Mini Review

Intravenous immunoglobulin suppresses B cell activities via antigen recognition region

Jun Tanaka

Central Research Laboratory, Japan Blood Products Organization, Kobe, Japan

Autoimmune diseases are characterized by the production of autoantibodies specific for self-antigens by activated B cells. However, the precise mechanism for the pathogenesis of autoimmunity remains unknown. Intravenous immunoglobulin (IVIg), which is manufactured from the pooled plasma of more than tens of thousands of healthy volunteers, is used successfully for the treatment of inflammatory and autoimmune disease, especially in case of resistance to conventional therapy such as steroid pulse therapy. Although the precise mechanisms by which IVIg acts in the treatment of autoimmune disease remain unclear, several groups have proposed various mechanisms that could play a role in modulating undesirable autoimmune responses. The IgG molecules in IVIg have various immunosuppressive effects, which are attributed to the Fc portion or antigen recognition region. Herein, I focus on the immunosuppressive effects of IVIg on activated B cells, mediated by the antigen recognition region.

Rec.11/27/2013, Acc.1/30/2014, pp134-139

Correspondence should be addressed to:
Jun Tanaka, 8F kobe KIMEC Center Building 1-5-2, Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan. Phone: +81-78-599-5095, Fax: +81-78-303-2561, E-mail: tanaka-jun@jbpo.or.jp

Key words intravenous immunoglobulin, autoimmune disease, F(ab')2 fragment, immune suppression, B cell

Introduction

The vertebrate immune system is maintained in a state of equilibrium by highly evolved, complex, and structured mechanisms. These mechanisms, if they are not tightly regulated, can cause immune-mediated disorders such as autoimmune diseases. Recently, the number of patients suffering from autoimmune diseases has increased. Autoimmune diseases are characterized by the production of pathogenic autoantibodies by activated B cells in response to a variety of self-antigens and/or organs. Therefore, autoantibody-producing activated B cells are the primary participants in the onset of pathology. B1 cells, which differ functionally from conventional B2 cells, spontaneously secrete natural antibodies, including autoantibodies, which account for most of the resting IgM levels and a large portion of the resting IgA levels found in normal serum[1-4]. Following Toll-like receptor 9 (TLR9) stimulation *via* a synthetic ligand, unmethylated CpG oligodeoxynucleotides (CpG), facilitate the expansion of B1 cell populations and the augmentation of autoantibody and interleukin (IL)-10 production[5, 6]. Although B1 cell population expansion has been reported in various mouse models of autoimmune

disease, such as the NZB/W F1 lupus model[7, 8], human B1 cell populations have only started to be defined recently[9], and the mechanism by which B1 cells control the development of autoimmune diseases in human is controversial. Although the primary producer of pathogenic autoantibodies has been debated, it is generally accepted that the suppression of autoantibody-producing cells, *i.e.*, activated B cells, is very important for autoimmune disease therapy.

Currently, intravenous immunoglobulin (IVIg) is being used increasingly for the treatment of a large number of autoimmune diseases. To replace deficiencies of immunoglobulins, IVIg was first used in 1952 for the prophylaxis of infections[10]. The use of IVIg for the treatment of autoimmune diseases was first described in 1981[11]. Pooled plasma from more than tens of thousands of healthy volunteers is used to manufacture IVIg, which contains a variety of specific antibodies to bacterial cells[12-14] or toxins[15-17]. The proposed underlying mechanisms of IVIg action in autoimmune diseases involve the modulation of Fc receptors, interference with the cytokine network and complements, provision of anti-idiotypic antibodies, suppression of lymphocyte effector function[18, 19], and the initial recognition of sialylated IgG by a carbohydrate receptor on sensor macrophages, which leads to the indirect up-regulation of an inhibitory IgG Fc receptor, FcγRIIB, on effector macrophages[20]. Furthermore, De Groot et al. identified and characterized several regulatory T cell epitopes, called Tregitope, that were discovered in the heavy and light chains of IgG[21]. The presentation of Tregitope on the surface of antigen presenting cells induces the activity of regulatory T cells. These studies represent the enhancement of research within the last decade regarding the role of IVIg for autoimmune disease treatment.

