Mouse Models of Rheumatoid Arthritis for Studies on Immunopathogenesis and Preclinical Testing of Fc Receptor-Targeting Biologics

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Rheumatoid arthritis · Mouse model · Crystallizable fragment gamma receptor · Intravenous immunoglobulin · Recombinant crystallizable fragment · rFc multimers

Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes inflammation, swelling, and pain in the joints and involves systemic complications. Mouse models of RA have been extensively used to model the pathogenesis of RA and to develop effective therapies. Although many components of the immune system have been studied in these models, the role of crystallizable fragment (Fc) gamma receptors (FcγRs) in RA has been sorely neglected. The aim of this review was to introduce the different mouse models of RA and to describe the different drug development strategies that have been tested in these models to target FcγR function, with the focus being on drugs that have been made from the Fc of immunoglobulin G (IgG). **Summary:** Evidence suggests that FcγRs play a major role in immune complex-induced inflammation in autoimmune diseases, such as RA. However, there is limited knowledge on the importance of FcγRs in the human disease even though there has been extensive work in mouse models of RA. Numerous mouse models of RA are available, with each model depicting certain aspects of the disease. Induced models of

RA have nonspecific immune activation with cartilage-directed autoimmunity, whereas spontaneous models of RA develop without immunization, which results in a more chronic form of arthritis. These models have been used to test FcγR-targeting monoclonal antibodies, intravenous immunoglobulin (IVIg), subcutaneously administered IVIg, and recombinant Fcs for their ability to interact with and modify FcγR function. Recombinant Fcs avidly bind FcγRs and exhibit enhanced therapeutic efficacy in mouse models of RA. **Key Message:** The therapeutic utility of targeting FcγRs with recombinant Fcs is great and should be explored in human clinical trials for autoimmune diseases, such as RA.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that primarily affects the joints of the hands and feet but can affect other joints and commonly involves systemic complications [1]. RA is a form of polyarthritis that affects the joints symmetrically. It is characterized by synovitis and synovial hyperplasia, cartilage and bone erosion, as well as systemic inflammation [2, 3]. This chronic inflammation and progressive tissue destruction lead to irreversible structural deformities and the eventual loss of function of the affected joints [4].

Animal models have been used extensively in studies of RA pathogenesis and have contributed to the development of several therapies [5–7]. Our understanding of the importance of pro-inflammatory cytokines, the adaptive immune system, autoantibodies, and crystallizable fragment (Fc) gamma receptors (FcγRs) in the pathogenesis of RA is due to studies in mouse models [8–14]. Monoclonal antibody therapies have been successfully developed in mouse models of RA. For example, infliximab is a monoclonal antibody that targets tumor necrosis factor- α (TNF- α) [8]. It has long been known that TNF- α plays a significant role in cartilage and bone erosion in RA because transgenic mice expressing human TNF- α experience a severe and erosive form of polyarthritis [8, 15]. Treatment of these arthritic mice with infliximab completely abrogates the disease [8]. Furthermore, the success of B-cell depletion therapy with rituximab, an anti-CD20 monoclonal antibody, was corroborated in both the collagen-induced arthritis (CIA) model and in K/BxN mice, which spontaneously develop arthritis [16, 17].

Although both mouse models and human studies have elucidated the importance of FcγRs in the pathogenesis of RA, these receptors have not been given the attention they deserve [18–21]. In RA, autoantibodies and immune complexes promote an inflammatory response by engaging FcγRs. This engagement can lead to a multitude of responses such as pro-inflammatory cytokine release, antibody-dependent cellular cytotoxicity (ADCC), phagocytosis, and respiratory burst. These responses lead to sustained inflammation and tissue destruction within the joint. Disrupting this interaction has been explored through multiple avenues such as monoclonal antibodies [22, 23], intravenous immunoglobulin (IVIg) [24, 25], and through the development of recombinant Fcs that are composed of the of immunoglobulin G (IgG) [26]. These proteins could potentially treat autoimmune diseases because of their ability to produce a range of different immunomodulatory effects [27]. This review provides an overview of the different mouse models of RA, and it discusses the use of different recombinant Fcs in these models to highlight the therapeutic utility of targeting FcγRs in RA.

