25]. Thus enhancement of ADCC technology by amino acid engineering plays an important role in development of the next generation of therapeutic monoclonal antibodies.

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ADCC enhancing glycosylation technology

Glycosylation of monoclonal antibodies is an extensive post-translational modification that has been associated with their biological activity in relation to Fc binding, conformation, stability, folding accuracy, pharmacokinetics and safety. Therapeutic antibodies produced in mammalian expression systems potentially bear two N-glycans at Asparagine residue of the N-X-S/T sequon (X can be any amino acid except proline) in CH2 domain of the Fc region[26]. The Fc fragment is involved in binding of Fc receptors, C1q for antibody effector functions and interaction with FcRn for pharmacokinetic properties. The N-glycans present in the Fc portion of IgG molecule consists of biantennary core sugar structure of N-acetyl glucosamine residues (GlcNAc) linked to Asparagine (Asn297) via an amide bond and three mannose (Man) residues. Variable additions of terminal and branching sugar residues to core structure such as *N*-acetylglucosamine, galactose, fucose and sialic acid (Fig-1) can influences significant role in binding of IgG to FcγRs and C1q. This structure gives rise to heterogeneity with a mixture of 30 or more glycoforms. Recent crystallographic studies demonstrated that glycane on Fc makes subtle conformational alterations in a limited region of Fc that leads to minor interactions with the amino acids of FcγRIIIA[27, 28]. Additionally, glycosylated IgGs are more resistant to proteases than aglycosylated and may play important role in antibody stability[29]. Structural studies indicate that the presence or absence of specific terminal sugars may affect hydrophilic and hydrophobic interactions between sugar residues and amino acid residues in the CH2 domain of the Fc fragment. These molecular interactions determine structural conformation of the IgG which in turn may impact on antibody effector functions and stability. Additionally some mabs also exhibit Fab glycosylation like cetuximab contains an N-glycan at Asn 99 of the VH region can influence the antigen binding affinity[30]

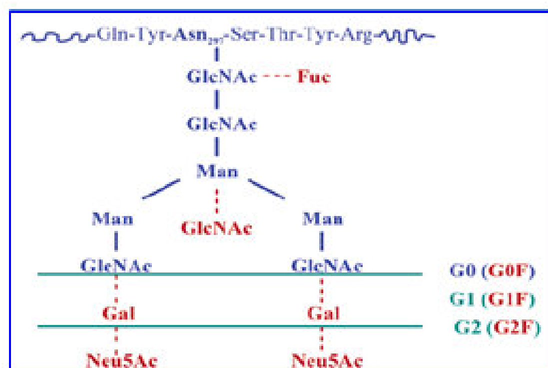

Figure-1

Biantenary carbohydrate moiety attached to Asn-297 of human IgG1-Fc.The oligosaccharide in blue represents heptasaccharide core and defines the G0 structure. The terminal residues that may be attached to the core confers the heterogeneity upon the structure is shown in red.

Most of the therapeutic antibodies produced in CHO cells and contain Fucose linked to core GlcNAc residue as the result of fucosyltransferase in the trans-Golgi. The absence of core Fucose, and terminal sialic acid results in significant increase in ADCC. Similarly presence of a bisecting N-acetylglucosamine in the Fc glycans substantially increases the ADCC activity of IgG1 by increasing binding affinity with FcγRIIIA receptor. Glycan at Asn 297 are packed between the two CH2 domains of Fc and Fuc residues close proximity to the FcγRIIIA-binding surface, may be significantly inhibit the contact with receptor[31]. It has been reported that glycosylation at Asn162 of FcγRIIIA may also plays significant role in interaction with IgG1-Fc, that is only accessible when Fc is nonfucosylated and thereby increases the binding affinity towards FcγRIIIA[32]. Structural studies of fucosylated and nonfucosylated Fc fragments reveal that the overall conformations of these glycoforms are similar, except for the hydration mode around Tyr296, as well as the observed differences in electron density maps around Asp280 and Asn297 residues. These minor differences in the Fc conformation between fucosylated and nonfucosylated antibodies may not completely explain the increased affinity of nonfucosylated antibody for the FcγRIIIA receptor[33]. Although the presence or absence of fucose definitely affects the interaction of an IgG with FcγRIIIA, whether this is due to direct interaction of the fucose with receptor or conformational influence on the IgG are unknown. Positive roles in the FcγR mediated activities of the nonfucosylated Fc region have been reported but remain controversial. One example is non fucosylated antibodies bind with lower affinity to FcγRIIIA F158 than to FcγRIIIA V158. As a resulting ADCC mediated by NK cells bearing the lower-affinity FcγRIIIA allotype is lower than that mediated by NK cells bearing the higher-affinity allotype. This report suggesting that non-fucosylated Fc may not achieve maximum ADCC activity for patients having the lower-affinity allotype[24]. Number of recombinant IgGs without core fucose or reduced levels are developed and currently in clinical trials for human therapeutics. In addition, decrease in sialic acid containing Fc glycans also plays role in elevating ADCC activity as terminal sialylation negatively affects antibody binding to the FcγRIIIA receptor. Sialic acid is negatively charged and bulkier than most monosaccharides, can form ionic, hydrophobic, and hydrophilic interactions with amino acid residues along with imposing spatial constraints because of its bulkiness. The charge and bulkiness of sialic acid residues might affect Fc conformation and antibody effector function[33]. Moreover the impact of sialic acid residues on the antibody function is not yet fully understood. Several promising technologies have been developed to improve the efficacy of therapeutic antibodies. Some approaches are Amino acid changes in Fc region, genetic modification of Fc glycosylation