In this mini review, I will present the results of several studies, including our own research, on the immunosuppressive effects of IVIg on activated B cells *via* an antigen recognition region.

F(ab')$_2$-mediated downregulation of IL-10 production

The roles of IL-10 in immune responses are considered to be multiple, IL-10 playing an inhibitory role in inflammation in some cases, while it can become harmful in inflammation in others[22, 23]. Specifically, IL-10 produced by B cells can serve as a growth factor for B cells themselves and induce their proliferation and differentiation. IL-10 also plays a critical role in some autoimmune diseases, such as systemic lupus erythematosus (SLE)[24] and Kawasaki disease[25].

Kessel et al. recently demonstrated that IL-10 and IL-6 production were reduced by IVIg treatment in cultured CpG-stimulated human B cells that were isolated from healthy volunteers as well as from SLE patients[26]. Our current study revealed that IL-10 production was significantly reduced in IVIg-treated CpG-activated mouse B1 cells *in vitro*. Moreover, we confirmed that the F(ab')$_2$ fragment of IgG is the effective portion of IVIg responsible for the suppression of CpG-induced mouse B1 cell activation[27]. This suppressive effect was almost diminished when the F(ab')$_2$ fragment was further split into monovalent Fab fragments, suggesting that the bivalent nature of the F(ab')$_2$ fragment is somehow important in suppression. Therefore, the suppressive effects of IVIg *via* an antigen recognition region seem to require the not only just binding the receptor but also the bivalent nature of IgG molecules.

Internalization into cytoplasm of IVIg

Several groups have demonstrated that IVIg exhibits idiotype-binding activity and an autoreactive nature toward various autologous molecules in humans, such as Fas, MHC class I and II, Fc receptors, CD5 and CD40[28, 29], presumably because IVIg is derived from several tens of thousands of healthy volunteers and largely comprises polyclonal or poly-reactive natural antibodies. Proulx et al. determined that IVIg preferentially binds to human B cells, rather than T cells, on the B cell antigen receptor (BCR) in addition to various other molecules on the human B cell surface *via* its anti-idiotypic activity[30]. Furthermore, we confirmed that IVIg binds to mouse B1 cells by using the anti-tetanus monoclonal human IgG as the negative control for IVIg, and that the binding capacity is dependent on IVIg's polyclonal F(ab')$_2$[27].

Moreover, several reports have indicated that the IVIg can be internalized to the cytoplasm of B cells *via* an as yet unknown mechanism[30, 31]. Our current study also demonstrated that a portion of the internalized IVIg or F(ab)$_2$ fragments co-localized with early endosome associated protein-1 (EEA-1), an early endosomal marker, suggesting that a substantial portion of the IVIg or F(ab')$_2$ fragments were incorporated into the endosomal fraction[27]. However, whether the internalization is necessary or dispensable for the inhibitory effect of IVIg is unknown, and will be one of the next subjects to be clarified.

Partial suppression of signaling pathway in IVIg-mediated suppression

Recently, our group revealed the signaling pathways that are suppressed by IVIg in activated human monocytic cell line (THP-1)[32] and primary mouse dendritic cells[33]. Although Séïté et al. indicated that IVIg suppresses the phosphorylation of extracellular-signal regulated kinase (ERK) in BCR-activated human B cells[34], little is known regarding the suppressive effects of IVIg on signaling pathways in activated B cells. Our present study elucidated the role of IVIg in TLR9-initiated signaling. Upon binding to CpG, TLR9 evokes several signaling pathways that culminate in the activation of four key molecules that are translocated into the nucleus and activate transcription of proinflammatory cytokines: NF-κB, ERK, c-Jun NH2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK). We confirmed that up-regulated levels of phospho-TAK1, phospho-p65, and phospho-ERK were vulnerable to IVIg, while IRAK-1 degradation and phospho-p38 MAPK levels did not change[27]. Therefore, these results indicated that IVIg partly suppressed pathways downstream of TLR9-initiated signaling (*i.e.*, the ERK and TAK1-NFκB pathways but not the IRAK1-p38 MAPK pathway). There are few reports that address the IVIg-mediated suppression of activation signals in immune cells. Therefore, further studies are required to identify the key target molecule of IVIg.

