Multimerized IgG1 Fc molecule as an anti-inflammatory agent

Emmanuel Stephen-Victor, Jagadeesh Bayry

AUTOIMMUNITY

Multimerized IgG1 Fc molecule as an anti-inflammatory agent

Emmanuel Stephen-Victor and Jagadeesh Bayry

The success of intravenous immunoglobulin immunotherapy in autoimmune diseases has inspired the development of novel Fc-based therapeutics, in particular, multimerized polyvalent Fc molecules. A new study reports that a recombinant IgG1 Fc hexamer alleviates acute and chronic autoimmune diseases in mice.

The generation of autoantibody-containing immune complexes is a hallmark of several autoimmune and inflammatory diseases. If not cleared efficiently from the body, these immune complexes could recruit and activate immune cells by engaging with diverse Fcγ receptors (FcγRs). FcγR-mediated signalling results in the activation of innate immune cells, platelets and endothelial cells, leading to effects that include the release of cytokines, induction of phagocytosis, degranulation and complement activation, all of which contribute to inflammation and tissue damage. Blocking effector functions mediated by FcγRs is therefore a promising approach in treating antibody-mediated autoimmune diseases. Ongoing research suggests that the Fc domain of IgG can recapitulate the therapeutic effects of intravenous immunoglobulin (IVIG) and that multimerized Fc in particular can potently inhibit FcγR-mediated signalling and complement activation[1].

High-dose (1–2 g/kg) IVIG, a pooled normal IgG preparation, remains a lifesaving therapeutic option for numerous autoimmune and inflammatory diseases[2,3]. The efficacy of this immunotherapy in diseases with various immunopathogenic mechanisms suggests that the therapeutic benefits of IVIG are mediated through diverse mechanisms, a notion confirmed by experimental and clinical reports[2,3]. The effects of IVIG can depend on the $F(ab)_2$ portion or the Fc fragment of the IgG molecule, or might require the entire molecule. One of the earliest mechanisms of IVIG to be described was the blockade of FcγRs, which was attributed solely to the Fc fragment of IgG. Hence, the use of the Fc fragment has been proposed as an alternative to IVIG therapy in immune complex-driven diseases. In fact, the Fc fragment was efficacious in treating children with acute immune thrombocytopenic purpura (ITP) in a clinical trial[4]. Despite these promising results, however, the expansion of FcγR-targeted therapeutics for the autoimmune and inflammatory diseases has been slow, mainly owing to incomplete knowledge of the functioning and diversity of Fc receptors, the molecular pathogenesis of these pathologies and protein engineering being in its infancy.

In the past decade, the development of Fc-based therapeutics, in particular, multimerized polyvalent forms, has gained momentum. The observation that human IgG2 in the serum can form hinge region-dependent dimers led to the design of fully recombinant IgG2a Fc multimers called stradomers; these stradomers demonstrated therapeutic efficacy in several models of autoimmune diseases, including collagen-induced arthritis (CIA), ITP and myasthenia gravis[5,6]. Multivalent Fc structures (containing two or three Fc domains) that exhibit avid FcγR binding without activating FcγR signalling have also been engineered; a trivalent Fc structure, Fc3Y, protected against animal models of autoimmune diseases in which immune complexes or FcγRs are implicated[7]. Additionally, biosynthesized hexameric IgG1 Fc molecules containing cysteine mutation at position 309 were shown to bind with high avidity to low-affinity inhibitory FcγRs, CD209 antigen (also known as DC-SIGN) and complement C1q, and induce complement activation[8], although these molecules were not tested for the therapy of autoimmune diseases and complement activation under physiological conditions. Together, these reports suggested that FcγR-mediated activation signals in autoimmune diseases could be blocked by using multimers of Fc domains.