Fc Receptors

Fc receptors are proteins expressed on the surface of myeloid cells, B cells, NK cells, NKT cells, and certain subsets of T cells that interact with the Fc of Igs as described in a thorough review of the topic [28]. Fc receptors are the link between antibodies and the innate im-

mune system. They can activate or inhibit cells, cause the release of pro-inflammatory cytokines, and they also participate in ADCC and phagocytosis [29]. FcγRs are part of a family of Fc receptors that recognize the Fc of IgG [28]. For human IgG, the activating receptors are FcγRI, FcγRIIA, FcγRIIC, FcγRIIIA, and FcγRIIIB [28]. FcγRI is a high-affinity activating receptor that binds monomeric IgG, whereas FcγRII and FcγRIII are low-affinity activating receptors that only bind aggregated IgG or immune complexes [28]. For mouse IgG, FcγRI, FcγRIII, and FcγRIV are the activating receptors [28]. FcγRI is also a high-affinity activating receptor in mice, whereas FcγRIII and FcγRIV are low-affinity activating receptors [28]. For both human and mouse IgG, FcγRIIB is the only inhibitory receptor [28]. In humans and mice, monocytes, macrophages, dendritic cells, and neutrophils express all types of activating FcγRs and the inhibitory FcγR, whereas B cells only express FcγRIIB [28].

The activating FcγRs have a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM), while the inhibitory FcγR has an immunoreceptor tyrosine-based inhibitory motif [28]. Initiation of the activation pathway leads to the phosphorylation of ITAM sequences by Src-family kinases, which recruits and activates the spleen tyrosine kinase. This results in the activation of downstream signaling pathways that are important for cellular proliferation, cytokine and chemokine production, ADCC, phagocytosis, and respiratory burst [21]. Initiation of the inhibitory pathway leads to the phosphorylation of immunoreceptor tyrosine-based inhibitory motif sequences, which prevents cellular proliferation, cytokine and chemokine production, ADCC, phagocytosis, and respiratory burst through the recruitment of Src-homology 2 domain tyrosine phosphatases, SHIP or SHP [21]. The crosslinking of FcγRs by IgG is critical for these downstream signaling pathways to occur [21]. Different IgG isotypes bind with different affinities to activating and inhibitory FcγRs, which can skew the activation/inhibition ratio toward cellular activation or inhibition [29]. The disequilibrium between activating and inhibitory FcγRs can result in uninhibited inflammatory responses, such as those seen in RA. Further understanding of this threshold of activation will promote the development of new therapeutic strategies that target FcγRs.

FcγRs in the Pathogenesis of RA

Evidence suggests that FcγRs play a major role in immune complex-induced inflammation in autoimmune diseases such as RA. During normal immune responses, the

FcγR-mediated antibody effector functions are fine-tuned by a balance between activating and inhibitory FcγRs. However, this balance is skewed toward activating functions of FcγRs in autoimmunity, which reduces the threshold for immune complex-mediated activation of inflammatory cells [19, 30]. In RA, this disequilibrium is caused by an up-regulation of activating FcγRs, which lowers the threshold for monocyte/macrophage activation, which are major contributors of inflammation and tissue damage [31].

Increases in the expression of activating FcγRs have been found on the peripheral blood monocytes of RA patients, and this correlates with inflammatory serological markers, such as erythrocyte sedimentation rate [32]. Additionally, treatment-naïve RA patients had significantly higher levels of activating FcγRIIA on their monocytes, macrophages, and NK cells in comparison to RA patients taking disease-modifying anti-rheumatic drugs. This suggests that there is a correlation between activating FcγR expression and disease activity [32, 33].