A possible role of SHP-1 in IVIg-mediated suppression

Our current study demonstrated that the stimulation of IVIg-bound B1 cells with CpG induced the aggregation of the bound IgG molecules into a few discrete patches. This aggregation reaction is similar to that when some cognate antigens cap the BCR. Moreover, the aggregated IgG molecules co-localized well with SHP-1 but not with SHIP[27]. Therefore, an immunoreceptor tyrosine-based inhibitory motif (ITIM)-harboring B cell inhibitory receptor that recruit SHP-1 but not SHIP may provide the molecular link between the binding of IgG to surface of B1 cells and TLR9 inhibition, in which IVIg targets an ITIM-harboring membrane-bound inhibitory receptor. B cells are known to express a series of SHP-1-recruiting inhibitory receptors on their surface including CD22 [or sialoglycoprotein-binding lectin (Siglec)-2][35], CD72[36, 37], Siglec-G[38], and PirB[39]. On the other hand, a major SHIP-recruiting receptor is FcγRIIB[40]. Kubo et al. noted that PirB, an ITIM-harboring membrane-bound inhibitory receptor that recruits SHP-1, suppresses TLR9 signaling in CpG-stimulated mouse B1 cells[6]. Séïté et al. showed that CD22 plays an important role as a target receptor of IVIg in the suppression of human B cells[34, 41]. However, by using CpG-activated B1 cells from gene-targeted mice deficient in CD22, PirB or FcγRIIB, our present study demonstrated that the suppressive effect of IVIg was not abolished even in the absence of each inhibitory receptor[27]. These data suggest that the IVIg-facilitated suppression of CpG-induced IL-10 production in mouse B1 cells is not, at least, solely mediated through PirB, CD22 or FcγRIIB.

Recently, the concept of inhibitory immunoreceptor tyrosine-based activation motif (ITAMi) has emerged as a new means to negatively regulate the immune response[42]. Although Aloulou et al. showed that IVIg controls inflammatory response by ITAMi signaling though FcγRIII in human FcγRIII transfected mouse monocyte/macrophages[43], no reports have yet established that the ITAMi signal is induced by IVIg *via* an antigen recognition region. Currently, the target receptor(s) and molecules that are related to the IVIg suppressive effects *via* an antigen recognition region essentially remain unknown.

Conclusion

IVIg has been used in the treatment of autoimmune diseases for the past 30 years. Although the mechanisms have not yet been elucidated fully, numerous mechanisms have been proposed to explain the immunosuppressive effects of IVIg. In this mini review, I have focused on the suppressive effect of IVIg on activated B cells *via* an antigen recognition region including the following four important features: (1) the suppression of IL-10 production, (2) the internalization into the cytoplasm, (3) the partial suppression of TLR9-mediated signaling pathways, and (4) the recruitment of SHP-1 (Fig.1). In this mini review, I have not addressed all of the effects of IVIg; moreover, there may be unknown effects or new properties of IVIg not considered here. However, several groups, including us, are currently working to provide evidence of immunosuppressive effects of IVIg in the near future. The enhanced knowledge of the underlying mechanisms of IVIg may be informative for not only the mechanism of action of IVIg, but also the mechanisms involved in the pathogenesis of autoimmune diseases.

