IgM Hexamers Utilization Over IgM Pentamers and IgG Monomers with Complement-Dependent Cytotoxicity

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Abstract

Antibodies are responsible for the immune system's response to pathogens or abnormal cells through recognition of specific components known as antigens. They activate complementary pathways that work to neutralize invading pathogens or malfunctioning cells. Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibody classes and their relation to Complement-Dependent Cytotoxicity (CDC) activation regarding C1q to fragment crystallizable (Fc) region interactions are examined to discuss the extent and reasons behind IgM hexamers demonstrating higher affinities for Fc-C1q interactions, which leads to CDC activity. Comparing and contrasting the structures of IgM hexamers, IgM pentamers, and IgG monomers will help better understand the underlying reasons for their differing affinities and potential applications in therapeutic settings. Furthermore, researching the CDC response to cancers and the potential for *in vitro* engineering of IgM hexamers is important, as IgM hexamers are typically unable to cross into the lumenal space due to structural abnormalities in comparison to IgM pentamers and IgG structures. This review discusses antibody structural conformations, the potential uses of IgM hexameric antibodies, and their respective impacts on the immune system to explore utilizing IgM Hexamers over traditional antibodies to offer a more effective therapeutic that could result in better targeted treatments.

1. Introduction

B cells produce antibodies via B-cell receptors that target certain pathogens or infected cells based on epitopes, which are regions on antigens recognized by immunoglobulins. Antibody specificity arises through several processes: Variable–diversity–joining rearrangement (V(D)J recombination), which rearranges certain genes; class switch recombination, which mutates the heavy chain to alter isotype; and somatic hypermutation, which introduces point mutations in a B cell capable of binding to an antigen (Hozumi and Tonegawa, 1976; Papavasiliou and Schatz, 2002). There are five known immunoglobulin isotypes: IgM, IgG, IgA, IgD, and IgE. These immunoglobulins have specific functions that serve as a crucial part of the immune response by alerting other immune cells about pathogens or cancerous tumor cells. Specifically, antibodies mediate antigen detection and effector functions, including the initiation of a complement pathway (Miletic and Frank, 1995). Antibodies are Y-shaped proteins composed of two pairs of light and heavy chains, which have variable regions located at the N-termini of each chain, that determine antigen specificity. The fragment antigen-binding (Fab) regions determine antigen specificity. Additionally, connected to the variable regions, the Fc or non-variable region of the antibody is consistent between a class of antibodies but not across the five isotypes (Charles A Janeway et al., 2001). Its primary function is the activation of a complement system immune response, such as CDC, where the Fc regions of IgM or IgG bind to a C1q protein, resulting in a cascade of protein interactions that lead to the lysis of a target cell (Diebolder et al., 2014). These components are relevant to the overall discussion of studies comparing the ability of CDC activation among IgM hexamers, pentamers, and IgG monomers.

2. Structural Differences of IgM Hexamers, Pentamers, and IgG Monomers

2.1 IgM Structural Overview

IgM has covalent disulfide bonds between monomeric subunits to take either pentameric or hexameric form, depending on the presence of a joining chain (J chain) (Müller et al., 2013;

Randall et al., 1992). The J chain functions in linking the heavy (μ) chains of IgM polymers together, but does not display binding to the light chains (Morrison and Koshland, 1972). The J chain is approximately 15kDa in size and has disulfide bonds with two μ chains in the terminal cysteine via Cys-575 residues to complete the pentameric polymer (Figure 1a), and Cys-414 residues in disulfide bonds are found between IgM monomers to stabilize the oligomer (Fazel et al., 1997). However, in IgMs without a J chain, the hexameric form of the polymer is favored (Figure 1b).(Randall et al., 1992) Hexameric IgM polymers possess Cμ4tp-Cμ4tp (Cμ4 tailpiece) dimers to compensate for the lack of J chain presence, where β-sheets form to complete the ring-shaped conformation, and every monomer possesses Cys-575 residues for disulfide bonds (Figure 1b) (Müller et al., 2013). The IgM Fc domain is comprised of three components: Cμ2, Cys-337 residue, Cμ3, Cys-414, Cμ4 residue, Cys-575 residue, and Cμ4's tailpiece at the C-terminal (Cμ4tp), which are bound together in disulfide bridges to form the polymeric structures (Figure 1c) (Müller et al., 2013; Sitia et al., 1990). IgM opsonizes, or increases the likelihood of mediated cell death, antigens via fixation of the complement system, and is especially effective with repeating epitopes present in foreign bodies (Schroeder and Cavacini, 2010).