Building on the aforementioned work, Spirig et al.[1] now report that a recombinant IgG1 Fc hexamer, termed Fc-μTP-L309C, potently inhibits FcγR-mediated activation signals and alleviates acute and chronic autoimmune diseases in mice. The recombinant hexamer was developed by fusing the μ-tailpiece from human IgM with the carboxy terminus of human IgG1. Furthermore, a point mutation at position 309 from leucine to cysteine was introduced that imparted stability to the hexamer by forming additional covalent bonds. Fc-μTP-L309C, but not the Fc monomer, avidly bound to low-affinity FcγRs such as CD16a, CD32a and CD32b/c as well as to the high-affinity receptor CD64 (REF.[1]). In

addition, Fc-μTP-L309C had greater avidity than IVIG for neonatal FcR (FcRn), a receptor implicated in prolonging the half-life of antibodies (Fig. 1). Functional assessments demonstrated that the hexamer inhibited antibody-dependent cell-mediated cytotoxicity and phagocytosis — crucial effector responses triggered by immune complexes upon binding to FcγRs. Of note, these effects of Fc-μTP-L309Cwere not associated with undesirable activation of immune cells.

Having established that Fc-μTP-L309C could inhibit FcγR-mediated functions in vitro, Spirig et al.[1] next evaluated the efficacy of the hexamer in vivo in an antibody-mediated model of acute arthritis. Treatment with a single dose of Fc-μTP-L309C (200 mg/kg; a dose 10-fold lower than that of IVIG) markedly decreased the formation of complement proteins C3 and C5a in the knee joints and also ameliorated bone degradation, restricted inflammatory cell infiltration and inhibited an array of pro-inflammatory cytokines and chemokines. The therapeutic effect of Fc-μTP-L309C was rapid (as early as 24 h after administration) and was sustained for up to 14 days. By contrast, IVIG had minimal effects even at day 8 and a clear therapeutic efficacy was observed only by day 14, reflecting the delayed kinetics of the response to IVIG in comparison with Fc-μTP-L309C. In a model of ITP, a single dose (200 mg/kg) of Fc-μTP-L309C sharply increased the platelet counts to levels comparable to those achieved by treatment with IVIG[1]. Similar protective effects of Fc-μTP-L309C in a rat model of neuromyelitis optica have also been reported by another group[9]. The rapid therapeutic effects of Fc-μTP-L309C in acute models of autoimmune diseases is not surprising as hexamers of IgG1 bind to FcγRs with higher avidity than the monomeric IgG in IVIG. Because of this binding, Fc-μTP-L309C inhibits pathogenic antibody-mediated inflammatory processes.

In a model of chronic arthritis, although Fc-μTP-L309C improved the clinical signs of CIA its effect waned over time and additional doses were required; by contrast, such additional therapy was not required for IVIG-treated mice[1]. Two main factors could explain these observations. First, the half-life of Fc-μTP-L309C is 3.1 hours in mice (for comparison, IgG molecules have a half-life of nearly 7 days in mice and 21 days in humans) so although an initial infusion of Fc-μTP-L309C would block immune complex-mediated activation of immune cells, sustained production of pathogenic antibodies and concomitant clearance of infused Fc-μTP-L309C would lead to flares of disease. Second, unlike Fc-μTP-L309C, the mechanism of action of IVIG is not dependent solely on FcγR blockade and inhibition of complement activation; instead, IVIG targets multiple components of the immune system, suppresses inflammatory cells and reciprocally enhances anti-inflammatory processes, including expansion of regulatory T cells[3,10].

Altogether, the results reported by Spirig et al.[1] could reinforce and propel the development of Fc-based therapeutics for autoimmune and inflammatory diseases. Although the Fc hexamers showed therapeutic effects in acute models of autoimmune diseases, the short half-life of this multimer and its limited mechanism of action (compared with IVIG) are major drawbacks. Also, whether Fc-based therapeutics would be beneficial in autoimmune diseases in which T cells are the major mediators of pathogenic responses remains to be determined. As with any recombinant protein, the immunogenicity of the hexamer following repeated therapy, and the role of glycans in its anti-inflammatory actions and binding properties, also need to be addressed. Alternatively, high-avidity binding properties of Fc hexamers and their inhibitory effects on classical and lectin pathways of complement activation could be exploited in the form of combination therapy with IVIG. Whereas Fc hexamers exert rapid suppressive effects on inflammation, the multi-pronged action of IVIG ensures durable therapeutic effects.

