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## **Intravenous Immunoglobulin: Mechanism of Action in Autoimmune and Inflammatory Conditions**

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26 **Abbreviations**

27 IVIG Intravenous Immunoglobulin

28 KD Kawasaki Disease

29 ITP Immune thrombocytopenic purpura

30 GBS Guillain–Barré syndrome GBS,

31 CIDP Chronic inflammatory demyelinating polyneuropathy

32 SLE Systemic lupus erythematosus

33 CIA Collagen Induced Arthritis

34 IL Interleukin

35 PBMC Peripheral blood mononuclear cells

36 DC Dendritic cells

37 pDC Plasmacytoid dendritic cells

38 EAE Experimental Autoimmune Encephalitis

39 MISC Multisystemic Inflammatory Syndrome in Children

40 NET Neutrophil extracellular traps

41

42 Key words: Intravenous immunoglobulin, inflammation, autoimmunity, innate immunity, adaptive

43 immunity, Regulatory T cells, IVIG

44 Clinical Commentary: *JACI in practice*

45

46 **Abstract:** IVIG is the mainstay of therapy for humoral immune deficiencies and numerous inflammatory  
47 disorders. Although the use of IVIG may be supplanted by several targeted therapies to cytokines, the  
48 ability of polyclonal IgG to not only act as an effector molecule but as a regulatory molecule is a clear  
49 example of the polyfunctionality of IVIG. This article will address the mechanism of action of IVIG in  
50 a number of important conditions that are otherwise resistant to treatment. In this commentary we will  
51 highlight mechanistic studies that shed light on the action of IVIG. This will be approached by identifying  
52 effects that are both common and disease specific, targeting actions that have been demonstrated on cells  
53 and processes that represent both innate and adaptive immune responses.

54

55

## 56 **Introduction**

57 IgG plays multiple roles in the immune system. Best known as an effector molecule in host defense,  
58 infusions of polyclonal IgG have been employed as the mainstay of treatment for patients with  
59 immunodeficiency diseases affecting the humoral immune system. Preparations of human IgG are  
60 available for intravenous (IVIG) or subcutaneous (SCIG) administration, which has allowed individuals  
61 with both primary and secondary immune defects to achieve much improved outcomes.<sup>1</sup> In addition,  
62 IVIG has been employed as a regulator of a large number of autoimmune and inflammatory conditions  
63 since the 1980's<sup>2</sup>. IVIG contains a broad spectrum of antibodies, as it is fractionated from plasma pools  
64 that include several thousand donors or more<sup>3</sup>. IVIG has been consistently and successfully used for  
65 numerous conditions, including Immune thrombocytopenic purpura (ITP), Kawasaki Disease (KD),  
66 Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy (CIDP), systemic lupus  
67 erythematosus, dermatomyositis, and other autoimmune and neurologic disorders<sup>4</sup>. Indeed, the number  
68 of conditions for which IVIG is used “off label” outnumbers those that have regulatory approval<sup>5,6</sup>.  
69 However, pressures on the plasma fractionation system leading to shortages of raw materials for IVIG,  
70 particularly during the recent pandemic period, demand that practitioners carefully scrutinize their use  
71 and employ caution both in prescribing, and in over-rationing this essential therapy, to the detriment of  
72 patients with primary antibody immune deficiency<sup>7</sup>. More thorough mechanistic understanding of the  
73 role of IVIG as an immune regulator can provide better rationale and determine the optimal use for this  
74 increasingly scarce resource.

75

76 IVIG has been used in two distinct dose regimes: low-dose (400-800 mg/kg) replacement therapy in  
77 primary immunodeficient patients and high-dose (1-2 g/kg) in autoimmune and inflammatory diseases.<sup>1</sup>  
78 As IVIG contains antibodies to diverse pathogens, the main goal of low-dose replacement therapy is to  
79 prevent recurrent infections in primary immunodeficient patients or in patients with recurrent infections  
80 with secondary immunoglobulin deficiencies. Several lines of evidence also suggest that low-dose IVIG

81 therapy can exert positive effects on the cellular immune compartment, depending on underlying  
82 immunodeficiency<sup>8-12</sup>. In contrast, most autoimmune conditions require high dose therapy. As will be  
83 discussed below, this is likely due to the need for specialized antibody contents that represent a small  
84 percentage of pooled IVIG, such as anti-idiotypic antibodies, fractions that have specific glycosylation,  
85 and other components<sup>2</sup>.

86

87 Autoimmune and inflammatory diseases are characterized by perturbed immune tolerance and aberrant  
88 activation of immune and nonimmune cells, inflammation, and tissue damage. Despite the significant  
89 number of novel, biological therapies that target cytokines and small-molecule inhibitors aimed at  
90 signaling pathways, IVIG continues to have an important therapeutic niche in these diseases. The  
91 rationale behind the extensive use of IVIG is due to a combination of relatively low therapeutic  
92 toxicity<sup>13,14</sup> with a very broad spectrum of immunoregulatory actions.