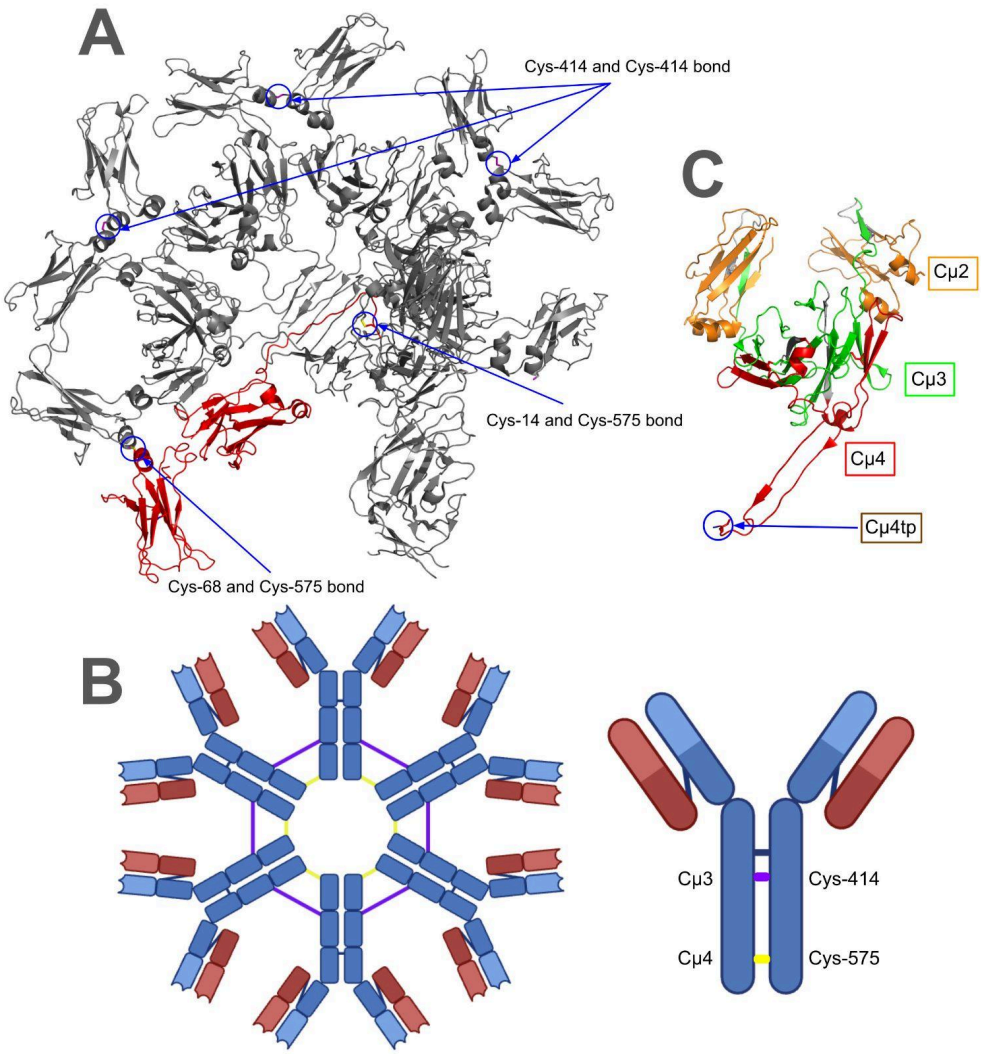

Figure 1. (A) Cryo-electron microscopy (cryo-EM) structure of a human IgM pentamer (green) with a J-chain (red) and disulfide bonds (yellow) at Cys-575 residues on two heavy chains with Cys-14 and Cys-68 residues on the J chain that allow the monomers to link together into an oligomer via intermediary bonds (PDB-6KXS) (Davis et al., 1989; Frutiger et al., 1992; Wiersma et al., 1997). Cys-414 disulfide bonds link together the individual monomers in both IgM pentamers and hexamers (magenta) (Pan et al., 2021). (B) A human IgM hexamer that lacks a J-chain, instead containing another IgM monomer. The IgM hexamer consists of monomers linked together via disulfide bonds with Cys-575 (yellow) and Cys-414 (purple) residues on different parts of their tailpieces, Cμ4 and Cμ3 domains, respectively (Müller et al., 2013; Randall et al., 1992). (C) Cryo-EM structure of a single IgM monomer's Fc region with the Cμ2 domain in orange, Cμ3 in green, Cμ4 in red, and the tailpiece of Cμ4 in brown (PDB-6KXS) (Li et al., 2020). Figures created with pymol Molecular Graphics System v.4.6.0 (Schrodinger LLC, New York, NY, USA) and BioRender.com.