(glycoengineering) and optimizing culture conditions for modifying glycosylation patterns of antibodies. Glycoengineering is becoming the preferred technology platform, because of low probability of immunogenicity and less impact on overall antibody structural stability[34]. Majority of the currently approved human recombinant IgGs are produced using either Chinese hamster ovary (CHO) cells or mouse myeloma-derived cells (SP2/0 or NS0 cells) and glycosylation patterns vary among species. Most of the therapeutic antibodies that are currently on the market are heavily fucosylated because they are produced by mammalian cell lines with intrinsic enzyme activity responsible for the core-fucosylation of the Fc N-glycans of the products[35]. Multiple strategies have been developed to reduce fucosylation of IgG by silencing the fucosyltransferase gene (*FUT8*) using RNAi methods or cloning and expression of N-acetylglucosaminyltransferase III (GnT-III) that adds bisecting GlcNAc residues to Fc result in a reduction of core fucose content. *FUT8* knockout CHO cells method is approved by regulatory authority and is considered to be the most feasible and reliable strategy for manufacturing fully non-fucosylated therapeutic antibodies. Additionally mouse myeloma-derived cells express active GnT-III enzyme that mediates the transfer of third GlcNAc arm (bisecting) from UDP-GlcNAc to N-glycane whereas CHO cells do not express an active GnT-III[36]. The presence of bisecting GlcNAc has been reported to improve antibody-dependent cellular cytotoxicity (ADCC). Biowa, developed *FUT8* gene knock-down technology to remove α(1,6)-linked Fuc sugars from *N*-glycans in a CHO cell line (Potelligent technology)that produce fucose-free antibodies with 50 to 100 times enhanced ADCC activity[37]. A similar improvement was reported by Genentech is that a mutant CHO cell line (LEC13) was used to produce trastuzumab which does not add fucose to the primary *N*-acetylglucosamine residue and they reported a 40- to 50-fold increase in the efficacy of FcγRIII-mediated ADCC[38]. The new CD-20 specific antibodies AME-133v (LY2469298-Ocaratuzumab), PRO131921, and GA101 (RO5072759, Obinutuzumab) are glycoengineered to lack of Fc fucosylation with higher affinity for FcγRIIIA and enhancement of ADCC. These three mabs are now undergoing active clinical development[56]. In addition to that, engineering of CHO cells with bacterial enzyme GDP-6-deoxy-D-lyxo-4-hexulose reductase can secrete antibodies lacking core fucose to N glycan with strongly enhanced ADCC effector function[39]. Recently Eureka therapeutics developed novel proprietary technology for enhancing ADCC through MAGE 1.5 (Magnified ADCC through Glycosylation Engineering) CHO cell line which produces single, uniform glucosylated glycan structure with increased affinity for FcγRIIIA[40]. All these approaches achieved dramatically enhanced ADCC activity in modified antibodies and glycoengineering offers a challenge, opportunity to biopharmaceutical industry in producing improved and consistent product.

CDC AS AN EFFECTOR FUNCTION OF MONOCLONAL ANTIBODIES

Another well-known immune effector function of IgG antibody is Complement Dependent Cytotoxicity (CDC). The formation of an antigen-antibody complex induces conformational changes in the Fc region of IgG molecule that expose a binding site for the C1q component of the complement system. CDC is mediated through series of complements triggered by the binding of C1q to the Fc region of antibody. Activation of C1q (close proximity to cell) results in the deposition of C3b, C5b, C6, C7, C8 subsequently leads to polymerization of C9 on cancer cell, which allows the formation of cytolytic Membrane Attack Complex (MAC). Once MAC forms on cell membrane induces the target cell lysis through influx of ions and water by generation of membrane penetrating pores[41, 42]. However, tumor cells are protected from complement by the expression of membrane-bound complement inhibitory proteins that function to limit complement activation and C3 cleavage (CD55 and CD46) or inhibit MAC formation (CD59)[43,44]. However, mabs still faces the enhancement of biological activity and several studies have been found that important to optimize, maximize the effector functions in improvement of antibody therapy. CDC also can be modulated by either Fc genetic mutation or Fc isotype engineering or Fc glycosylation modifications.