93

94 IgG molecules are complex glycoproteins, structured to both interact with target antigens via their  
95 variable regions, and with cells that express Fc receptors via their constant regions (Figure 1). These are  
96 complemented by multiple glycosylation sites which increase the mobility of the molecule and mediate  
97 interaction between IgG and lectin receptors on cells in the immune system. As demonstrated in Figure  
98 1, IVIG has been implicated in multiple critical immune processes that can mitigate inflammatory  
99 responses in autoimmune diseases. These actions encompass both the innate and adaptive immune  
100 systems. In this commentary we will address several of the key mechanisms of action which can provide  
101 direction for the continued use of IVIG and assist in potentially developing therapeutic substitutes for  
102 this critical therapy.

103

104 **IVIG modulates structural cells**

105 Structural cells like epithelial cells, fibroblasts and endothelial cells express a wide range of immune  
106 genes and respond to the inflammatory stimuli. Stevens-Johnson syndrome (SJS), toxic epidermal  
107 necrolysis (TEN), and SJS/TEN overlap syndrome are rare severe skin reactions, in most cases triggered  
108 by medications, with high morbidity and mortality of up to 40% for TEN. IVIG is one of several  
109 therapies, utilized after corticosteroids, which have been shown to improve outcomes, reduce hospital  
110 stays and decrease time for the skin to heal.<sup>15,16</sup> The therapeutic benefits of IVIG in TEN is suggested to  
111 be due to inhibition of Fas-mediated keratinocyte death<sup>17,18</sup>. A different mechanism is seen in  
112 experimental models of bullous pemphigoid, an autoimmune blistering disease, for which IVIG  
113 suppressed inflammatory cytokines like IL-6 from keratinocytes<sup>19</sup>. In pathologies associated with fibrosis  
114 such as systemic lupus erythematosus and Sjögren's syndrome, IVIG therapy may reverse fibroblast  
115 proliferation<sup>20</sup>, and also inhibited early fibrogenic changes in experimental models of Systemic  
116 Sclerosis<sup>21</sup>.

117

118 Endothelial cells function as a barrier between the bloodstream and tissue. They actively contribute to  
119 inflammatory processes by secretion of cytokines and chemokines, and by regulating the adhesion and  
120 mobility of various immune cells. By activating mitochondrial apoptotic signalling pathways, IVIG  
121 induced apoptosis of TNF- $\alpha$ -stimulated umbilical vein endothelial cells<sup>22</sup>. IVIG inhibited TNF- $\alpha$ -  
122 induced activation of NF- $\kappa$ B<sup>23</sup> and as a consequence inhibited inflammatory cytokine-mediated  
123 proliferation of endothelial cells, and expression of adhesion molecules, inflammatory cytokines and  
124 chemokines<sup>24-26</sup>. Similarly, in a murine model of stroke, IVIG suppressed ischemia-induced enhancement  
125 of markers of endothelial cell adhesion and lymphocyte infiltration<sup>27</sup>.

126

127 IVIG can inhibit inflammatory processes of endothelial cells via specific antibodies in its repertoire that  
128 interact with target molecules. Specifically, anti-IL-1 $\alpha$  IgG antibodies in IVIG have been shown to inhibit  
129 IL-1 $\alpha$ -mediated activation of endothelium and consequently, reduce neutrophil adhesion<sup>28</sup>. In a murine

130 model of antiphospholipid antibody syndrome, IVIG inhibited antiphospholipid antibodies-induced  
131 endothelial cell activation and thrombosis *in vivo*<sup>29</sup>. IVIG also increased HLA-DR expression in  
132 endothelial cells, decreased IL-6 and promoted endothelial cell amplification of Treg cells, all of which  
133 may assist in maintenance of allograft tolerance<sup>30</sup>. Thus, by targeting endothelial cells, IVIG not only  
134 reduces endothelial cell function but also mitigates the influx of immune cells to sites of inflammation.

135

### 136 **Innate immunity and IVIG**

137 The innate immune compartment, including soluble factors such as complement molecules and innate  
138 immune cells, plays a key role in the initiation and propagation of pathogenic immune responses through  
139 the secretion of inflammatory mediators like cytokines and chemokines, recruiting effector cells,  
140 mediating T cell differentiation and programming, and by causing tissue damage. Innate immune cells  
141 include antigen presenting cells such as dendritic cells (DC), monocyte/macrophages; NK cells, and  
142 granulocytes like neutrophils, eosinophils, and basophils. IVIG actively regulates several key  
143 components of the innate immune system.

144

### 145 ***IVIG and complement pathways***

146 The complement pathway is composed of a complex network of proteins that interact with each other in  
147 a sequential manner to produce a variety of biological responses. Well known for its crucial role in host  
148 defense against infections, the complement pathway also contributes to a range of diseases. IVIG  
149 contains antibodies that exert complement scavenging effects<sup>27,31-33</sup>. By interacting with C3b  
150 complement components and preventing the binding of activated C3 to C5 convertase, IVIG inhibited  
151 the deposition of C5b-C9 membrane attack complexes on endomysial capillaries, restoring the capillary  
152 network and reducing microvasculopathy, a characteristic feature of dermatomyositis<sup>31</sup>. Another report  
153 showed that IVIG diminished complement amplification in dermatomyositis patients by reducing the



154 concentration of C3 convertase precursors in blood<sup>32</sup>. In both dermatomyositis and KD patients, IVIG  
155 therapy suppressed expression of multiple genes for complement products and their receptors<sup>34,35</sup>.