2.2 IgG Structural Overview

IgG is naturally present as monomers in four different subclasses, IgG1, IgG2, IgG3, and IgG4, that can aggregate into higher-order oligomers, such as hexamers, upon attachment to antigens to activate the complement system (Diebolder et al., 2014; Schroeder and Cavacini, 2010). The basic structure is consistent with IgM and is comprised of two heavy and light chains linked together via disulfide bonds (Porter, 1959). IgG contains a hinge region between the Fab regions and the Fc portion of the antibody that allows for rotation of the antigen-binding sites for greater avidity and immune system collaboration (Figure 2a) (Deveuve et al., 2019). In IgG1, the hinge is approximately 15 amino acids in size with two disulfide bonds; IgG2 has an approximately 12 amino acid hinge with four disulfide bonds providing a more rigid structure; the IgG3 hinge is the larger at approximately 62 amino acids and 11 disulfide bonds, which causes a much shorter lifespan of about seven days; IgG4 contains a hinge region with approximately 12 amino acids and two disulfide bonds, and has a serine residue instead of proline at residue 228 to promote a phenomenon known as Fab-arm exchange that causes light and heavy chain recombination causing more possible antigen binding, and IgG4 has the least stable structure compared to IgG1-3 (Figure 2c-f) (Frangione et al., 1969; Liu and May, 2012). The IgG3 has a longer hinge region, which results in greater effector function than IgG1 (Vidarsson et al., 2014; Zwarthoff et al., 2021). IgG1 and IgG3 have the greatest affinity for CDC and are most prevalent in the resulting lysis of target cells (Zwarthoff et al., 2021). The IgG Fc region has CH2 and CH3 (Cγ2–Cγ3) domains, stabilized by non-covalent interactions and glycosylated at Asn-297 (CH2), which enhances C1q and Fc receptor binding (Figure 2b) (Edelman et al., 1969; Frangione et al., 1969).

