Intravenous immunoglobulin (IVIg) is used as treatment for several autoimmune and inflammatory conditions, but its specific mechanisms are not fully understood. Herein, we aimed to evaluate, using systems biology and artificial intelligence techniques, the differences in the pathophysiological pathways of autoimmune and inflammatory conditions that show diverse responses to IVIg treatment. We also intended to determine the targets of IVIg involved in the best treatment response of the evaluated diseases. Our selection and classification of diseases was based on a previously published systematic review, and we performed the disease characterization through manual curation of the literature. Furthermore, we undertook the mechanistic evaluation with artificial neural networks and pathway enrichment analyses. A set of 26 diseases was selected, classified, and compared. Our results indicated that diseases clearly benefiting from IVIg treatment were mainly characterized by deregulated processes in B cells and the complement system. Indeed, our results show that proteins related to B-cell and complement system pathways, which are targeted by IVIg, are involved in the clinical response. In addition, targets related to other immune processes may also play an important role in the IVIg response, supporting its wide range of actions through several mechanisms. Although B-cell responses and complement system have a key role in diseases benefiting from IVIg, protein targets involved in such processes are not necessarily the same in those diseases. Therefore, IVIg appeared to have a pleiotropic effect that may involve the collaborative participation of several proteins. This broad spectrum of targets and ‘non-specificity’ of IVIg could be key to its efficacy in very different diseases.
Introduction

Intravenous immunoglobulin (IVIg) is a preparation of normal human IgG derived from large pools of healthy human plasma (1). IVIg is used as replacement therapy to prevent infections in patients with primary or secondary immune deficiency syndromes and, at high doses, exerts immunomodulatory effects to treat autoimmune and inflammatory disorders (2). Indeed, IVIg has been approved by the European Medicines Agency (EMA) for several autoimmune conditions, such as immune thrombocytopenia (ITP), chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré syndrome (GBS), Kawasaki disease (KD), and multifocal motor neuropathy (MMN) (3). In addition, the off-label uses of IVIg are growing rapidly, and several other autoimmune and inflammatory conditions have been suggested as potential indications (4). Nonetheless, limited clinical evidence indicates that the successful response to IVIg observed in some autoimmune conditions may not necessarily and systematically be extended to others. For instance, adrenoleukodystrophy and inclusion body myositis do not respond well to this treatment (2, 5, 6).

The beneficial effect of IVIg is explained by mechanisms involving either its F(ab′)_2 (7) or Fc domain (8) or both. The immunomodulatory effects of IVIg are complex and intricate; IVIg has been proved to modulate B and T cells, phagocytosis, complement activity, cytokine production, and the properties of dendritic cells (DCs), among many others (1, 9–11). This wide range of cellular and molecular targets can trigger pleiotropic effects in the immune system (1, 9). The specific immune and inflammatory processes modulated by IVIg depend on the pathophysiological process driving each disease (10, 12). Besides, the molecular mechanisms may also depend on the dose of IVIg and the window during the immunopathogenesis when IVIG is administered (13). Although IVIg has been shown to modulate a large number of immunological processes, the complete understanding of the molecular mechanisms defining treatment response remains unclear for some of them.

Systems biology and artificial intelligence tools have been used in the past to unveil the mechanism of action of a variety of drugs for hematological or cardiovascular diseases, among others (14, 15). However, this approach has been rarely used on IVIg, and the few reported examples were centered on the treatment of a specific disease (16–19). Therefore, we considered that the abovementioned tools could be applied in a larger setting to shed some light on the mechanisms of action of IVIg and their diversity depending on the treated disease. By means of system biology and machine learning models based on Artificial Neural Networks (ANNs) (20–22), we evaluated the differences in the pathophysiological pathways of autoimmune and inflammatory conditions that show diversity in responses to treatment with IVIg. Also, we aimed at determining the targets of IVIg involved in the best treatment response of the evaluated diseases.