Several other studies have showed the importance of activating FcγRs in patients with RA. In one study, monocytes from RA patients secreted TNF-α, interleukin (IL)-1β, and IL-8 upon stimulation with immobilized immune complexes. The release of these pro-inflammatory cytokines was inhibited by blocking FcγRIIA with a monoclonal antibody [34]. In another study, immune complexes that were isolated from the synovial fluid of RA patients induced the same response from monocytes, which was also blocked by a monoclonal antibody to FcγRIIA [35]. These results were reproduced in healthy subjects; the stimulation of healthy monocytes and macrophages with immune complexes formed by anti-citrullinated protein antibodies from RA patient sera or synovial fluid resulted in TNF-α secretion via engagement of FcγRIIA [31, 35, 36]. When the RA synovium was compared with a healthy synovium, there was increased expression of activating FcγRs in the RA synovium, and this correlated with an increased number of synovial macrophages that expressed TNF-α [37, 38].

Thus, FcγRs are potential targets for the treatment of antibody-mediated autoimmune diseases.

Targeting FcγRs

Historically, FcγRs have been blocked with mouse antihuman monoclonal antibodies that target the IgG-binding site of FcγRs, as well as with IVIg. More recently, recombinant Fcs derived from the Fc of IgG have been used to block autoantibody and immune complex engagement with FcγRs to prevent a range of cellular responses.

Anti-FcγR Monoclonal Antibodies

Monoclonal antibodies directed against FcγRs have been used to block immune complex-mediated activation of these receptors. Mouse antihuman monoclonal antibodies have been made against each of the activating and inhibitory FcγRs [39, 40]. The mouse monoclonal antibody 3G8 targets human FcγRIII and has been used in humans to block the clearance of antibody-coated platelets in patients with immune thrombocytopenia (ITP) [41, 42]. ITP is an autoimmune disease whereby platelets are targeted by the humoral system and destroyed through FcγR-mediated phagocytosis in the spleen and liver [43]. 3G8 successfully prevented platelet loss in human clinical trials for ITP [43]. The high-affinity FcγRI has also been targeted by monoclonal antibodies in clinical trials for ITP. Monoclonal antibody 197 abrogated IgG binding to FcγRI, and it modulated the expression of FcγRI in humans [44].

Although the role of FcγRIII in human ITP is apparent, the role of this FcγR and others has not been elucidated in more complex autoimmune diseases, such as RA. There are significant challenges when selecting which FcγRs to target in RA because leukocytes express several FcγRs at the same time. It is not clear which FcγRs on which cell types are involved in the pathogenesis of RA and whether there is redundancy between FcγRs or whether some FcγRs are more important than others. Thus, the development of anti-FcγR monoclonal antibodies for the treatment of complex autoimmune diseases in humans has been limited.

Intravenous Immunoglobulin

IVIg is the pooled plasma from tens of thousands of donors and it is mainly composed of polyvalent IgG antibodies, with trace amounts of IgA and IgM [45–47]. IVIg was initially indicated to treat primary immunodeficiency; however, it is now used as a therapy for several autoimmune diseases and inflammatory conditions [48, 49]. Additionally, high-dose IVIg has become a first-line therapy for autoimmune diseases such as ITP and Kawasaki disease [50, 51]. IVIg was first given at a high dose of 400 mg/kg over 5 consecutive days to raise platelet counts in pediatric patients with ITP [52]. Later, it was found to be effective in treating adult ITP with over 80% of patients responding to IVIg shortly after treatment [53–57].