156

157 In a murine model of stroke, IVIG protected against experimental stroke by scavenging C3b and  
158 preventing complement-mediated neuronal cell death<sup>27</sup>. IVIG also neutralized anaphylatoxins C3a and  
159 C5a, and suppressed their effector functions both in vitro and in vivo animal models<sup>33</sup>. Thus, IVIG exerts  
160 diverse actions on the complement system to attenuate inflammation.

161

### 162 *Monocytes/Macrophages and Dendritic cells:*

163 IVIG inhibited activation of monocytes and macrophages both in mice and humans, and induced anti-  
164 inflammatory cytokines like IL-1 receptor antagonist (IL-1RA), TGF- $\beta$  and IL-10<sup>36-41</sup>. IVIG induced  
165 Fas-mediated apoptosis of innate cells and neutralized various innate inflammatory cytokines by virtue  
166 of high-affinity anti-cytokine IgG antibodies<sup>42</sup>. IVIG also promoted an expansion of monocytic myeloid-  
167 derived suppressor cells<sup>43</sup>. Interestingly, induction of IL-10 by IVIG in TLR-4 activated monocytes is  
168 dependent on Fc $\gamma$ RI (CD64) and Fc $\gamma$ RIIb (CD32B), and is impaired in high affinity genetic FCGR1IA  
169 risk variants (H131R polymorphism, rs1801274)<sup>38</sup>.

170

171 The effect of IVIG therapy on monocytes may be a biomarker in KD. Single cell RNA sequencing-based  
172 profiling of PBMCs from acute KD patients revealed that monocytes are the major source of  
173 inflammatory mediators in these patients<sup>35</sup>. IVIG therapy reduced CD14<sup>+</sup> monocytes/macrophages and  
174 CD16<sup>+</sup> positive inflammatory monocytes in circulation<sup>35,44-46</sup>, as well as expression of calgranulin genes  
175<sup>35,47</sup> and high affinity Fc $\gamma$ RI receptors<sup>45</sup>. Microarray data confirmed that IVIG therapy downregulated  
176 *MAPK14*, *TLR5* and *MYD88*, the signaling and adapter proteins involved in TLR and IL-1 receptor  
177 signaling<sup>48</sup> which affects multiple signal transduction pathways<sup>38,49,50</sup>. In line with these observations,  
178 analyses of M1(inflammatory macrophages which cause tissue damage) and M2 (regulatory

179 macrophages which induce tissue repair) macrophages in KD patients revealed that during acute phases  
180 of the disease, transcripts of both M1 and M2 markers were increased, then declined following IVIG  
181 therapy<sup>51</sup>. IVIG mediated epigenetic regulation of target genes in macrophages via hypermethylation of  
182 CpG sites at its promoter region<sup>51</sup>.

183

184 DC are the major professional antigen presenting cells which direct both immune tolerance and primary  
185 and memory T-cell responses. IVIG suppressed expression of DC co-stimulatory molecules CD40, CD80  
186 and CD86, and HLA-DR in vitro <sup>52</sup>, leading to a tolerogenic DC phenotype. Adoptive transfer of IVIG-  
187 treated CD11c<sup>+</sup> DC led to amelioration of ITP in mouse<sup>53</sup>. IVIG therapy in CIDP patients reduced levels  
188 of inflammatory CD16<sup>+</sup> myeloid DC<sup>54</sup>, and reduced inflammatory cytokines like IL-12 and TNF<sup>52,55</sup>,  
189 while enhancing IL-10<sup>52</sup>. IL-10 was also induced by IVIG in two myeloid DC subsets in KD patients in  
190 the subacute phase of recovery<sup>56</sup>. IVIG suppressed IFN $\alpha$  production in pDC via two mechanisms: in SLE  
191 patients, IVIG inhibited Fc $\gamma$ RIIa and IFN $\alpha$  production induced by SLE immune complexes; additionally  
192 IVIG contained F(ab')<sub>2</sub> residues which induced PGE<sub>2</sub> in monocytes, leading to suppression of TLR-7 or  
193 TLR-9 agonist-induced IFN $\alpha$  production<sup>57</sup>.

194

195 Initial reports on successful clinical use of Fc fragments of IVIG for the treatment of ITP suggested that  
196 IVIG blocked Fc $\gamma$  receptors and hence prevented immune complex-mediated activation of innate  
197 immune cells<sup>58</sup>. Subsequent studies, particularly in experimental animal models, reported that terminal  
198  $\alpha$ 2,6-sialic acid-linked residues on the Fc portion of IgG may mediate some of these immunoregulatory  
199 functions of IVIG (Figure 1), suggesting possible enrichment of IgG preparations for sialic acid  
200 containing fractions, and thus more targeted usage. However, the importance of the  $\alpha$ 2,6-sialic acid  
201 linked residues appears to be disease and possibly model specific. Murine studies suggest that the  $\alpha$ 2,6-  
202 sialic acid portion of IVIG enhances the inhibitory Fc $\gamma$ RIIb in effector splenic macrophages <sup>59-62</sup>.  $\alpha$ 2,6-  
203 sialic acid linkages may induce IL-33 in marginal-zone macrophages via SIGN-R1 signaling (or in