Materials and methods

Selection and classification of diseases

To compare the heterogeneous efficacy response to IVIg treatment, we resorted to a previously published systematic and evidence-based classification of neurological and autoimmune diseases (2). We further classified the diseases according to their response to IVIg in four clusters, as previously reported (2): ‘definitely beneficial’ (DB), ‘probably beneficial’ (PB), ‘may provide benefit’ (MPB), and ‘unlikely to provide benefit’ (UPB) (Table 1, left column). The level and strength of evidence supported this classification according to: (i) evidence category, in groups Ia, Ib, IIA, IIB, III, and IV (e.g., group Ia referred to evidence obtained from meta-analysis of randomized controlled trials, while group IV to those obtained from expert committee reports, opinions or clinical experience of respected authorities of both), and (ii) strength of recommendation, which estimated the strength of the evidence to assign the condition to an IVIg response cluster, and ranged from A (strongest support) to D (weakest support) (2). For our study, we only included disorders for which the benefit/no benefit of IVIg treatment had been established with enough clinical evidence, i.e. those with a level of evidence Ia, Ib, IIA, or IIB (supported by clinical trials) and a strength of recommendation of A or B (2). We considered ‘IgM anti-myelin-associated glycoprotein (anti-MAG) paraprotein-associated peripheral neuropathy’ and ‘demyelinating neuropathy associated with monoclonal IgM’...
| IVIg response cluster | Disorders | Pathophysiological processes | Number of effectors |
|-----------------------|-----------|-----------------------------|--------------------|
| Definitely Beneficial (DB) | Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) | 2- T cell-mediated response  
1- Dysregulated B cell response  
4- Complement system  
3- Myelin damage by macrophages | 70 |
| | Multifocal Motor Neuropathy (MMN) | 1- Dysregulated B cell response  
4- Complement system  
5- Myelin damage and axonal | 26 |
| | Guillain-Barre Syndrome (GBS) | 2- T cell-mediated response  
1- Dysregulated B cell response  
4- Complement system  
3- Myelin damage by macrophages | 67 |
| | Graves ophthalmopathy (GO) | 2- T cell-mediated response and inflammation by fibroblasts  
5- Orbital fibroblast proliferation and migration  
1- Dysregulated B cell response  
5- Production of ECM components by fibroblasts  
3- Adipogenic and myofibroblastic differentiation | 52 |
| | Immune thrombocytopenic purpura (ITP) | 1- Dysregulated B cell response  
2- T cell-mediated response  
4- Complement system  
5- Suppression of megakaryocyte proliferation and maturation  
5 - Dysfunctional mesenchymal stem cells (MSCs) | 61 |
| | Kawasaki Disease (KD) | 2- T cell-mediated response  
1- Dysregulated B cell response  
4- Complement system  
3- Exaggerated innate immune response - Systemic inflammation  
5- Aneurysm formation and angiogenesis | 95 |
| | Myasthenia Gravis (MG) | 1- Dysregulated B cell response  
2- T cell-mediated response  
4- Complement system  
5- Synaptic dysfunction  
5- Muscular atrophy | 67 |
| | Probably Beneficial (PB) | Ig M anti-MAG paraprotein-associated peripheral neuropathy (anti-MAG IgM MGUS) | 1- Dysregulated B cell response  
4- Complement system | 22 |
| | | Lambert-Eaton Myasthenic Syndrome (LEMS) | 1- Dysregulated B cell response  
4- Complement system  
5- Synaptic dysfunction  
5- Muscular atrophy | 29 |
| | | Stiff-Person Syndrome (SPS) | 1- Dysregulated B cell response  
2- T cell-mediated response  
5- Synaptic dysfunction | 24 |
| | | Dermatomyositis (DM) | 3- Exaggerated innate immune response  
2- T cell-mediated response  
4- Complement system  
5- Skin and muscle atrophy | 61 |