There are several mechanisms of action that have been proposed for the immunomodulatory effects of IVIg; how-

ever, this topic is still heavily debated [57]. One of the earliest proposed mechanisms of IVIg includes the blockade of activating FcγRs on macrophages by IVIg [56, 58]. Fehr et al. [56] showed that the clearance of radiolabeled erythrocytes was delayed by IVIg administration, suggesting that IVIg worked to treat ITP by blocking FcγRs on macrophages and inhibiting platelet clearance. This theory was corroborated by Salama et al. [59] who showed that there was increased hemolysis after IVIg therapy. Additional evidence for the involvement of FcγRs was demonstrated by Aloulou et al. [60] who showed that binding of IVIg to FcγRIIIA induced inhibitory ITAM signaling and contributed to the anti-inflammatory activity of IVIg. There is also evidence showing a role for the antibody-binding Fab region of IVIg in its immunomodulatory effects. Investigators have shown that the antigen-binding fragments (F[ab']₂) of IVIg could ameliorate ITP in human patients [61, 62]. In addition, anti-Siglec-8 autoantibodies found in IVIg preparations were shown to have anti-inflammatory effects [63, 64]. One group found that sialylation of the Fab, not the Fc, was associated with the anti-inflammatory activity [65].

The main hypotheses as to how IVIg works as an anti-inflammatory biologic come from the group at the Rockefeller University headed by Jeffrey Ravetch [66–68]. One theory proposed involves the upregulation of inhibitory FcγRIIB on effector cells, which increases their threshold for activation [66, 68]. Using an antibody-induced passive mouse model of ITP in FcγRI, FcγRIIB, and FcγRIII knockout mice, Samuelsson et al. [66] showed that IVIg reduced platelet clearance through the concurrent inhibition of FcγRIII with the upregulation of FcγRIIB. Additionally, IVIg was shown to be ineffective at treating ITP in FcγRIIB knockout mice [66]. However, other investigators could not replicate this work [69]. One study found that IVIg specifically downregulated activating FcγRIIIA on CD11c⁺ dendritic cells, which had no effect on FcγRIIB expression [69]. Other studies showed that FcγRIIB was unnecessary for IVIg efficacy using different strains of FcγRIIB knockout mice [69–72].

In accordance with their theory that IVIg upregulates FcγRIIB as part of its mechanism, Ravetch et al. [73] proposed that the Fcγ-sialylated fraction of IgG is responsible for the immunomodulatory properties of IVIg [24, 73–75]. This model proposed that a small fraction (10%) of total IVIg sialylated in the Fcγ domain engages the cluster of differentiation 209, also known as DC-SIGN, on regulatory macrophages and dendritic cells, which causes them to release IL-33 [68, 75]. IL-33 is a Th2-polarizing cytokine that would then promote the release of IL-4 from basophils at sites of inflammation. IL-4 acts on

macrophages to upregulate the expression of FcγRIIB, which lowers inflammation [68]. This model proposes that the subsequent upregulation of FcγRIIB on effector cells raises their threshold for activation, thus allowing for a reduction in inflammation [67, 68, 75].

However, this model has not been supported by other investigators. Despite a recent report that IVIg induces IL-4 in human basophils, which supports the “Ravetch model” [76], other investigators have shown that although IL-4 is induced by IVIg treatment in mouse models [77], neither IL-4 nor basophils are required for the anti-inflammatory effects of IVIg [78–80]. In addition, although a recent report indicates that Fc sialylation may play a role in the mechanism of action of IVIg in certain conditions [81], other groups have not been able to confirm a role for Fc sialylation [82–86]. Additional studies also suggest that there is not a role for either DC-SIGN [87] or IL-33 [77, 88] in the anti-inflammatory mechanism of IVIg. These discrepancies between the model of IVIg action proposed by the Ravetch group and the findings by other scientists in the field led to a rebuttal article by von Gunten et al. [89] published in 2014.

Other possible mechanisms of action of IVIg have been proposed. Production of prostaglandin E₂ (PGE₂) by IVIg binding to dendritic cells, which then induces an increase in T-regulatory cells has been reported [90, 91]. Also, it was recently published that IVIg induces autophagy which regulates the inflammatory response [92]. A definitive mechanism for the anti-inflammatory activity induced by IVIg awaits resolution. Its mechanism of action appears complicated, and it may work differently depending on the autoimmune model that is used.

