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Intravenous Immunoglobulin: Mechanism of Action in Autoimmune and Inflammatory Conditions

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

4
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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.¹ In addition,
62 IVIG has been employed as a regulator of a large number of autoimmune and inflammatory conditions
63 since the 1980's². IVIG contains a broad spectrum of antibodies, as it is fractionated from plasma pools
64 that include several thousand donors or more³. 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 disorders⁴. Indeed, the number
68 of conditions for which IVIG is used “off label” outnumbers those that have regulatory approval^{5,6}.
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 deficiency⁷. 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.¹
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 immunodeficiency⁸⁻¹². 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 components².

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^{13,14} 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.^{15,16} The therapeutic benefits of IVIG in TEN is suggested to
111 be due to inhibition of Fas-mediated keratinocyte death^{17,18}. 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 keratinocytes¹⁹. In pathologies associated with fibrosis
114 such as systemic lupus erythematosus and Sjögren's syndrome, IVIG therapy may reverse fibroblast
115 proliferation²⁰, and also inhibited early fibrogenic changes in experimental models of Systemic
116 Sclerosis²¹.

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- α -stimulated umbilical vein endothelial cells²². IVIG inhibited TNF- α -
122 induced activation of NF- κ B²³ and as a consequence inhibited inflammatory cytokine-mediated
123 proliferation of endothelial cells, and expression of adhesion molecules, inflammatory cytokines and
124 chemokines²⁴⁻²⁶. Similarly, in a murine model of stroke, IVIG suppressed ischemia-induced enhancement
125 of markers of endothelial cell adhesion and lymphocyte infiltration²⁷.

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 α IgG antibodies in IVIG have been shown to inhibit
129 IL-1 α -mediated activation of endothelium and consequently, reduce neutrophil adhesion²⁸. In a murine

130 model of antiphospholipid antibody syndrome, IVIG inhibited antiphospholipid antibodies-induced
131 endothelial cell activation and thrombosis *in vivo*²⁹. 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 tolerance³⁰. 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^{27,31-33}. 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 dermatomyositis³¹. Another report
153 showed that IVIG diminished complement amplification in dermatomyositis patients by reducing the

154 concentration of C3 convertase precursors in blood³². In both dermatomyositis and KD patients, IVIG
155 therapy suppressed expression of multiple genes for complement products and their receptors^{34,35}.

156

157 In a murine model of stroke, IVIG protected against experimental stroke by scavenging C3b and
158 preventing complement-mediated neuronal cell death²⁷. IVIG also neutralized anaphylatoxins C3a and
159 C5a, and suppressed their effector functions both in vitro and in vivo animal models³³. 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- β and IL-10³⁶⁻⁴¹. IVIG induced
165 Fas-mediated apoptosis of innate cells and neutralized various innate inflammatory cytokines by virtue
166 of high-affinity anti-cytokine IgG antibodies⁴². IVIG also promoted an expansion of monocytic myeloid-
167 derived suppressor cells⁴³. Interestingly, induction of IL-10 by IVIG in TLR-4 activated monocytes is
168 dependent on Fc γ RI (CD64) and Fc γ RIIb (CD32B), and is impaired in high affinity genetic FCGR1IA
169 risk variants (H131R polymorphism, rs1801274)³⁸.

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 patients³⁵. IVIG therapy reduced CD14⁺ monocytes/macrophages and
174 CD16⁺ positive inflammatory monocytes in circulation^{35,44-46}, as well as expression of calgranulin genes
175^{35,47} and high affinity Fc γ RI receptors⁴⁵. 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 signaling⁴⁸ which affects multiple signal transduction pathways^{38,49,50}. 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 therapy⁵¹. IVIG mediated epigenetic regulation of target genes in macrophages via hypermethylation of
182 CpG sites at its promoter region⁵¹.

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 ⁵², leading to a tolerogenic DC phenotype. Adoptive transfer of IVIG-
187 treated CD11c⁺ DC led to amelioration of ITP in mouse⁵³. IVIG therapy in CIDP patients reduced levels
188 of inflammatory CD16⁺ myeloid DC⁵⁴, and reduced inflammatory cytokines like IL-12 and TNF^{52,55},
189 while enhancing IL-10⁵². IL-10 was also induced by IVIG in two myeloid DC subsets in KD patients in
190 the subacute phase of recovery⁵⁶. IVIG suppressed IFN α production in pDC via two mechanisms: in SLE
191 patients, IVIG inhibited Fc γ RIIa and IFN α production induced by SLE immune complexes; additionally
192 IVIG contained F(ab')₂ residues which induced PGE₂ in monocytes, leading to suppression of TLR-7 or
193 TLR-9 agonist-induced IFN α production⁵⁷.