204 humans, DC-SIGN) or CD23<sup>59,63,64</sup>. IL-33 activates basophils via the ST2 receptor to induce IL-4<sup>63,64</sup>  
205 which in turn enhances FcγRIIb expression on effector splenic macrophages. Several animal models such  
206 as K/BxN-induced arthritis, experimental autoimmune encephalomyelitis (EAE), ITP and experimental  
207 allergic bronchopulmonary aspergillosis (ABPA) have validated the requirement of sialylated Fc region  
208 or sialylated IgG in imparting protective effects<sup>61,63-69</sup>. In allergic airways disease, a second sialic acid  
209 receptor, DCIR, was shown to mediate the effects of sialylated IgG in abrogating airway inflammation<sup>70</sup>.  
210 In contrast, models of autoimmune diseases such as K/BxN serum transfer arthritis, collagen-induced  
211 arthritis (CIA), ITP and EAE reported that neither sialylation of Fc fragments nor FcγRIIb are mandatory  
212 for the anti-inflammatory effects of IVIG<sup>71-74</sup>

213

214 In human studies have also not been as conclusive. Flow cytometry and cellular surface plasmon  
215 resonance imaging did not find evidence to support CD23 or DC-SIGN as receptors for human IgG  
216 irrespective of glycosylation properties on F(ab')<sub>2</sub> or Fc<sup>75</sup>. Both FcγRIIb or Fc-sialylation were  
217 dispensable for IVIG to inhibit IgG-mediated phagocytosis by human macrophages<sup>76</sup>. Although IL-33  
218 was induced by IVIG in autoimmune patients, it was not produced by DC-SIGN<sup>+</sup> innate cells<sup>77</sup>. IL-33  
219 did not induce activation of human basophils nor production of IL-4<sup>78</sup>, suggesting that the action of IVIG  
220 modulating human basophils would be via different mechanisms. Sialic acid moieties on IgG were also  
221 not required for activation of the Wnt/β-catenin pathway, autophagy and immune complex-mediated  
222 induction of type I IFN by human pDC<sup>57,79,80</sup>. DC-SIGN on human monocyte-derived DC played a key  
223 role in inducing COX-2-mediated PGE<sub>2</sub> production and regulatory T cell (Treg) expansion<sup>81</sup>. But unlike  
224 mice, interaction with DC-SIGN was mediated by F(ab')<sub>2</sub> fragments rather than Fc, suggesting that either  
225 sialic acid molecules on Fab or anti-DC-SIGN IgG antibodies could mediate these effects. More work is  
226 needed to define the role of sialylated Fc fragments in mediating immunoregulatory functions of IVIG.

227

228 ***Granulocytes:***

229 *Neutrophils:* Neutrophils have a role in inflammatory diseases such as KD through recruiting other  
230 innate immune cells to the site of inflammation, secreting inflammatory mediators and causing tissue  
231 damage. IVIG therapy exerted cytotoxic effects on neutrophils in KD patients<sup>82,83</sup> possibly through anti-  
232 Fas and anti-Siglec9 IgG via caspase-dependent and caspase-independent pathways, respectively<sup>84</sup>. IVIG  
233 also reduced neutrophil nitric oxide in KD patients<sup>85</sup>. In multisystem inflammatory syndrome in children  
234 (MIS-C)<sup>86</sup>, IVIG targeted IL-1 $\beta$ <sup>+</sup> neutrophils via PI3K- and NADPH oxidase-dependent cytotoxicity, and  
235 suppressed their activation<sup>82</sup>. IVIG inhibited neutrophil extracellular trap (NET) formation in anti-  
236 neutrophil cytoplasmic antibody (ANCA)-associated vasculitis *in vivo*<sup>87</sup>. This may be due to IVIG  
237 inducing lactoferrin in neutrophils that negatively regulates NET formation<sup>87,88</sup>.

238

239 The immunoregulatory role of IVIG on neutrophils goes beyond cytotoxicity. In a mouse model of sickle  
240 cell disease, IVIG interfered with recruitment of neutrophils in inflamed venules by increasing rolling  
241 velocity of granulocytes and reducing adhesion to venules<sup>89</sup>. Using a neutrophil-mediated acute vascular  
242 injury model the effect of IVIG on neutrophil adhesion and activation was dependent on Fc $\gamma$ RIII via  
243 recruitment of SHP-1<sup>90</sup>.

244

245 *Basophils:* IVIG induces the activation marker CD69 as well as IL-4 and other cytokines in IL-3-primed  
246 human basophils via F(ab')<sub>2</sub>- and Syk-dependent mechanisms by interacting with surface-bound IgE<sup>78</sup>.  
247 Induction of CD69 was also observed in IVIG-treated myopathy patients<sup>78</sup>. IL-4 produced by basophils  
248 might dampen inflammation by enhancing Fc $\gamma$ RIIb and antagonizing Th1 and Th17.

249

250 *Eosinophils:* IVIG induces ROS-dependent cytotoxic effects on eosinophils in the presence of  
251 inflammatory cytokines both by caspase-dependent and caspase-independent pathways, via anti-Siglec-  
252 8 IgG<sup>91</sup>. IVIG therapy in Churg-Strauss syndrome patients decreased CD69<sup>+</sup> activated eosinophils<sup>92</sup>

253 suggesting functional anti-Siglec-8 IgG-mediated cytolysis. Similarly, in moderate to severe childhood  
254 atopic dermatitis patients, IVIG therapy caused a decline in peripheral blood eosinophil counts<sup>93</sup>.