Limitations of IVIg

IVIg is generally considered to be a safe drug because it has minimal side effects and most of these side effects are benign, with chills and headaches being most common. More serious adverse events such as thrombosis and hemolysis are very rare [93–99]. IVIg also has a very favorable safety profile because it is immunomodulatory rather than immunosuppressive.

Despite this, there are serious challenges associated with the manufacturing and application of this drug. First, the manufacturing of IVIg requires highly specialized production facilities, which are very expensive to maintain [100]. Patients who receive IVIg are at risk of contracting blood-borne pathogens, particularly newly emerging infections, so careful attention must be paid to

pathogen safety. Not only is the cost of these production facilities expensive, but IVIg itself is very expensive because its supply is dependent on the availability and the collection of human plasma. With an increase in the demand for IVIg to treat a variety of autoimmune and inflammatory conditions and a decrease in the supply of human plasma donations, the price of IVIg has drastically increased to pay for the high doses required for immunomodulation [101].

IVIg in RA

IVIg has been increasingly used for the treatment of patients with autoimmune and inflammatory diseases, such as ITP, Guillain-Barré syndrome, Kawasaki disease, and several other rare diseases [102]. Additionally, IVIg is used off-label in many other diseases and is currently under evaluation for the treatment of RA [103].

However, there is a limited amount of research performed on the therapeutic efficacy of IVIg in RA [104–108]. The human trials that were done to evaluate this were not controlled and were performed in the prebiologic era; thus, the results were equivocal [104–108]. Additionally, the patient cohort for these studies was small, with some studies having less than 20 patients enrolled [108, 109]. A high-dose IVIg protocol of 1–2 g/kg of IVIg per month for a minimum of 6 months is critical for effectively treating systemic autoimmune diseases [47]. However, these studies used lower doses of 400 mg/kg of IVIg without any long-term follow-up [108, 109].

There has been an increasing amount of evidence that IVIg works to treat RA in various mouse models through the FcγR blockade [22, 89, 110–112]. However, the numerous challenges associated with IVIg production and its application has provided incentives to develop an alternative therapy with similar efficacy but with improved characteristics. This particularly applies for indications where the effector mechanism(s) of IVIg are dependent on the Fc of IgG. The mechanism of IVIg in mouse models of RA has been proposed to be mediated by the Fc of IgG [22, 89, 110–112]. Accordingly, many researchers have investigated recombinant Fcs as a replacement for IVIg.

Mouse Models of RA

Several mouse models have progressed our understanding of the immunopathogenesis of RA and have contributed to the development of several therapies. Most

of these mouse models have a role for FcγRs in their pathogenesis and are being used to investigate recombinant Fcs as a replacement for IVIg.

Induced Mouse Models of RA

The CIA mouse model is the gold standard in vivo model for RA because its pathological features closely resemble those in humans [113, 114]. CIA is a form of polyarthritis with synovial inflammation, cartilage destruction, and bone erosion [113]. In CIA, susceptible mouse strains such as DBA/1, B10.Q, and B10.RIII DBA/1 are immunized against type II collagen (CII) in complete Freund's adjuvant [114, 115]. The clinical signs of arthritis usually appear 3–5 weeks after immunization [115, 116]. The pathogenesis of CIA is dominated by Th1 and Th17 cells, which provide B-cell help such that autoantibodies to CII are made [117].

The collagen antibody-induced arthritis (CAIA) mouse model represents the effector phase of arthritis [118, 119]. A cocktail of anti-CII monoclonal antibodies is administered to induce arthritis in mice [120]. LPS is used to enhance the incidence and severity of the arthritis. There are many susceptible mouse strains that can be used for this model, and the clinical signs of arthritis generally appears a few days after antibody administration [121]. Immune complexes initiate inflammation by activating the classical complement pathway or by engaging FcγRs on immune cells [119, 121–123].