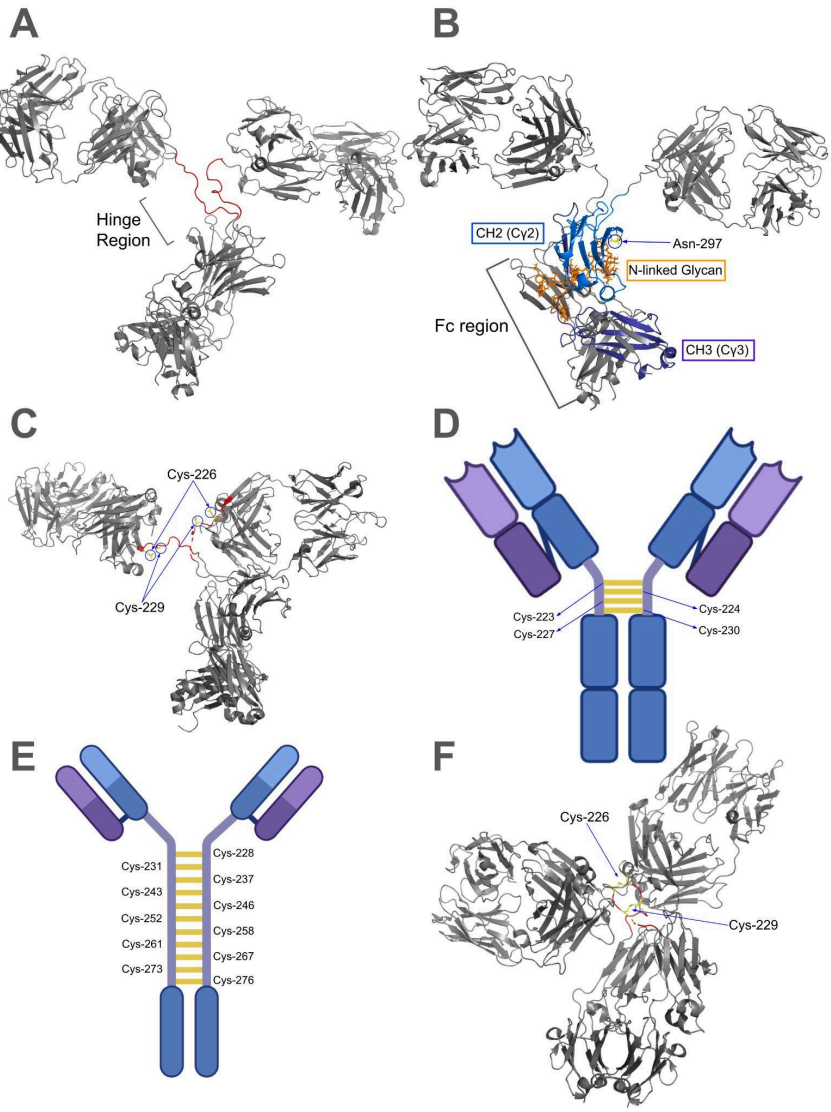

Figure 2. (A) An X-ray diffraction analysis shows an IgG monomer with its hinge region colored red (PDB-1IGT) (Harris et al., 1997). The hinge region provides flexibility to create greater Fab region affinity by conforming to a separated epitope (Deveuve et al., 2019). (B) The CH2 (lightblue) and CH3 (darkblue) domains of the Fc region on IgG that stabilize with C1q receptor binding with an N-linked glycan (orange) on the Asn-297 residue (yellow) (PDB-1IGT) (Boune et al., 2020; Harris et al., 1997; van Osch et al., 2021). (C, D, E, F) X-ray diffraction analyses show IgG1 and IgG4 with schematics modeling IgG2 and IgG3, which demonstrate the differing hinge regions and disulfide bonds. A lack of data regarding the IgG2 and IgG3 hinge region is the reason for the schematics in (D) and (E). IgG1 has Cys-226 and Cys-229 residues (yellow) for heavy chain binding in the hinge region (red) that form disulfide bonds, but do not in this figure (PDB-1HZH) (Saphire et al., 2001); IgG2 has 4 disulfide bonds Cys-223, Cys-224, Cys-227, and Cys-230 residues (yellow) (Dillon et al., 2008); IgG3 has 11 total disulfide bonds, which are Cys228, Cys231, Cys237, Cys243, Cys246, Cys252, Cys258, Cys261, Cys267, Cys273, and Cys276 residues (yellow) (Michaelsen et al., 1977; Spiteri et al., 2021); IgG4 has the same residues as IgG1 with Cys-226 and Cys-229 (yellow) across the hinge region (red) (PDB-5DK3)

(Liu and May, 2012; Michaelsen et al., 1977; Scapin et al., 2015). The overall Fab and Fc region, excluding the hinge region and CH2 domain, of IgG1-4 is very similar. Figures created with PyMOL Molecular Graphics System v.4.6.0 (Schrodinger LLC, New York, NY, USA) and BioRender.com.

3. Complement Activation with C1 Complex via IgM Hexamers, Pentamers, and IgG Oligomers

3.1. Structural Components of IgM and IgG for C1 Complex Binding

Antigen binding sites for IgM and IgG differ in structure, but both classes of immunoglobulins share the F(ab′)2, Fab′, Fab, and Fv fragments (Coulter and Harris, 1983; Diebolder et al., 2014). IgM is found with 10-12 Fab regions, due to its polymeric state, instead of the typical 2 in IgG, which provides a higher avidity for antigen response (Klimovich, 2011). Within the Fab regions are CDRs (Complementarity-Determining Regions) for hyperspecific antigen binding, comprising a total of six CDRs: three in both the heavy and light chains (Kelow et al., 2020). In relation to variable regions, CDRs provide the functionality for binding to epitopes on antigens with CDR1, CDR2, and CDR3 loops possessing unique sequences for affinity (Kelow et al., 2020). The classical complement system consists of circulating C1 complexes of size 790kDa with subunits C1q, C1r, and C1s (Figure 3a) (Cruz et al., 2021; Venkatraman Girija et al., 2013). To initiate the cascade, C1 must first bind to antibody complexes on antigens using the recognition site C1q: C1q has a disulfide-linked hexameric structure with globular heads splaying out from an N-terminal stalk, forming 6 collagenous arms (Cruz et al., 2021; Venkatraman Girija et al., 2013). Each gC1q (the globular heads) contains three chains (A, B, C) (Figure 3b) that bind to Fc regions of antibodies (Figure 3c,d) (Sharp et al., 2019).