*Emmanuel Stephen-Victor[1] and Jagadeesh Bayry[2]**
[1]Boston Children's Hospital, Boston, MA, USA.
[2]Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Paris, France.
**e-mail: jagadeesh.bayry@crc.jussieu.fr*

1. Spirig, R. et al. rIgG1 Fc hexamer inhibits antibody-mediated autoimmune disease via effects on complement and FcγRs. *J. Immunol.* **200**, 2542–2553 (2018).
2. Gelfand, E. W. Intravenous immune globulin in autoimmune and inflammatory diseases. *N. Engl. J. Med.* **367**, 2015–2025 (2012).
3. Galeotti, C., Kaveri, S. V. & Bayry, J. IVIG-mediated effector functions in autoimmune and inflammatory diseases. *Int. Immunol.* **29**, 491–498 (2017).
4. Debre, M. et al. Infusion of Fcγ fragments for treatment of children with acute immune thrombocytopenic purpura. *Lancet* **342**, 945–949 (1993).
5. Jain, A. et al. Fully recombinant IgG2a Fc multimers (stradomers) effectively treat collagen-induced arthritis and prevent idiopathic thrombocytopenic purpura in mice. *Arthritis Res. Ther.* **14**, R192 (2012).
6. Thiruppathi, M., Sheng, J. R., Li, L., Prabhakar, B. S. & Meriggioli, M. N. Recombinant IgG2a Fc (M045) multimers effectively suppress experimental autoimmune myasthenia gravis. *J. Autoimmun.* **52**, 64–73 (2014).
7. Ortiz, D. F. et al. Elucidating the interplay between IgG-Fc valency and FcγR activation for the design of immune complex inhibitors. *Sci. Transl. Med.* **8**, 365ra158 (2016).
8. Czajkowsky, D. M. et al. Developing the IVIG biomimetic, hexa-Fc, for drug and vaccine applications. *Sci. Rep.* **5**, 9526 (2015).
9. Tradtrantip, L., Felix, C. M., Spirig, R., Morelli, A. B. & Verkman, A. S. Recombinant IgG1 Fc hexamers block cytotoxicity and pathological changes in experimental in vitro and rat models of neuromyelitisoptica. *Neuropharmacology* **133**, 345–353 (2018).
10. Maddur, M. S., Kaveri, S. V. & Bayry, J. Circulating normal IgG as stimulator of regulatory T cells: lessons from intravenous immunoglobulin. *Trends Immunol.* **38**, 789–792 (2017).

Acknowledgements
The authors' work is supported by the Institut National de le Santé et de la Recherche Médicale, Sorbonne Université and Université Paris Descartes.

Competing interests
J.B. has in the past received research support and has collaborated with CSL Behring, Bern for the exploration of the mechanisms of action of intravenous immunoglobulin and pooled normal IgA. E.S.-V. declares no competing interests.

Fig. 1 | **Anti-inflammatory mechanisms of a recombinant IgG1 Fc hexamer.** The recombinant IgG1 Fc hexamer Fc-μTP-L309C could inhibit immune complex (IC)-mediated inflammatory processes by blocking Fcγ receptors (Fcγ Rs), thus suppressing FcγR-mediated activation signals and consequently processes such as phagocytosis, respiratory burst and antibody-dependent cell-mediated cytotoxicity (ADCC). Fc-μTP-L309C might also enhance catabolism of autoantibodies by blocking neonatal FcR (FcRn) and inhibit the classical and lectin complement pathways by blocking the cleavage of C2 and by depletion of C4, respectively.