(Continued)
| IVlg response cluster | Disorders | Pathophysiological processes | Number of effectors |
|-----------------------|-----------|-----------------------------|---------------------|
| **Probably Beneficial** | Birdshot retinochoroidopathy (BSRC) | 1 - Dysregulated B cell response | |
| | | 2 - Abnormal T cell activation | |
| | | 3 - T cell-mediated response | |
| | | 3 - and inflammatory mediators’ perpetuation | 23 |
| | Henoch-Schonlein purpura (HSP) | 3 - Exaggerated innate immune response | |
| | | 1 - Dysregulated B cell response | |
| | | 2 - T cell-mediated response | |
| | | 4 - Complement system | |
| | | 5 - Accelerated extracellular matrix breakdown | |
| **May Provide Benefit** (MPB) | Relapsing-Remitting Multiple Sclerosis (RRMS) | 3 - Exaggerated innate immune response | |
| | | 2 - T cell-mediated response | |
| | | 1 - Dysregulated B cell response | |
| | | 4 - Complement system | |
| | | 5 - Impaired neurotransmission | 109 |
| | Intractable childhood epilepsy (ICE) | 5 - Drug-resistance | |
| | | 5 - Ion and neurotransmitter imbalance | |
| | | 3 - Neuroinflammation by microglia and astrocytes | |
| | Postpolio syndrome (PPS) | 3 - Systemic inflammation | |
| | | 5 - Sympathetic toxicity (induced by inflammatory mediators) | |
| | | 5 - Muscular atrophy and inflammatory response | 19 |
| | Juvenile idiopathic arthritis (JIA) | 3 - Exaggerated innate immune response | |
| | | 2 - T cell-mediated response | |
| | | 1 - Dysregulated B cell response | |
| | | 3 - NK cells dysfunction | |
| | | 2 - Defective Tregs immunoregulation | |
| | | 4 - Complement system | |
| | | 5 - Joint damage | 87 |
| | Anti-phospholipid antibody syndrome in pregnancy (APS) | 5 - Abnormal placental development | |
| | | 3 - Innate Immune Response | |
| | | 1 - Dysregulated B cell response | |
| | | 4 - Complement system | |
| | | 5 - Thrombosis factor dysregulation | 45 |
| | Severe rheumatoid arthritis (sRA) | 2 - T cell-mediated response | |
| | | 1 - Dysregulated B cell response | |
| | | 3 - Synovial inflammation | |
| | | 5 - Articular destruction | |
| | | 5 - Bone erosion | 148 |
| | Still disease (SD) | 2 - T cell-mediated response | |
| | | 3 - Exaggerated innate immune response | |
| | | 3 - NK cells dysfunction | |
| | | 5 - Neutropenia | 63 |
| | Felty’s syndrome (FS) | 3 - Exaggerated innate immune response | |
| | | 1 - Dysregulated B cell response | |
| | | 5 - Neutropenia | 21 |
| | Macrophage activation syndrome (MAS) | 3 - Exaggerated innate immune response | |
| | | 2 - T cell-mediated response | |
| | | 3 - NK cells dysfunction | |
| | | 3 - Cell death by activated macrophages | 29 |
as the same disease because of their degree of similarity and lack of differential molecular information available in scientific literature. Therefore, we hereafter referred to these two diseases as ‘IgM anti-MAG paraprotein-associated peripheral neuropathy’. To increase the number of analyzed disorders, we added two other autoimmune diseases with a well-established level of clinical response to IVIg: KD and Crohn’s disease (CD). IVIg has been approved by the US Food and Drug Administration (23) and the EMA (24) to treat KD according to strong evidence supporting its positive response (note that, in the abovementioned systematic review (2), KD was classified as an infection-related disease and not an autoimmune disease). Therefore, we incorporated KD into the DB cluster. On the contrary, unclear clinical evidence on the impact of IVIg on CD prompted us to include it in the UPB cluster in our study. We only reclassified one condition in our study, myasthenia gravis (MG), an approved indication for IVIg by the EMA. MG was moved from the PB to the DB cluster on the basis of the extended clinical use of IVIg in this condition and its positive response according to clinical evidence (25–27).

Disease characterization

We characterized the selected conditions molecularly through manual curation of the current scientific literature, as previously reported (21). First, we searched for reviews on the molecular pathogenesis, pathophysiology, and molecular mechanisms of the conditions in the PubMed database. Second, we retrieved the publications identified in these searches and assessed them at the title and abstract level. If molecular information describing the condition pathophysiology was present, we thoroughly reviewed full texts seeking to identify the main pathophysiological processes involved in the condition. We referred to these processes as ‘pathophysiological processes’. Third, we further characterized each pathophysiological process at the protein level (Supplementary Table S1 in the S1 File). We performed our searches in June 2020, limited them to 10 years before that date, and expanded them by reviewing relevant references in the results. We only included candidate articles with references more recent than year 2000, with the exception of references for anti-MAG, for which very little molecular information was found with this protocol, and for references for immunoglobulin heavy
Inhibition