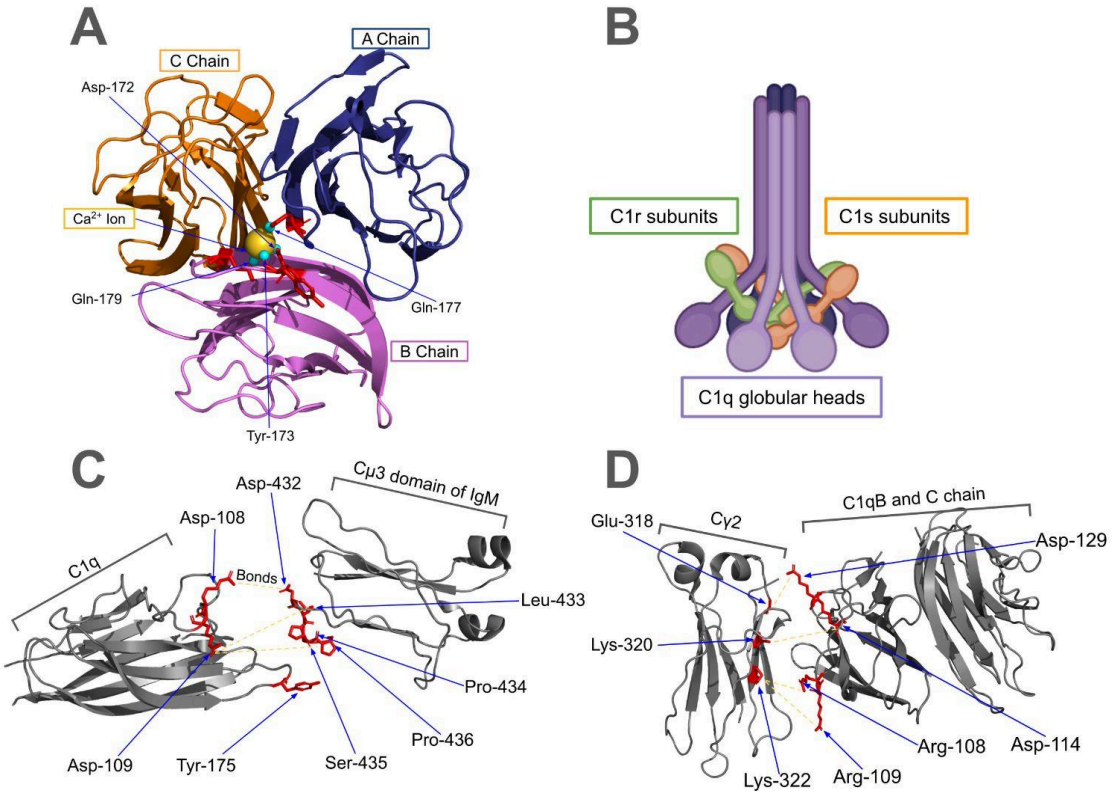

Figure 3. C1 Complex binding with IgG and IgM. (A) A schematic of the C1 complex with subunits C1q (purple globular heads), C1r (green), and C1s (orange) (BioRender). (B) An X-ray diffraction analysis shows the C1q globular heads with the A (orange), B (violet), and C (dark blue) chains capable of binding to Fc regions (PDB-1PK6) (Gaboriaud et al., 2003). C1q subunits contain a central calcium ion (Ca^{2+}, gold) that functions to hold together the three chains of C1q and mediate interactions with antibodies IgG and IgM, which is coordinated by oxygen ligands (cyan) contributed by the side-chain oxygen of residues on A and B chains: Asp-172, the side-chain carbonyls of Gln-177 and Gln-179, the main-chain carbonyl of Tyr-173, and two waters (not shown) (Gaboriaud et al., 2003; Roumenina et al., 2005). (C) X-ray diffraction analyses visualize C1q binding to IgM via IgM's Cμ3 domain (Cμ3 residues DLPSP, Asp-Leu-Pro-Ser-Pro residues 432–436) and C1q's B chain (C1q residue Arg-108, Arg-109, Tyr-175) (PDB-6KXS, 1PK6) (Gaboriaud et al., 2003; Li et al., 2020; Sharp et al., 2019). (D) X-ray diffraction analyses visualize IgG interacting with C1q via IgG's Cγ2 domain (Cγ2 residues Glu-318, Lys-320, and Lys-322) and C1q's B and C chains (C1q B chain residues Arg-108, Arg-109, Arg-114, and Arg-129, C chain residues Lys-170 and Asp-195) (PDB-1PK6, 1H3Y) (Bally et al., 2019; Gaboriaud et al., 2003; Kaul and Loos, 1997; Krapp et al., 2003; Sharp et al., 2019). Figures created with PyMOL Molecular Graphics System v.4.6.0 (Schrodinger LLC, New York, NY, USA) and BioRender.com.