255

256 Other positive effects of IVIG on eosinophils have also been observed. Eosinophil levels are frequently  
257 significantly higher in KD patients compared to control subjects<sup>94</sup>. In work by Kuo et al, IVIG therapy  
258 induced IL-5 and elevated eosinophil counts, which were positively correlated with successful IVIG  
259 therapy<sup>95</sup>. Mechanistically, increased IL-5 (or other eosinophil chemotactic factors) without increased  
260 eosinophil activation factors was correlated with post-IVIG therapy eosinophilia<sup>96</sup> and mitigated Th1  
261 inflammation. Th2 cytokines following IVIG therapy were proposed to also help decrease coronary  
262 artery lesions.

263

#### 264 ***Natural Killer cells:***

265 Classically known for their ability to kill malignant and virus-infected cells by cytotoxic effects, Natural  
266 Killer (NK) cell activation also leads to secretion of pro-inflammatory cytokines. IVIG inhibits direct  
267 cytotoxicity and ADCC function of human NK cells in vitro<sup>97</sup> associated with apoptotic cell death in  
268 CD56<sup>dim</sup> NK cells<sup>98</sup>. Reduced NK cell function following IVIG therapy was reported in ITP<sup>99</sup>,  
269 CIDP<sup>100,101</sup>, and KD, all associated with reduced cytotoxic CD56<sup>dim</sup> NK cell subsets, while preserving or  
270 increasing regulatory CD56<sup>bright</sup> NK cells<sup>101,102</sup>.

271

272 Some women with multiple high-risk pregnancies have elevated preconception peripheral NK cells; trials  
273 of IVIG therapy significantly improved the delivery birthweight of babies born to women with high risk  
274 of low birthweight infants<sup>103</sup>. A murine model of recurrent pregnancy loss was associated with increased  
275 CD44<sup>bright</sup> NK cells; IVIG reduced spontaneous abortion rates while suppressing increases in the  
276 CD44<sup>bright</sup> NK cell subset<sup>104</sup>. Women with recurrent spontaneous abortion similarly display increased NK  
277 cells but exhibit reduced NK cell cytotoxicity; IVIG therapy significantly increased the live birth rate<sup>105</sup>-

278 <sup>108</sup>, as well as increasing expression of inhibitory receptors and decreased activating receptors of NK  
279 cells<sup>105</sup>. Further detailed investigation on the regulation of NK cells by IVIG is needed.

280

### 281 **Adaptive Immunity: Human studies**

282

283 *Treg/Th17 axis*: CD4<sup>+</sup> T cells are heterogenous and various subsets have been identified. Tregs are  
284 necessary for the control of inflammation, while, aside from controlling infection, Th1, Th2 and Th17  
285 cells can promote tissue damage, and are associated with autoimmunity<sup>109,110</sup>. Early studies indicated that  
286 IVIG therapy balances Th1 and Th2 cells<sup>111</sup>. Experimental studies have further reported that IVIG  
287 suppressed the differentiation, expansion and function of human Th17 cells in an F(ab')<sub>2</sub>-dependent  
288 manner by inhibiting STAT-3 phosphorylation<sup>112</sup>. KD has been a paradigm for understanding the role of  
289 IVIG in the Treg/Th17 axis. While Th17 cells, as well as cytokines IL-17, IL-22, and IL-23, can be  
290 elevated in acute KD, these cytokines were downregulated up to eight weeks following IVIG therapy<sup>113</sup>.  
291 Analyses of mRNA in a group of KD subjects revealed that there were no significant changes in the  
292 frequency of Th17 cells before and after IVIG therapy; however, Treg-related IL-10 and FoxP3 levels  
293 increased 3 days after IVIG, and plasma IL-17 levels significantly decreased after 3 weeks<sup>114</sup>. Single-  
294 cell RNA sequencing has also demonstrated increased *FOXP3* mRNA levels after IVIG treatment<sup>35</sup>.  
295 Franco et al.<sup>115</sup> found that two weeks after IVIG therapy, KD patients without coronary artery lesions  
296 presented an expansion of a Treg population that produced IL-10 and low amounts of IL-4 but no TGF-  
297  $\beta$ . In contrast, patients with arterial inflammation did not exhibit this profile, reinforcing the idea that  
298 Tregs are key for controlling the vascular inflammation and may be associated with KD resolution<sup>115</sup>.  
299 Additionally, two myeloid DC subsets (CD14<sup>+</sup> cDC2 and ILT-4<sup>+</sup> CD4<sup>+</sup> tmDC) from KD patients  
300 internalized IgG in vitro through Fc $\gamma$ R, secreted IL-10 and expanded Fc-specific Tregs<sup>56</sup>.