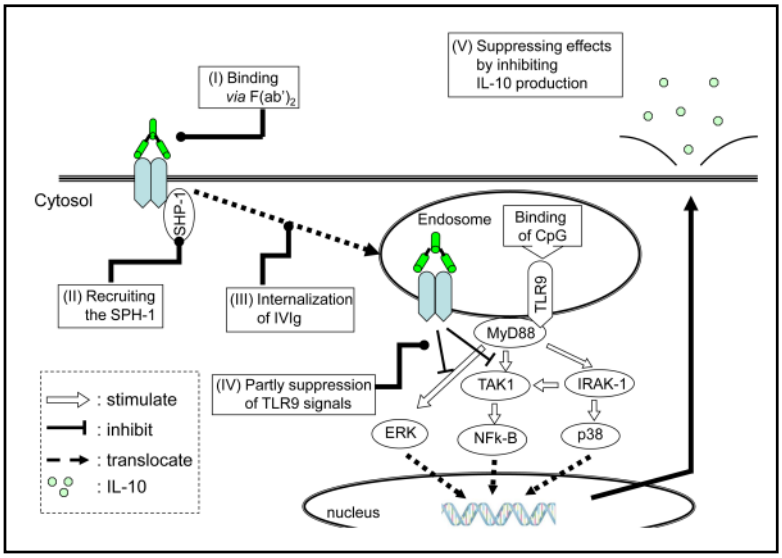

Fig.1 Immunosuppressive effects of IVIg on activated B cells effected by the antigen recognition region

IVIg suppresses B cell activation by (I) binding the receptor via F(ab')2, (II) recruiting SHP-1, (III) internalizing to the endosome, (IV) partially suppressing the TLR9 signaling pathways, and (V) inhibiting IL-10 production.

Acknowledgement

The author would like to express the deepest appreciation to Professor Toshiyuki Takai (Institute of Development, Aging and cancer, Tohoku University, Japan) for his critical reviewing of this manuscript.

Conflict of Interests

Funding: No funding sources.

Competing interests: None declared.

The author is employed by Japan Blood Products Organization.

References

1) Holodick NE, Repetny K, Zhong X, Rothstein TL: Adult BM generates CD5+ B1 cells containing abundant N-region additions. Eur J Immunol. 2009; 39: 2383-2394.
2) Sidman CL, Shultz LD, Hardy RR, Hayakawa K, Herzenberg LA: Production of immunoglobulin isotypes by Ly-1+ B cells in viable motheaten and normal mice. Science. 1986; 232: 1423-1425.
3) Forster I, Rajewsky K: Expansion and functional activity of Ly-1+ B cells upon transfer of peritoneal cells into allotype-congenic, newborn mice. Eur J Immunol. 1987; 17: 521-528.
4) Ishida H, Hastings R, Kearney J, Howard M: Continuous anti-interleukin 10 antibody administration depletes mice of Ly-1 B cells but not conventional B cells. J Exp Med. 1992; 175: 1213-1220.
5) Murakami M, Yoshioka H, Shirai T, Tsubata T, Honjo T: Prevention of autoimmune symptoms in autoimmune-prone mice by elimination of B-1 cells. Int Immunol. 1995; 7: 877-882.
6) Kubo T, Uchida Y, Watanabe Y, Abe M, Nakamura A, Ono M, Akira S, Takai T: Augmented TLR9-induced Btk activation in PIR-B-deficient B-1 cells provokes excessive autoantibody production and autoimmunity. J Exp Med. 2009; 206: 1971-1982.
7) Hayakawa K, Hardy RR, Parks DR, Herzenberg LA: The "Ly-1 B" cell subpopulation in normal immunodefective, and autoimmune mice. J Exp Med. 1983; 157: 202-218.
8) Morshed SR, Mannoor K, Halder RC, Kawamura H, Bannai M, Sekikawa H, Watanabe H, Abo T: Tissue-specific expansion of NKT and CD5+B cells at the onset of autoimmune disease in (NZBxNZW)F1 mice. Eur J Immunol. 2002; 32: 2551-2561.
9) Griffin DO, Rothstein TL: Human b1 cell frequency: isolation and analysis of human b1 cells. Front Immunol. 2012; 3: 122.
10) Bruton OC: Agammaglobulinemia. Pediatrics. 1952; 9: 722-728.
11) Imbach P, Barandun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schoni M, Vest M, Wagner HP: High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. Lancet. 1981; 1: 1228-1231.
12) Mikolajczyk MG, Concepcion NF, Wang T, Frazier D, Golding B, Frasch CE, Scott DE: Characterization of antibodies to capsular polysaccharide antigens of Haemophilus influenzae type b and Streptococcus pneumoniae in human immune globulin intravenous