3.2 Overview of CDC Pathway

Cryo-EM data show that the entire C1 complex undergoes conformational changes after binding to the antibodies to accommodate C1r and C1s dimers (Mortensen et al., 2017). C1r and C1s are serine proteases, which hydrolyze peptide bonds, that assemble in a tetrameric shape with the serine protease domains at the end of the C1r2s2 tetramer (Sharp et al., 2019; Weiss et al., 1986). Each C1r autolytically cleaves the other, a process called autoactivation, to activate the C1 complex by then cleaving C1s (Arlaud et al., 2002; Cruz et al., 2021). C1s is then able to cleave the C4 protein into C4a, an anaphylatoxin, and C4b, which recruits the C2 protein that C1s cleaves into C2a, a serine protease, and C2b (Merle et al., 2015; Walport, 2001). C4b and C2a form C3 convertase (C4b2a) that cleaves the C3 protein into C3a, an anaphylatoxin for inflammation, and C3b (Rawal and Pangburn, 2003). C3b binds to the target cell to amplify opsonization and continues to interact with C4b2a to form C5 convertase (C4b2a3b), which cleaves C5 into C5a, an anaphylatoxin for immune cell recruitment, and C5b. C5b recruits C6, C7, C8, and multiple C9 molecules to form the membrane attack complex (MAC), C5b–C9, a pore-like structure approximately 10 nm in diameter, which disrupts the target cell membrane, ultimately causing osmotic lysis of the target cell (Merle et al., 2015; Rawal and Pangburn, 2003; Ricklin et al., 2010).

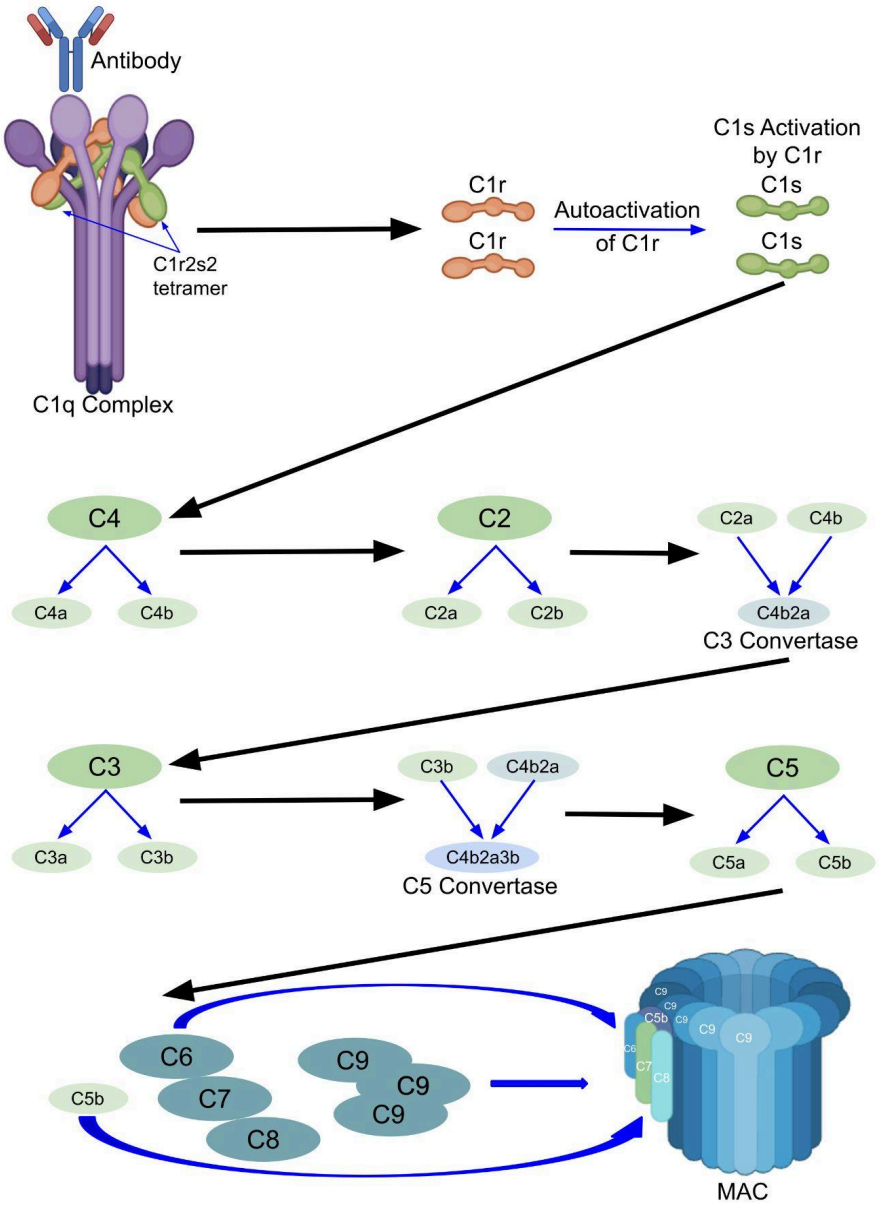

Figure 4. Schematic for visualization of the CDC activation process beginning from the initial C1 Complex binding to an antibody to the MAC complex, enabling cell lysis (Noris and Remuzzi, 2013). Figure created with BioRender.com.