Approaches to improving CDC

CDC is initiated by binding of antibody to antigen with subsequent binding and activation of several complement cascade proteins and rank order of CDC potencies are IgG3 ≥ IgG1 ≥ IgG2 = IgG4. Human IgG isotype of the IgG1 and IgG3 are very important for their biological functions such as ADCC or CDC. IgG3s are generally not selected for therapeutic development mainly because the plasmatic half-life is shorter than other three isotypes. Several studies indicate that two domains in the human IgG1 Fc region comprise 233-236 and 327-331involved in the interaction with FcγR and C1q of the complement[45]. Few residues, L235, D265 and P331 in CH2 domain has been shown to be important for C1q binding and CDC activity in human IgG. Later group demonstrated human C1q binding epicentre of human IgG1 is centered around these four spatially close residues, D270, K322, P329, and P331. Single point substitutions of these residues with Alanine significantly decreased C1q binding or complement activation and confirmed D265, P331 plays important role in C1q binding, CDC activity[46]. Esohe and Pin Yee et al identified residues K326 and E333 in CH2 domain of IgG1 plays significant role in interaction of the antibody with C1q and are potential sites for improving the efficacy of CDC. They reported that single substitution at residue K326W in Rituximab showed increase in C1q binding affinity and CDC activity. Further increase in CDC activity was observed by double mutations, K326W and E333S over wild type antibody. These significant improved CDC may be due to conformational alteration of antibody structure[47]. The Xencor group has engineered a series of Fc variants with enhanced complement- and FcγR-mediated activities in humanized anti-CD20 monoclonal IgG1 antibody Ocrelizumab. Triple substitution S267E/H268F/S324T (EFT) variant gained greatest increase in CDC activity but displayed reduced FcγRIIIa affinity with lower ADCC activity. Combination of EFT CDC-enhancing variant with two more substitutions, S267E/H268F/S324T/G236A/I332E(EFTAE) providing additional increase in CDC activity with higher FcγRIIA affinity, slightly better FcγRIIIA binding than native IgG1. These results demonstrated that the identified substitutions are improved the binding affinity to C1q with enhanced CDC and showed can be transferable to other antibodies targeting different antigens[48]. One study reported that an anti-CEA human mab of IgG4 was made to genetically convert its isotype from IgG4 to IgG1 by cloning VH and VL genes from the parental antibody (IgG4) to constant region of the human IgG1. The resulting engineered antibody exhibited significantly higher CDC and ADCC activity against CEA-expressing tumor cells over parental antibody[49]. Mutational studies have identified several 'hotspots' in human IgG1 for binding to the C1q and FcγR receptors. In particular L234, L235, P331 in Fc shown to have a strong modulating effect on both ADCC and CDC activities[50]. Apart from amino acid substitution, Akito and Mika et al identified unique approach called Complegent technology to enhance CDC of CD-20 antibody by structural shuffling between isotypes. These shuffled antibody heavy chain variant consisting of CH1, hinge region from human IgG1 and Fc from IgG3 showed a remarkable increase in CDC than wild type antibody[51]. CDC enhancing activity of IgG1/IgG3 chimeric variant was further improved by fucose removal of ADCC maximising potelligent approach. The successful combination of these two enhancing modifications in anti-CD20 antibody showed comparable level of ADCC and CDC those seen for Potelligent or Complegent alone[7]. The core C1q binding site in human IgG1 is dependent on source of complement and concentration may also play important role, there are species differences in complement activation. One example is mutation at P331 (P331A) in human IgG1 resulted in a molecule that was unable to confer complement lysis with guinea pig complement. However with either rabbit or human complement, a substantial amount of CDC activity was observed for the P331A mutant. Rituximab appeared to be less effective in conferring lysis when probed with guinea pig complement than with human or rabbit complement[46]. Several studies indicated that over expression of complement regulatory proteins (CRPs) on tumour cells controls the CDC by different steps of C cascade and prevents membrane attack complex (MAC) mediated cell lysis[52]. CRPs are CD46 (membrane cofactor protein), CD55 (decay

accelerating factor) inhibits the complement C3 generation, C5 convertase activity and CD59 prevents pore formation by MAC. One way of improve the CDC activity of mabs would be neutralization of C regulatory proteins by various cytokines like IL-1β, IL-4 and single chain fragments[53]. These kind of advances are being made to increase the anti-tumour activity of mab by modification in Fc region and neutralizing the inhibitory effect of CRPs are seems to be promising strategies. The positive correlation between CDC activity and galactose has been found in the anti-CD20 antibody Rituximab, where C1q binding to the Fc region increases with the percentage of galactosylation, and similar effects have been found in other CDC-dependent therapeutic antibodies[54]. Terminal galactose residues do not affect antibody binding to the FcγRIIIA receptor and ADCC activity. Removal of terminal galactose residues from Fc glycans reduces complement-dependent cytotoxicity (CDC) activity. Depending on the absence or presence of galactose on one or both branches of the sugar moiety, three subfamilies called IgG-G0 (no galactose), IgG-G1 (galactose on one arm), and IgG-G2 (galactose on both arms) have been defined. C1q binding is impacted by terminal galactose content, as G2 glycoform shows about 2 fold higher C1q binding and CDC activity compared to the G0 glycoform[55].

CONCLUSION

Over the past decade, tremendous progress has been made in the enhancement of effector functions of mabs. As discussed in this review, several candidates established Fc, glycoengineering approaches for the next generation therapeutic antibodies. It is hoped that next approaches is to optimize the therapeutic antibodies with multiple enhancing modifications into a single antibody platform.

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