301

302 The effects of IVIG on Treg are not restricted to KD. Women with recurrent pregnancy loss (RPL), ITP  
303 patients successfully treated with IVIG, or ex vivo IVIG-treated healthy donor T cells, showed increased  
304 Tregs as well as enhanced in vitro Treg activation and increased suppressive function<sup>35,116-118</sup>. In GBS  
305 patients, IVIG reciprocally regulated Th1/Th17 and Tregs<sup>119</sup> suggesting that Treg frequency represents  
306 a potential immunological biomarker to predict clinical response to IVIG therapy<sup>120</sup>. Similarly, patients  
307 with CIDP and dermatomyositis showed increased frequency of Tregs following IVIG<sup>102</sup>. In vitro  
308 stimulation with IVIG of PBMC from GBS patients resulted in increased in vitro secretion of IL-10 and  
309 TGF- $\beta$ 1<sup>121</sup> and expansion of Tregs<sup>121</sup>. Reduced frequency of circulating Tregs in myasthenia gravis was  
310 corrected by IVIG and induced expansion of circulating CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> and CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>  
311 CTLA-4<sup>+</sup> T cells.

312

313 *B cells and humoral antibody responses:*

314

315 Potential mechanisms through which IVIG regulates the humoral immune system include the (i)  
316 neutralization of pathogenetic autoantibodies via anti-idiotypic antibodies<sup>122</sup>, (ii) acceleration of the  
317 catabolism of pathogenic autoantibodies by saturation of FcRn<sup>123</sup>, (iii) interaction with inhibitory Fc  
318 receptors, (iv) the reset of immunoglobulin repertoires<sup>124</sup>, and (v) inhibition of activation and  
319 proliferation of B-cells by recruiting phosphatases<sup>125,126</sup>.

320

321 IVIG suppressed B-cell activation and proliferation through agonistic binding to inhibitory receptors  
322 such as CD22 and Fc $\gamma$ RIIb, while antagonizing signaling through BCR or TLRs<sup>126</sup>, although this is not  
323 a consistent finding in human B-cells<sup>105</sup>. Compared to healthy controls, patients with CIDP display  
324 reduced expression of Fc $\gamma$ RIIb on the surface of naïve and memory B-cells; this can be rescued following  
325 treatment with IVIG, resulting in upregulation of Fc $\gamma$ RIIb on both B-cell subsets<sup>124</sup>. Treatment of GBS

326 with IVIG promoted rapid expansion of plasmablasts one week after onset of treatment<sup>124</sup>. In addition,  
327 IVIG may reduce B-cell survival by neutralization of BAFF, as demonstrated in CIDP patients<sup>127,128</sup>.

328

### 329 **Adaptive Immunity: Murine studies**

330 Using a collagen induced arthritis (CIA) model, it was demonstrated that IVIG affected T-cell and  
331 germinal center responses<sup>129</sup>, and that IVIG-mediated attenuation of CIA was IL-10 dependent and  
332 associated with increased frequencies of Tregs and decreased Th17 in the spleen, coupled with a decrease  
333 in splenic germinal center B- and T-follicular helper (Tfh) cells. Further, IVIG attenuates murine allergic  
334 airways disease (AAD) by inducing highly suppressive antigen specific Tregs<sup>130-132</sup>. This entails  
335 modification of DC and is driven at least in part by Fc-sialic acid residues<sup>70,130,133</sup>. IgG-derived Tregitopes  
336 (T-regulatory epitopes), which can be produced synthetically<sup>134</sup>, can reproduce the effects of IVIG in  
337 allergic airways disease<sup>135</sup>. IVIG had a positive effect on proliferation of natural Tregs<sup>136</sup> and reciprocally  
338 regulated pathogenic Th1/Th17 in experimental models of autoimmune diseases like EAE by regulating  
339 T-cell trafficking<sup>73</sup>; this effect was independent of IgG sialylation<sup>74</sup>. Other mechanisms including  
340 modulation of prostaglandin E2 have been reported by which IVIG induces and /or expands Tregs<sup>70,81,134</sup>.

341

342 Anti-idiotypic antibodies are naturally occurring antibodies against various molecules including normal  
343 cytokines, receptors and pathogenic autoantibodies; anti-idiotypic antibodies in IVIG may help in  
344 regulating inflammatory responses. From as early as 1984, with the discovery of anti-idiotypic antibodies  
345 in IVIG against idiotypes of anti-VIII autoantibodies, multiple candidate anti-idiotypic antibodies have  
346 surfaced as highly relevant molecules<sup>52,122,137-139</sup>. For example, anti-anti-citrullinated-protein antibodies  
347 fractionated from commercial IgG (ACPA-sIVIG) was as effective as high-dose IVIG at Treg induction,  
348 reduced anti-collagen and anti-ACPA antibody responses, increased anti-inflammatory cytokine (IL-10  
349 and TGF- $\beta$ ), and decreased pro-inflammatory cytokine (TNF $\alpha$  and IL1 $\beta$ ) production in the CIA model  
350 <sup>140</sup>. Similarly, another study showed that anti-anti-  $\beta_2$ GPI specific fraction of IVIG, was highly effective



351 at preventing fetal loss and repairing fecundity in mice with experimental antiphospholipid syndrome  
352 (APS)<sup>141</sup>. These studies provide insight into the need to understand potential bioactive fractions within  
353 normal human immune globulin that can mitigate disease.