194

195 Initial reports on successful clinical use of Fc fragments of IVIG for the treatment of ITP suggested that
196 IVIG blocked Fc γ receptors and hence prevented immune complex-mediated activation of innate
197 immune cells⁵⁸. Subsequent studies, particularly in experimental animal models, reported that terminal
198 α 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 α 2,6-sialic acid
201 linked residues appears to be disease and possibly model specific. Murine studies suggest that the α 2,6-
202 sialic acid portion of IVIG enhances the inhibitory Fc γ RIIb in effector splenic macrophages ⁵⁹⁻⁶². α 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^{59,63,64}. IL-33 activates basophils via the ST2 receptor to induce IL-4^{63,64}
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^{61,63-69}. In allergic airways disease, a second sialic acid
209 receptor, DCIR, was shown to mediate the effects of sialylated IgG in abrogating airway inflammation⁷⁰.
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 IVIG⁷¹⁻⁷⁴

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')₂ or Fc⁷⁵. Both FcγRIIb or Fc-sialylation were
217 dispensable for IVIG to inhibit IgG-mediated phagocytosis by human macrophages⁷⁶. Although IL-33
218 was induced by IVIG in autoimmune patients, it was not produced by DC-SIGN⁺ innate cells⁷⁷. IL-33
219 did not induce activation of human basophils nor production of IL-4⁷⁸, 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^{57,79,80}. DC-SIGN on human monocyte-derived DC played a key
223 role in inducing COX-2-mediated PGE₂ production and regulatory T cell (Treg) expansion⁸¹. But unlike
224 mice, interaction with DC-SIGN was mediated by F(ab')₂ 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^{82,83} possibly through anti-
232 Fas and anti-Siglec9 IgG via caspase-dependent and caspase-independent pathways, respectively⁸⁴. IVIG
233 also reduced neutrophil nitric oxide in KD patients⁸⁵. In multisystem inflammatory syndrome in children
234 (MIS-C)⁸⁶, IVIG targeted IL-1 β ⁺ neutrophils via PI3K- and NADPH oxidase-dependent cytotoxicity, and
235 suppressed their activation⁸². IVIG inhibited neutrophil extracellular trap (NET) formation in anti-
236 neutrophil cytoplasmic antibody (ANCA)-associated vasculitis *in vivo*⁸⁷. This may be due to IVIG
237 inducing lactoferrin in neutrophils that negatively regulates NET formation^{87,88}.

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 venules⁸⁹. Using a neutrophil-mediated acute vascular
242 injury model the effect of IVIG on neutrophil adhesion and activation was dependent on Fc γ RIII via
243 recruitment of SHP-1⁹⁰.

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')₂- and Syk-dependent mechanisms by interacting with surface-bound IgE⁷⁸.
247 Induction of CD69 was also observed in IVIG-treated myopathy patients⁷⁸. IL-4 produced by basophils
248 might dampen inflammation by enhancing Fc γ 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 IgG⁹¹. IVIG therapy in Churg-Strauss syndrome patients decreased CD69⁺ activated eosinophils⁹²

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 counts⁹³.

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 subjects⁹⁴. In work by Kuo et al, IVIG therapy
258 induced IL-5 and elevated eosinophil counts, which were positively correlated with successful IVIG
259 therapy⁹⁵. Mechanistically, increased IL-5 (or other eosinophil chemotactic factors) without increased
260 eosinophil activation factors was correlated with post-IVIG therapy eosinophilia⁹⁶ 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 vitro⁹⁷ associated with apoptotic cell death in
268 CD56^{dim} NK cells⁹⁸. Reduced NK cell function following IVIG therapy was reported in ITP⁹⁹,
269 CIDP^{100,101}, and KD, all associated with reduced cytotoxic CD56^{dim} NK cell subsets, while preserving or
270 increasing regulatory CD56^{bright} NK cells^{101,102}.

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 infants¹⁰³. A murine model of recurrent pregnancy loss was associated with increased
275 CD44^{bright} NK cells; IVIG reduced spontaneous abortion rates while suppressing increases in the
276 CD44^{bright} NK cell subset¹⁰⁴. Women with recurrent spontaneous abortion similarly display increased NK
277 cells but exhibit reduced NK cell cytotoxicity; IVIG therapy significantly increased the live birth rate¹⁰⁵-

278 ¹⁰⁸, as well as increasing expression of inhibitory receptors and decreased activating receptors of NK
279 cells¹⁰⁵. 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⁺ 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^{109,110}. Early studies indicated that
286 IVIG therapy balances Th1 and Th2 cells¹¹¹. Experimental studies have further reported that IVIG
287 suppressed the differentiation, expansion and function of human Th17 cells in an F(ab')₂-dependent
288 manner by inhibiting STAT-3 phosphorylation¹¹². 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 therapy¹¹³.
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 weeks¹¹⁴. Single-
294 cell RNA sequencing has also demonstrated increased *FOXP3* mRNA levels after IVIG treatment³⁵.
295 Franco et al.¹¹⁵ 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 β . 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 resolution¹¹⁵.
299 Additionally, two myeloid DC subsets (CD14⁺ cDC2 and ILT-4⁺ CD4⁺ tmDC) from KD patients
300 internalized IgG in vitro through Fc γ R, secreted IL-10 and expanded Fc-specific Tregs⁵⁶.