Spontaneous Mouse Models of RA

Human TNF-α-transgenic mice spontaneously develop arthritis [124]. After 3–4 weeks of age, synovial hyperplasia and inflammatory cell infiltrates are observed in the joint, and at around 10 weeks of age, the mice exhibit pannus formation, cartilage destruction, and bone erosion [125]. Synovial fibroblasts are the main drivers of arthritis in this model, rather than immune cells [126]. This model elucidated the importance of TNF-α in the pathogenesis of arthritis and was instrumental in the development of TNF inhibitors [124].

IL-1 receptor antagonist knockout mice also develop spontaneous arthritis. Interestingly, these mice generate antibodies against CII, double-stranded DNA, and IgM RF [9]. After 5 weeks of age, synovial inflammation ensues, and at 8 weeks of age, mice experience higher rates of morbidity. Given the fact the anti-IL-1β therapies such as Anakinra, a molecule targeting the human IL-1 receptor antagonist, have only worked in a minor subset of RA patients; this mouse model is not commonly used to study this disease [127].

K/BxN mice also develop arthritis spontaneously. K/BxN mice are made by crossing T-cell receptor (TCR)-transgenic KRN mice with nonobese diabetic mice [105]. Arthritis progression in K/BxN mice is driven by the activation of T cells expressing the KRN TCR that recognizes a self-peptide bound to the nonobese diabetic-derived MHC class II I-Ag7 molecule on antigen-presenting cells [128]. The peptide recognized by the K/BxN TCR is glucose-6-phosphate isomerase (G6PI), a cytosolic glycolytic enzyme catalyzing the inter-conversion of d-glucose-6-phosphate to d-fructose-6-phosphate [13, 129]. Pathogenic autoantibodies to G6PI are created when activated T cells give help to B cells through CD40:CD40-ligand (L) engagement [12, 13, 127–131]. The serum from K/BxN mice contains anti-G6PI autoantibodies and, thus, can be transferred to a variety of mouse strains, including B- and T-cell-deficient mice, to induce arthritis [12, 130, 131]. This arthritis lasts for ~2–4 weeks; however, repeated administration of the serum can prolong the arthritis [132, 133].

Many researchers use the K/BxN serum transfer model to study arthritis even though there is no breach in tolerance. Although this model is a useful tool to understand how autoantibodies drive the progression of arthritis by interacting with downstream components of the innate immune system, there are some major differences between the serum transfer model and the human disease. These include differences in antibody specificities and function, and differences in immune cells that drive pathogenesis [12, 13]. Considering this, studies have been done to treat K/BxN mice endogenously because the immune cells involved in driving the inflammatory response in K/BxN mice more tightly mimic those experienced in the human disease [110].

Recombinant Fcs Successfully Ameliorate RA in Mouse Models

Many researchers have investigated recombinant Fcs as a replacement for IVIg. First, monomeric Fc derived from the plasma of donors successfully treated autoimmune diseases such as ITP and Kawasaki disease [134, 135]. However, there were issues that arose from the small molecular weight of these plasma-derived Fcs, such as rapid clearance from the body. Additionally, these potential replacements did not resolve the issues associated with obtaining human plasma. Researchers focused on the future of immunoglobulin-based therapies [136, 137],

and this has led to the recent development of recombinant Fcs [26, 27, 138, 139]. This alternative would preclude the use of human plasma.

Many strategies have been used to multimerize the Fc of IgG in order to produce polyvalent Fc molecules [26, 27, 138, 139]. First, stradomers were made through the fusion of the human IgG2 hinge region to human IgG1 Fc or to mouse IgG2a Fc [140]. This led to the production of stradomerized Fcs that bound FcγRs with high avidity [141]. These stradomers demonstrated therapeutic efficacy in the CIA model and in animal models of ITP, inflammatory neuropathy, and myasthenia gravis [141–143]. Importantly, efficacy was achieved at approximately 20–40-fold lower doses than IVIg.