354

### 355 **Conclusion**

356 There has been extensive mechanistic study in animal models of disease and observation in IVIG-treated  
357 individuals. In this clinical commentary, we addressed pertinent studies that provide clues to biomarkers  
358 that track the effects of IVIG in autoimmune and inflammatory conditions. IVIG therapy can be best  
359 utilized if there will be clearer guidance for ancillary measures of immunological effectiveness to  
360 complement clinical observations. To summarize over 30 years of use of this therapy in a brief  
361 commentary does not do justice to the extensive amount of work that has been performed. However, the  
362 take home message is that there has been significant animal and human study of IVIG mechanistic  
363 biomarkers that we can use for clinical application. For example, measuring monocyte subsets or NK  
364 cells, as has been demonstrated in KD, in arthritis models and in high-risk pregnancies, may give  
365 practitioners more information regarding the likelihood of treatment success. Moreover, the accumulated  
366 evidence on induction of Tregs by IVIG suggests that there is a role for monitoring Treg in patients for  
367 whom there are questions on the effectiveness of IVIG therapies; this could be a target for validation in  
368 larger cohorts. Considering IVIG as a scarce resource argues for development of distinct guidelines not  
369 simply for disease indications, but for baseline evaluation and follow-up of individuals who have IVIG  
370 therapy initiated for autoimmune and inflammatory diseases. This will not only provide a method of  
371 monitoring success or failure of therapy but will allow for accrual of evidence that can advance the care  
372 of those who are treated with human immunoglobulin.

373

374 Further mechanistic study will also improve the chances of understanding various fractions of IVIG that  
375 have specific bioactivity. The study of sialic acid linkages may address a need for a fraction of IgG that

376 can target specific conditions, but it also has increased the sophistication of preparation of other antibody  
377 therapies, which require proper glycosylation to have maximum effect. Other modalities such as  
378 Tregitopes or anti-idiotypic antibodies such as targeted anti-endothelial antibodies, as examples, can  
379 reduce reliance on the plasma supply. Until such time as a true substitute is found through clinical trials,  
380 IVIG will continue to be a mainstay of therapy for multiple autoimmune conditions.  
381

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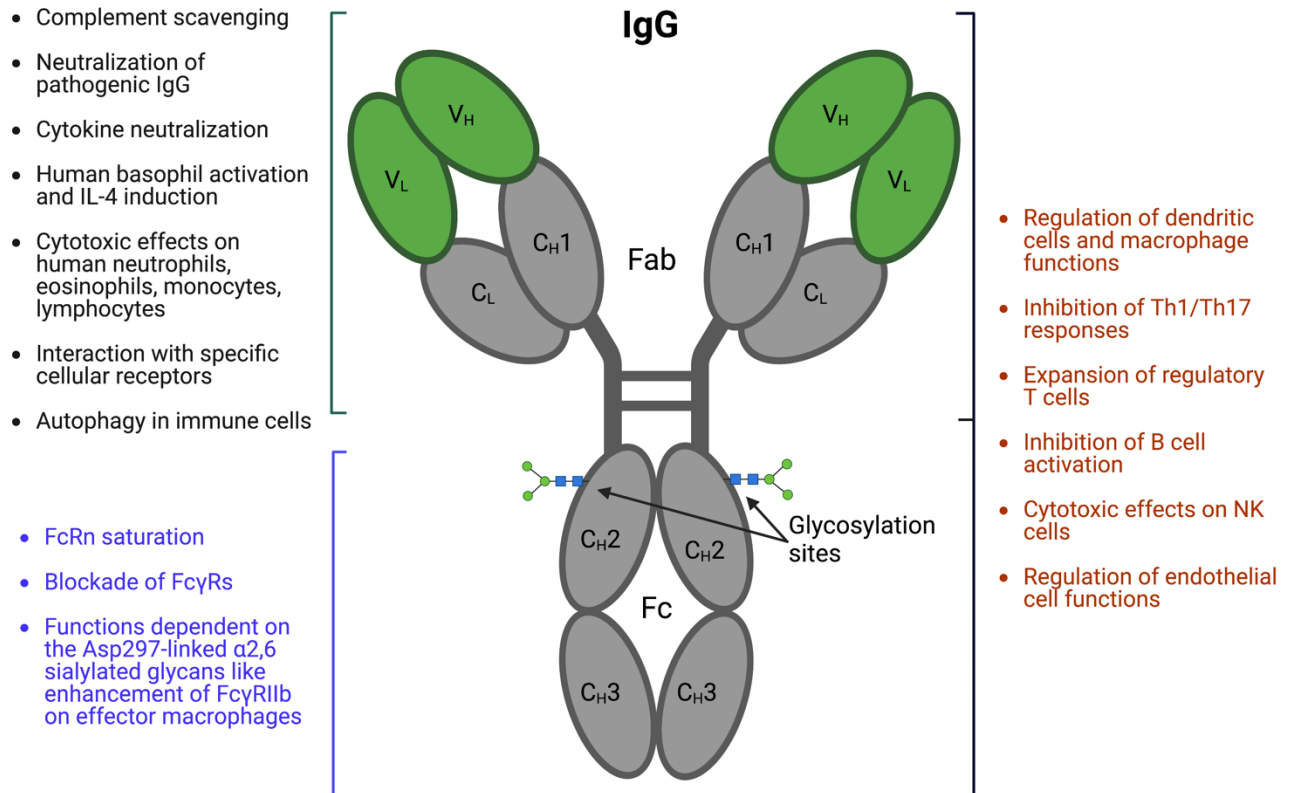
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801 **Figure Legend:**