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^{35,116-118}. In GBS
305 patients, IVIG reciprocally regulated Th1/Th17 and Tregs¹¹⁹ suggesting that Treg frequency represents
306 a potential immunological biomarker to predict clinical response to IVIG therapy¹²⁰. Similarly, patients
307 with CIDP and dermatomyositis showed increased frequency of Tregs following IVIG¹⁰². In vitro
308 stimulation with IVIG of PBMC from GBS patients resulted in increased in vitro secretion of IL-10 and
309 TGF- β 1¹²¹ and expansion of Tregs¹²¹. Reduced frequency of circulating Tregs in myasthenia gravis was
310 corrected by IVIG and induced expansion of circulating CD4⁺CD25⁺FoxP3⁺ and CD4⁺CD25⁺FoxP3⁺
311 CTLA-4⁺ 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 antibodies¹²², (ii) acceleration of the
317 catabolism of pathogenic autoantibodies by saturation of FcRn¹²³, (iii) interaction with inhibitory Fc
318 receptors, (iv) the reset of immunoglobulin repertoires¹²⁴, and (v) inhibition of activation and
319 proliferation of B-cells by recruiting phosphatases^{125,126}.

320

321 IVIG suppressed B-cell activation and proliferation through agonistic binding to inhibitory receptors
322 such as CD22 and Fc γ RIIb, while antagonizing signaling through BCR or TLRs¹²⁶, although this is not
323 a consistent finding in human B-cells¹⁰⁵. Compared to healthy controls, patients with CIDP display
324 reduced expression of Fc γ RIIb on the surface of naïve and memory B-cells; this can be rescued following
325 treatment with IVIG, resulting in upregulation of Fc γ RIIb on both B-cell subsets¹²⁴. Treatment of GBS

326 with IVIG promoted rapid expansion of plasmablasts one week after onset of treatment¹²⁴. In addition,
327 IVIG may reduce B-cell survival by neutralization of BAFF, as demonstrated in CIDP patients^{127,128}.

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 responses¹²⁹, 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 Tregs¹³⁰⁻¹³². This entails
335 modification of DC and is driven at least in part by Fc-sialic acid residues^{70,130,133}. IgG-derived Tregitopes
336 (T-regulatory epitopes), which can be produced synthetically¹³⁴, can reproduce the effects of IVIG in
337 allergic airways disease¹³⁵. IVIG had a positive effect on proliferation of natural Tregs¹³⁶ and reciprocally
338 regulated pathogenic Th1/Th17 in experimental models of autoimmune diseases like EAE by regulating
339 T-cell trafficking⁷³; this effect was independent of IgG sialylation⁷⁴. Other mechanisms including
340 modulation of prostaglandin E2 have been reported by which IVIG induces and /or expands Tregs^{70,81,134}.