3.3 IgG CDC Pathway Differences

IgG initiates CDC via a different mechanism due to its structural differences. A cryo-electron tomography and atomic force microscopy study demonstrated that IgG creates a hexameric binding platform on the antigen to bind six C1q subunits (Cruz et al., 2021; Zeller et al., 2022). The hexameric compound normally forms via Fc-Fc interactions of IgG, but in the absence of IgG:C1q complexes forming, which was found in a study using SpA (Staphylococcal Protein A) binding competitively to Fc regions on IgG, Fc-Fc interactions are prevented resulting in no hexamerization, so complement activation does not occur (Cruz et al., 2021; Sharp et al., 2019).

4. Characteristics of antibody reaction to low-antigen dense tumors

4.1.1 CDC Activity Comparison

Due to the naturally multivalent structure of IgM (ranging from 10 to 12 binding sites), it is much more likely to bind to epitopes on antigens compared to IgG, which has two binding sites. A study done by Oskam et al. in 2022 measured the effectiveness of IgG1, IgM pentamers, and IgM hexamers by producing antibodies of IgG1, IgM pentamers, and IgM hexamers specific for biotin and citrullinated proteins. With the produced antibodies, ELISAs were used to measure the amount of complement components, such as C1q, C4b, and C3b, produced after using human serum to incubate antigen-coated plates with varying densities of biotinylated human serum albumin (HSA-bt) exposed to the antibodies (Oskam et al., 2022). Oskam et al. also utilized a CDC assay to measure the endpoint outcome of cell lysis achieved on biotinylated red blood cells (RBCs) incubated with the antibodies to measure low antigen density; lysis was quantified by spectrophotometric measurement of released hemoglobin (Oskam et al., 2022). The results show that at high antigen densities, both IgM and IgG1 configurations induced similar CDC potency; at lower antigen densities, both IgM forms outperform IgG1 at a significant rate; at critically low antigen densities, IgM hexamers demonstrate higher potency than even IgM pentamers (Oskam et al., 2022).

Table 1. ***Complement C3b Deposition on HSA-bt in ELISA***

Epitopes per HSA (Approximate Density)	IgM Hexamer	IgM Pentamer	IgG1 Monomer
14 (high)	90-95%	85-90%	70-80%
8 (Moderate)	85-90%	70-80%	30-40%
3 (Low)	80%	40-50%	10-15%
1 (Critically Low)	50-60%	20-30%	0-5%

[a]Antibody effectiveness represented through complement component deposition rates estimated from Oskam et al. ELISA assays on HSA-coated plates (Oskam et al., 2022).

4.1.2. CDC Activity Differences From Structural Differences

The greater cell lysis rate can be attributed to the higher number of Fab regions in IgM molecules, which leads to a greater stability and arrangement of Fc regions for C1 complex binding. Compared to the two Fab regions of IgG and the need for two to six separate immunoglobulins to form aggregates for C1 complex binding, IgM achieves greater efficacy for antigen binding and complement pathway activation (Perelson and Wiegel, 1979). IgM hexamers outperform IgM pentamers since the hexameric configurations do not need to undergo conformation change for efficient C1q binding; the C1 complex binding is demonstrated by the effectiveness of IgM hexamers in Oskam et al's study through the greater percentage of complement system component C3b deposition rates at low antigen densities, proving greater complement system activation (Sharp et al., 2019).