802 **Figure 1: The current knowledge on the implication of either F(ab')<sub>2</sub>, Fc or both in the mechanisms**  
803 **of action of IVIG.** IgG contain Fab and Fc regions. Several mechanisms of IVIG are mediated by F(ab')<sub>2</sub>  
804 fragments. Some of the Fc-mediated functions also implicit the involvement of α<sub>2</sub>,6-sialic acid linkages  
805 at Asn297. However, mechanisms of IVIG for dendritic cells, various T cell subsets and B lymphocytes  
806 are dependent on both F(ab')<sub>2</sub> and Fc fragments. V<sub>H</sub>, heavy chain variable domain; V<sub>L</sub>, light chain  
807 variable domain; C<sub>H</sub>, heavy chain constant domain; C<sub>L</sub>, light chain constant domain. Figure created in  
808 BioRender.com.

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832 **Table 1: Landmark studies on the mechanisms of action of IVIG**  
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Innate Immune Compartment	References
Blockade of Fc $\gamma$ receptors	Debré et al. 1993 <sup>58</sup>
Induction of apoptosis of immune cells by Fas apoptosis pathway	Prasad et al. 1998 <sup>142</sup>
Induction of anti-inflammatory IL-1 receptor antagonist (IL-1RA) in monocytes	Ruiz de Souza et al. 1995 <sup>36</sup>
Suppression of an array of immune activation genes in monocytes of Kawasaki disease	Abe et al. 2005 <sup>47</sup>
Regulation of dendritic cell functions	Bayry et al. 2003 <sup>52,55</sup> Siragam et al. 2006 <sup>53</sup> Wiedeman et al. 2013 <sup>57</sup>
Inhibition of NK cytotoxicity	Ruiz et al. 1996 <sup>108</sup>
Cytotoxic effects on neutrophils by anti-Siglec-9 autoantibodies	von Gunten et al. 2006 <sup>84</sup>
Inhibition of neutrophil extracellular trap (NET)	Uozumi et al. 2020 <sup>87</sup>
Cytotoxic effects on eosinophils by anti-Siglec-8 autoantibodies	von Gunten et al. 2007 <sup>91</sup>
IL-3-dependent induction of human basophil activation and IL-4 secretion via anti-IgE IgG	Galeotti et al. 2019 <sup>78</sup>
Fc-Sialylation-dependent anti-inflammatory mechanisms in Mice	Kaneko et al. 2006 <sup>61</sup> Anthony et al. 2011 <sup>63</sup> Fiebiger et al. 2015 <sup>64</sup>
Identification of receptors for sialylated Fc fragments of IgG	Anthony et al. 2008 <sup>143</sup> Séité et al. 2010 <sup>126</sup> Massoud et al 2014 <sup>70</sup> Fiebiger et al. 2015 <sup>64</sup>
Induction of inhibitory ITAM signaling through Fc $\gamma$ RIII	Aloulou et al. 2012 <sup>144</sup>
Induction of autophagy in innate immune cells	Das et al. 2020 <sup>87</sup>
Epigenetic regulation of macrophages	Guo et al. 2020 <sup>51</sup>

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Innate Immune Compartment	References
Regulation of Th1/Th2 balance	Graphou et al 2003 <sup>111</sup>
Inhibition of Th17 differentiation, expansion and function	Maddur et al. 2011 <sup>112</sup>
Enhancement of regulatory T cells	Kessel et al. 2007 <sup>118</sup> Ephrem et al. 2008 <sup>136</sup>
Reciprocal regulation of Th17/Treg cells	Othy et al. 2013 <sup>73</sup> Lee et al 2014 <sup>129</sup> Guo et al. 2015 <sup>114</sup>

Identification of mechanisms of Treg expansion in human and mouse	De Groot et al. 2008 <sup>134</sup> Trinath et al. 2013 <sup>81</sup> Massoud et al 2014 <sup>70</sup> Fiebiger et al. 2015 <sup>64</sup>
Suppression of IL-4- and CD40-induced B-lymphocyte activation	Zhuang et al. 2002 <sup>125</sup>
Inhibition of TLR9 signaling by recruiting phosphatases	Séité et al. 2011 <sup>126</sup>

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<b>Soluble/Humoral Factors</b>	<b>References</b>
Neutralization of pathogenic autoantibodies by anti-idiotypic antibodies	Sultan et al. 1984 <sup>122</sup>
Neutralization of various cytokines by virtue of high-affinity anti-cytokine IgG antibodies	Svenson et al. 1993 <sup>42</sup>
Complement scavenging effects	Basta and Dalakas. 1994 <sup>31</sup> Basta et al. 2003 <sup>33</sup>

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<b>Structural Cells</b>	
Modulation of endothelial functions	Xu et al. 1998 <sup>25</sup>
Inhibition of toxic epidermal necrolysis by blockade of Fas-mediated keratinocyte death	Viard et al. 1998 <sup>18</sup>
Saturation of FcRn	Akilesh et al. 2004 <sup>123</sup>
Modulation of immunoregulatory or structural muscle genes in the patients with inflammatory myopathies	Raju and Dalakas 2005 <sup>34</sup>

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