A trivalent multimer was also made and compared to multimers with increasing and decreasing valences [144]. The trimer, M230, was composed of 3 IgG1 Fcs that were stabilized by “knobs-into-holes” technology developed by Ridgway et al. [145] in 1996. This study showed that any molecule with a valency greater than 3 bound FcγRs with high avidity and initiated activating signals, whereas any molecule with a valency of 3 or less bound FcγRs with high avidity without triggering activating signals [144]. Results from both the CIA and the CAIA models showed that M230 was more efficacious than IVIg at 10-fold lower doses [144]. In the ITP mouse model, it was most effective as it raised platelet counts at 100-fold lower doses than IVIg [144]. Additionally, M230 ameliorated epidermolysis bullosa acquisita at doses as low as 25 mg/kg [144]. This study addressed the importance of monitoring adverse events after multimeric Fc administration. Like immune complexes, Fc multimers can crosslink FcγRs and lead to subsequent activation. The consequences of this would be a severe inflammatory response, which would ultimately be counterintuitive to the objective of treatment with the Fc.

A hexameric-Fc molecule called HexaGard was developed using the technology developed by Smith et al. [146] who showed that the addition of the C-terminal 18 aa tailpiece of IgM lead to hexamerization of IgG. This technology was applied to hexamerize human IgG1 Fcs to form HexaGard [147]. HexaGard showed high avidity for FcγRs and for cluster of differentiation 209 and showed efficacy in a mouse model of ITP. However, future studies on HexaGard were discontinued because it appeared to have unwanted side effects, such as the activation of complement through its enhanced avidity to C1q, which lead to C5b-9 deposition [147].

In addition to HexaGard, multiple hexameric-Fc fusion proteins were engineered to be tested in multiple in

vitro and in vivo systems to determine the consequences of multivalent FcγR engagement [148]. One hexameric-Fc was composed of an IgG4 and IgG1 entitled γ4eng-hexameric-Fc. The second hexameric-Fc was a mutant version of this protein containing L234F/P331S mutations to reduce platelet and complement activation, entitled γ1eng-hexameric-Fc. Finally, the third hexameric-Fc was another mutant which had F234L/F296Y mutations introduced to increase its ability to block FcγR-dependent phagocytosis, entitled γ4eng-F234L F296Y hexameric-Fc [149].

The major finding of this study was that the hexameric-Fcs bound avidly to FcγRs, and this interaction caused the rapid internalization of these hexameric-Fcs and the subsequent degradation of activating but not inhibitory FcγRs. This effect was prolonged and occurred in both in vitro and in vivo systems. This effect disrupted FcγR function and lead to the potent inhibition of phagocytosis. The hexameric-Fcs were further tested in a mouse model of ITP and were found to be effective at protecting platelets from phagocytic degradation for a prolonged period. This study highlighted the therapeutic utility for hexameric-Fcs in diseases involving FcγR-mediated phagocytosis, such as ITP. However, this study also highlighted the potential challenges for the clinical development of hexameric-Fc-based therapeutics because of their rapid clearance in in vivo systems.

More recently, much attention has been given to a recombinant human IgG1 Fc multimer, GL-2045 [138–143]. GL-2045 is composed of the human IgG1 Fc with its C-terminus fused to the human IgG2 hinge region to facilitate the formation of stradamers [150, 151]. When this molecule was first designed, it was shown to be a potent inhibitor of complement activation [151]. However, a more recent article published by the same group focused on its potential as a replacement for IVIg for the treatment of IgG-mediated autoimmune diseases because of its ability to efficiently mimic the anti-inflammatory and immunomodulatory properties of Ig Fc aggregates [111].