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^{52,122,137-139}. 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- β), and decreased pro-inflammatory cytokine (TNF α and IL1 β) production in the CIA model
350 ¹⁴⁰. Similarly, another study showed that anti-anti- β_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)¹⁴¹. 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.
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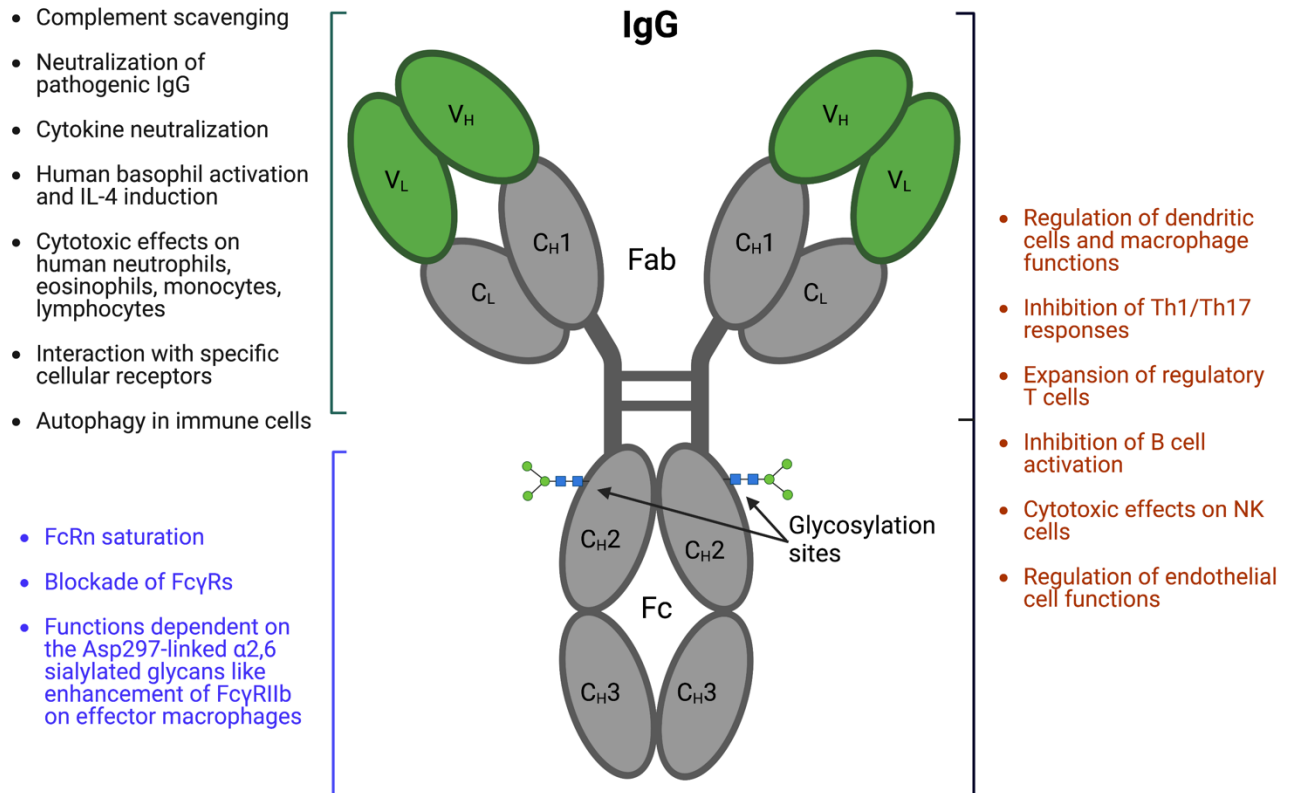
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801 **Figure Legend:**

802 **Figure 1: The current knowledge on the implication of either F(ab')₂, 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')₂
804 fragments. Some of the Fc-mediated functions also implicit the involvement of α₂,6-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')₂ and Fc fragments. V_H, heavy chain variable domain; V_L, light chain
807 variable domain; C_H, heavy chain constant domain; C_L, 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 γ receptors	Debré et al. 1993 ⁵⁸
Induction of apoptosis of immune cells by Fas apoptosis pathway	Prasad et al. 1998 ¹⁴²
Induction of anti-inflammatory IL-1 receptor antagonist (IL-1RA) in monocytes	Ruiz de Souza et al. 1995 ³⁶
Suppression of an array of immune activation genes in monocytes of Kawasaki disease	Abe et al. 2005 ⁴⁷
Regulation of dendritic cell functions	Bayry et al. 2003 ^{52,55} Siragam et al. 2006 ⁵³ Wiedeman et al. 2013 ⁵⁷
Inhibition of NK cytotoxicity	Ruiz et al. 1996 ¹⁰⁸
Cytotoxic effects on neutrophils by anti-Siglec-9 autoantibodies	von Gunten et al. 2006 ⁸⁴
Inhibition of neutrophil extracellular trap (NET)	Uozumi et al. 2020 ⁸⁷
Cytotoxic effects on eosinophils by anti-Siglec-8 autoantibodies	von Gunten et al. 2007 ⁹¹
IL-3-dependent induction of human basophil activation and IL-4 secretion via anti-IgE IgG	Galeotti et al. 2019 ⁷⁸
Fc-Sialylation-dependent anti-inflammatory mechanisms in Mice	Kaneko et al. 2006 ⁶¹ Anthony et al. 2011 ⁶³ Fiebiger et al. 2015 ⁶⁴
Identification of receptors for sialylated Fc fragments of IgG	Anthony et al. 2008 ¹⁴³ Séité et al. 2010 ¹²⁶ Massoud et al 2014 ⁷⁰ Fiebiger et al. 2015 ⁶⁴
Induction of inhibitory ITAM signaling through Fc γ RIII	Aloulou et al. 2012 ¹⁴⁴
Induction of autophagy in innate immune cells	Das et al. 2020 ⁸⁷
Epigenetic regulation of macrophages	Guo et al. 2020 ⁵¹

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

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

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

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

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