Mini Review

Intravenous immunoglobulin suppresses B cell activities via antigen recognition region

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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 (IVlg), 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 IVlg 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 IVlg have various immunosuppressive effects, which are attributed to the Fc portion or antigen recognition region. Herein, I focus on the immunosuppressive effects of IVlg on activated B cells, mediated by the antigen recognition region.

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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. 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. Although B1 cell population expansion has been reported in various mouse models of autoimmune disease.
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 (IVlg) is being used increasingly for the treatment of a large number of autoimmune diseases. To replace deficiencies of immunoglobulins, IVlg was first used in 1952 for the prophylaxis of infections \(^10\). The use of IVlg 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 IVlg, which contains a variety of specific antibodies to bacterial cells \(^12,^14\) or toxins \(^15-17\). The proposed underlying mechanisms of IVlg 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 IVlg 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 IVlg 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 IVlg 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 IVlg-treated CpG-activated mouse B1 cells in vitro. Moreover, we confirmed that the F(ab')\(_2\) fragment of IgG is the effective portion of IVlg 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 IVlg 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 IVlg**

Several groups have demonstrated that IVlg 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 IVlg is derived from several tens of thousands of healthy volunteers and largely comprises polyclonal or poly-reactive natural antibodies. Proulx et al. determined that IVlg 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 IVlg binds to mouse B1 cells by using the anti-tetanus monoclonal human IgG as the negative control for IVlg, and that the binding capacity is dependent on IVlg’s polyclonal F(ab')\(_2\) \(^27\).

Moreover, several reports have indicated that the IVlg 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 IVlg 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 IVlg 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 IVlg is unknown, and will be one of the next subjects to be clarified.
Mini Review  
Immunomodulation by Intravenous Immunoglobulin 
Inflammation and Regeneration  Vol.34  No.3    May  2014  

136

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) and primary mouse dendritic cells. Although Séité et al. indicated that IVIg suppresses the phosphorylation of extracellular-signal regulated kinase (ERK) in BCR-activated human B cells, 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. 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. 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], CD72, Siglec-G, and PirB. On the other hand, a major SHIP-recruiting receptor is FcγRIIB. 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. Séité et al. showed that CD22 plays an important role as a target receptor of IVIg in the suppression of human B cells. 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. 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 (ITAM) has emerged as a new means to negatively regulate the immune response. Although Aloulou et al. showed that IVIg controls inflammatory response by ITAM signaling through FcγRII in human FcγRII transfected mouse monocyte/macrophages, no reports have yet established that the ITAM 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. 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 not only for the mechanism of action of IVIg, but also for the mechanisms involved in the pathogenesis of autoimmune diseases.
Mini Review Immunomodulation by Intravenous Immunoglobulin

Inflammation and Regeneration Vol.34 No.3 May 2014

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.

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Conflict of Interests
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