The article [111] showed that GL-2045 was more efficient at blocking the binding of immune complexes from patients with RA to FcγRs, and it was more efficient at inhibiting phagocytosis in comparison to IVIg. Additionally, GL-2045 efficiently ameliorated ITP in a mouse model, and both prevented and suppressed arthritis in the CIA model. GL-2045 also reduced the systemic levels of potent inflammatory cytokines such as IL-6. As of 2018, GL-2045 has been in phase 1 clinical trials for the treatment of unspecified autoimmune diseases.

Two hexameric recombinant Fc molecules entitled Fc-μTP and Fc-μTP-L309C were designed as potential replacements for IVIg [152]. Fc-μTP was generated by fusing the 18 aa residues of the human IgM tailpiece to the C-terminus of a wild-type human IgG1 Fc, Fc-μTP, or a variant with a point mutation changing the leucine residue at position 309 to a cysteine residue, Fc-μTP-L309C. The same technology that was used to create HexaGard was also used to create Fc-μTP and Fc-μTP-L309C [148, 149].

This resulted in the formation of 2 recombinant Fc hexamers, with the point mutations in Fc-μTP-L309C, providing a more stable structure than Fc-μTP, because of the formation of disulfide bonds between the Fc molecules [152]. Fc-μTP-L309C bound FcγRs with high avidity and inhibited FcγR-mediated effector functions such as ADCC, phagocytosis, and respiratory burst [152]. In addition, Fc-μTP-L309C prevented full activation of the classical complement pathway by blocking C2 cleavage, which avoided the generation of inflammatory downstream products such as the potent anaphylatoxin C5a or the membrane attack complex. Fc-μTP-L309C suppressed CIA, CAIA, K/BxN serum transfer, and K/BxN endogenous arthritis in mice when given therapeutically at approximately a 10-fold lower dose than IVIg [78, 110]. This was associated with reduced inflammatory cytokine production and complement activation [152].

The potential advantages of replacing IVIg with recombinant Fcs are evident. First, they allow for the reproducible manufacturing of high amounts of recombinant product with consistent quality using processes that are independent of plasma supply. A reduction in the need for plasma would provide massive benefits for healthcare systems because IVIg is expensive to produce and shortages are common. Importantly, the potential for higher efficacy may allow for significantly lower doses for patients such that they could be administered subcutaneously from the comfort of one's home. This could drastically increase the quality of life for patients who receive regular IVIg infusions.

Conclusion

In addition to RA, there are many other autoantibody-mediated autoimmune diseases and their prevalence is increasing worldwide [153–155]. Unfortunately, systemic immunosuppression with steroids is a reality for most patients and they suffer from severe adverse effects, con-

comitant morbidities, and an increased mortality [156]. Thus, there is a need for the development of novel treatments for patients suffering from autoantibody-mediated autoimmune diseases that do not impose serious negative effects.

Activating FcγRs initiate and maintain inflammatory responses induced by immune complexes. This has been illustrated in mouse models of RA, such as CIA [157]. Additionally, transgenic mice expressing human FcγRIIA are highly susceptible to tissue damage by immune complexes, which further implicates FcγRs as central mediators of inflammation in human disease [158]. In these animal models, immune complex binding to FcγRs activates inflammatory cells, resulting in pro-inflammatory mediator release and tissue damage [158].

Several studies have reported the importance of activating FcγRs in patients with RA. Blocking FcγRIIA engagement with immune complexes from the sera or synovial fluid of RA patients with monoclonal antibodies prevented the release of pro-inflammatory mediators. Thus, FcγRs are potential targets for the treatment of autoimmune diseases involving pathogenic autoantibodies, and recombinant Fcs is a therapeutic strategy that should be considered.

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Author Contributions

B.J.B.L. is a graduate student who wrote the manuscript and performed specific experiments cited in the manuscript; D.R.B. is the principal investigator who co-wrote and edited the manuscript. The authors satisfy the ICMJE criteria for authorship and have read and approved the manuscript.

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