4.2. CDC for Tumors Comparison

A greater affinity for low-antigen density conditions is especially relevant for tumor relapse, which becomes more prevalent in tumors that exhibit lower antigen expression and experience antigen escape to evade the immune system (Lu and Zhao, 2025; Olson and McNeel, 2012). IgM hexamers' high avidity for antigens, even in low-density conditions, reduces the capacity of antigen escape, which has clinical usage for the prevention of tumor relapse (Wang et al., 2016). In 2007, Azuma et al. conducted the first report of a majority hexameric IgM solution that was shown to mitigate cancer approximately 5 to 10 times more effectively than its counterpart of majority pentameric IgM for melanoma metastases (Azuma et al., 2007). By creating GM3-positive human melanoma and GM3-positive B16F10 mouse melanoma cell lines with only the GM3 ganglioside, the monoclonal L612 IgM, CJ45 IgM, and CA19 IgM specific for GM3, but with differing amounts of hexameric and pentameric IgM concentrations, were tested for CDC activity against the melanoma (Azuma et al., 2007). L612 contained 19.8% hexameric and 73.6% pentameric forms of IgM, CJ45 contained 4.5% hexameric and 91.5% pentameric forms of IgM, and CA19 had the largest concentration of hexameric IgM at 79.2% with 9.3% pentameric IgM; the remaining concentrations were tetrameric and aggregate forms of IgM. A Chromium-51 release assay was used to measure antibody-induced CDC activity against the tumor cell lines (Azuma et al., 2007). At an antibody concentration of 5 μg/mL, CA19, CJ45, and L612 demonstrated similar levels of CDC activity against B16F10; however, at lower antibody concentrations, approximately 0.04 to 0.2 μg/mL, CA19 demonstrated greater levels of CDC than CJ45 and L612. CA19's antibody concentration necessary to achieve 50% specific cytotoxicity was found to be approximately 5 to 10 times lower than CJ45 and L612 (Azuma et al., 2007). For M1 human melanoma, CA19 demonstrated similar results of cytotoxic activity at differing antibody concentrations compared to CJ45 and L612, as in the B16F10 test (Azuma et al., 2007). This demonstrates that a higher concentration of hexameric IgM results in a more effective monoclonal antibody treatment, as CA19 showed greater cytotoxicity than its counterparts, CJ45 and L612, which contain more pentameric IgM, at lower antibody concentrations.

5. Synthetic Production of IgM Hexamers

Typically, antibodies pass through the epithelial barrier to reach organs and bind to pathogens, alerting the immune system to respond (Rojas and Apodaca, 2002). IgM hexamers are unable to pass through the epithelia of the bloodstream because they do not possess the J-chain, which is used to bind to polymeric Ig receptor (pIgR) to allow IgM to become exocrine secretions (Johansen et al., 2000). To rectify this issue, supplementing patients with artificially produced IgM hexamers could be used, which would allow the use of higher-affinity antibodies to treat low-antigen-density tumors. IgM hexamers can be produced using mammalian cells as cell hosts, which preserves glycosylation patterns for accurate production of monoclonal antibodies (Al Ojaimi et al., 2022). The specific lines of host mammalian cells used for recombinant production are human embryonic kidney cells (HEK293), mouse bone marrow cells (NS0), mouse spleen cells (Sp2/0), Chinese hamster ovary cells (CHO), and human embryonic retinal cells (PER.C6) (Ho et al., 2012; Jones et al., 2003; Kunert and Reinhart, 2016; Melixetian et al., 2003). Transport of artificially produced antibodies can bypass the epithelium via a subcutaneous injection (Al Ojaimi et al., 2022). Intratumoral injection directly into a tumor can also be used to create a more effective therapeutic response with IgM hexamers (Baniel et al., 2020; Hong et al., 2020). This method can be used to bypass the restriction that a J-chain-lacking IgM hexamer possesses.

Conclusion

This research paper has discussed the structural distinctions among IgM hexamers, IgM pentamers, and IgG monomers, focusing on their influence on Fc-C1q interactions and CDC in low-antigen-density tumors. IgM hexamers, characterized by the absence of a J-chain and stabilized by Cμ4tp-Cμ4tp dimers, possess a structural advantage allowing for enhanced CDC efficacy. IgM hexamers achieve greater cell lysis at critically low antigen densities, compared to pentamers and for IgG. The multivalent nature of IgM hexamers (10–12 Fab regions) enables efficient C1q recruitment without the Fc-Fc aggregation required by IgG, making them highly effective against tumors prone to antigen escape and relapse. However, limitations such as restricted tumor penetration due to high affinity and the inability to cross epithelial barriers due to the absence of J-chain-mediated pIgR binding pose challenges for natural IgM hexamers in therapeutic settings.

In vitro production of IgM hexamers offers a potential solution using recombinant techniques in mammalian cells to produce hexamer antibodies, which can be paired with subcutaneous or intratumoral injections to bypass epithelial restrictions. These approaches amplify CDC and can be utilized in therapeutics to optimize tumor penetration. By harnessing these advancements, IgM hexamers offer a transformative approach to combat low-antigen-density cancers, potentially reducing relapse rates and demonstrating the potential for personalized immunotherapies.

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