preparations. Clin Diagn Lab Immunol. 2004; 11: 1158-1164.
13) Pollack M: Antibody activity against Pseudomonas aeruginosa in immune globulins prepared for intravenous use in humans. J Infect Dis. 1983; 147: 1090-1098.
14) Tanaka J, Nakae T, Onoe T, Horiuchi Y, Miyamoto H, Adan-Kubo J, Adachi H, Ono Y: Complement-mediated bacteriolysis after binding of specific antibodies to drug-resistant Pseudomonas aeruginosa: morphological changes observed by using a field emission scanning electron microscope. J Infect Chemother. 2010; 16: 383-387.
15) Nakae T, Hirayama F, Hashimoto M: [Neutralizing activity of human immunoglobulin preparation against toxic shock syndrome toxin-1]. Kansenshogaku Zasshi. 2002; 76: 195-202.
16) Norrby-Teglund A, Basma H, Andersson J, McGeer A, Low DE, Kotb M: Varying titers of neutralizing antibodies to streptococcal superantigens in different preparations of normal polyspecific immunoglobulin G: implications for therapeutic efficacy. Clin Infect Dis. 1998; 26: 631-638.
17) Yanagisawa C, Hanaki H, Natae T, Sunakawa K: Neutralization of staphylococcal exotoxins in vitro by human-origin intravenous immunoglobulin. J Infect Chemother. 2007; 13: 368-372.
18) Ballow M: The IgG molecule as a biological immune response modifier: mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory disorders. J Allergy Clin Immunol. 2011; 127: 315-323; quiz 324-325.
19) Ibanez C, Montoro-Ronsano JB: Intravenous immunoglobulin preparations and autoimmune disorders: mechanisms of action. Curr Pharm Biotechnol. 2003; 4: 239-247.
20) Anthony RM, Wermeling F, Ravetch JV: Novel roles for the IgG Fc glycan. Ann N Y Acad Sci. 2012; 1253: 170-180.
21) De Groot AS, Moise L, McMurry JA, Wambre E, Van Overtvelt L, Moingeon P, Scott DW, Martin W: Activation of natural regulatory T cells by IgG Fc-derived peptide "Tregitopes". Blood. 2008; 112: 3303-3311.
22) Saraiva M, O'Garra A: The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010; 10: 170-181.
23) O'Garra A, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C: Strategies for use of IL-10 or its antagonists in human disease. Immunol Rev. 2008; 223: 114-131.
24) Llorente L, Zou W, Levy Y, Richaud-Patin Y, Wijdenes J, Alcocer-Varela J, Morel-Fourrier B, Brouet JC, Alarcon-Segovia D, Galanaud P, Emilie D: Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. J Exp Med. 1995; 181: 839-844.
25) Noh GW, Lee WG, Lee W, Lee K: Effects of intravenous immunoglobulin on plasma interleukin-10 levels in Kawasaki disease. Immunol Lett. 1998; 62: 19-24.
26) Kessel A, Peri R, Haj T, Snir A, Slobodin G, Sabo E, Rosner I, Shoenfeld Y, Toubi E: IVIg attenuates TLR-9 activation in B cells from SLE patients. J Clin Immunol. 2011; 31: 30-38.
27) Tanaka J: Intravenous immunoglobulin suppresses IL-10 production by activated B cells in vitro. Open J Immunol. 2012; 2: 149-160.
28) Lemieux R, Bazin R, Neron S: Therapeutic intravenous immunoglobulins. Mol Immunol. 2005; 42: 839-848.
29) Leucht S, Uttenreuther-Fischer MM, Gaedicke G, Fischer P: The B cell superantigen-like interaction of intravenous immunoglobin (IVIG) with Fab fragments of V(H) 3-23 and 3-30/3-30.5 germline gene origin cloned from a patient with Kawasaki disease is enhanced after IVIG therapy. Clin Immunol. 2001; 99: 18-29.
30) Proulx DP, Aubin E, Lemieux R, Bazin R: Spontaneous internalization of IVIg in activated B cells. Immunol Lett. 2009; 124: 18-26.
31) Paquin Proulx D, Aubin E, Lemieux R, Bazin R: Inhibition of B cell-mediated antigen presentation by intravenous immunoglobulins (IVIg). Clin Immunol. 2010; 135: 422-429.
32) Murakami K, Suzuki C, Kobayashi F, Nakano A, Fujii A, Sakai K, Imada T: Intravenous immunoglobulin preparation attenuates LPS-induced production of pro-inflammatory cytokines in human monocytic cells by modulating TLR4-mediated signaling pathways. Naunyn Schmiedebergs Arch Pharmacol. 2012; 385: 891-898.
33) Fujii A, Kase Y, Suzuki C, Kamizono A, Imada T: An fc gamma receptor-mediated upregulation of the production of interleukin 10 by intravenous immunoglobulin

in bone-marrow-derived mouse dendritic cells stimulated with lipopolysaccharide in vitro. J Signal Transduct. 2013; 2013: 239320.
34) Seite JF, Cornec D, Renaudineau Y, Youinou P, Mageed RA, Hillion S: IVIg modulates BCR signaling through CD22 and promotes apoptosis in mature human B lymphocytes. Blood. 2010; 116: 1698-1704.
35) Nitschke L, Carsetti R, Ocker B, Kohler G, Lamers MC: CD22 is a negative regulator of B-cell receptor signalling. Curr Biol. 1997; 7: 133-143.
36) Parnes JR, Pan C: CD72, a negative regulator of B-cell responsiveness. Immunol Rev. 2000; 176: 75-85.
37) Li DH, Winslow MM, Cao TM, Chen AH, Davis CR, Mellins ED, Utz PJ, Crabtree GR, Parnes JR: Modulation of peripheral B cell tolerance by CD72 in a murine model. Arthritis Rheum. 2008; 58: 3192-3204.
38) Hoffmann A, Kerr S, Jellusova J, Zhang J, Weisel F, Wellmann U, Winkler TH, Kneitz B, Crocker PR, Nitschke L: Siglec-G is a B1 cell-inhibitory receptor that controls expansion and calcium signaling of the B1 cell population. Nat Immunol. 2007; 8: 695-704.
39) Ujike A, Takeda K, Nakamura A, Ebihara S, Akiyama K, Takai T: Impaired dendritic cell maturation and increased T(H)2 responses in PIR-B(-/-) mice. Nat Immunol. 2002; 3: 542-548.
40) Ono M, Bolland S, Tempst P, Ravetch JV: Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. Nature. 1996; 383: 263-266.
41) Seite JF, Guerrier T, Cornec D, Jamin C, Youinou P, Hillion S: TLR9 responses of B cells are repressed by intravenous immunoglobulin through the recruitment of phosphatase. J Autoimmun. 2011; 37: 190-197.
42) Pasquier B, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffie C, Henin D, Benhamou M, Pretolani M, Blank U, Monteiro RC: Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. Immunity. 2005; 22: 31-42.
43) Aloulou M, Ben Mkaddem S, Biarnes-Pelicot M, Boussetta T, Souchet H, Rossato E, Benhamou M, Crestani B, Zhu Z, Blank U, Launay P, Monteiro RC: IgG1 and IVIg induce inhibitory ITAM signaling through FcgammaRIII controlling inflammatory responses. Blood. 2012; 119: 3084-3096.