P06127 T-cell surface glycoprotein CD5 CD5 T-cell related ↓ Q9NNX6 CD209 antigen CD209, P25445 Tumor necrosis factor receptor superfamily member 6 FAS Apoptosis ↑ ↓ P51681 C-C chemokine receptor type 5 CCR5 Innate immunity, T-cell related ↓ Q9UMR7 C-type lectin domain family 4 member A CLEC4A (61)

Pathophysiology

P01730 T-cell surface glycoprotein CD4 CD4 T-cell related ↓ P02745 Complement C1q subcomponent subunit A C1QA Complement system ↓ P01024 Complement C3 C3 Complement system ↓ P00736 Complement C1r subcomponent chain C1R Complement system ↓ P09871 Complement C1s subcomponent C1S Complement system ↓ P01031 Complement C5 C5 Complement system ↓ P09025 Complement C4-B C4B Complement system ↓ ↓ HLA class I and II (includes 21 proteins) HLA HLA ↓ P25445 Tumor necrosis factor receptor superfamily member 6 FAS Apoptosis ↑ ↓ Q9NNX6 CD209 antigen CD209, DQ-SIGN Innate immunity ↑ ↓ Q9UMR7 C-type lectin domain family 4 member A CLEC4A, DCR1 Innate immunity ↑ ↓ P51681 C-C chemokine receptor type 5 CCR5 Innate immunity, T-cell related ↓ P0I702 M1-specific T-cell receptor beta chain TRB T-cell related ↓ P06127 T-cell surface glycoprotein CD5 CD5 T-cell related ↓ P01730 T-cell surface glycoprotein CD4 CD4 T-cell related ↓ HLA, Human leukocyte antigen; IVIg, Intravenous immunoglobulin; Ref., Reference.

1. Inhibition; 2. Activation.

TABLE 2 List of IVIg protein targets (direct targets) identified by literature search and classified according to their related immunological function.

| UniProt ID | Protein Name | Gene Name | Functional group | IVIg effect | Ref. |
|------------|--------------|-----------|------------------|-------------|-----|
| P02735     | B-cell receptor CD22 | CD22       | B-cell related   | ↑           | (31) |
| P08637     | Low affinity immunoglobulin gamma Fc region receptor III-A | FCGR3A/CD16a | B-cell related | ↓ | (32) |
| O75015     | Low affinity immunoglobulin gamma Fc region receptor III-B | FCGR3B/CD16b | B-cell related | ↓ | (32) |
| P125318    | Low affinity immunoglobulin gamma Fc region receptor II-a | FCGR2A/CD32 | B-cell related | ↓ | (32) |
| P55899     | IgG receptor FcRn large subunit p51 | FCGR7 | B-cell related | ↓ | (33–35) |
| Q9Y275     | Tumor necrosis factor ligand superfamily member 13B | TNFSF13B, BAFF | B-cell related | ↓ | (36–38) |
| O75888     | Tumor necrosis factor ligand superfamily member 13 | TNFSF13, APRIL | B-cell related | ↓ | (37) |
| Q9NYZ4     | Sialic acid-binding Ig-like lectin 8 | SIGLEC8 | Antigen presentation | ↑ | (39) |
| Q9Y336     | Sialic acid-binding Ig-like lectin 9 | SIGLEC9 | Antigen presentation | ↑ | (40) |
| P02745     | Complement C1q subcomponent subunit A | C1QA | Complement system | ↓ | (41) |
| P00736     | Complement C1r subcomponent chain | C1R | Complement system | ↓ | (42, 43) |
| P09871     | Complement C1s subcomponent | C1S | Complement system | ↓ | (42, 43) |
| P01024     | Complement C3 | C3 | Complement system | ↓ | (44–46) |
| P01031     | Complement C5 | C5 | Complement system | ↓ | (47, 48) |
| P09205     | Complement C4-B | C4B | Complement system | ↓ | (46, 49) |
| – HLA class I and II (includes 21 proteins) | HLA | HLA | ↓ | (50) |
| P25445     | Tumor necrosis factor receptor superfamily member 6 | FAS | Apoptosis | ↑ | (51) |
| Q9NNX6     | CD209 antigen | CD209, DQ-SIGN | Innate immunity | ↑ | (51–55) |
| Q9UMR7     | C-type lectin domain family 4 member A | CLEC4A, DCR1 | Innate immunity | ↑ | (56, 57) |
| P51681     | C-C chemokine receptor type 5 | CCR5 | Innate immunity, T-cell related | ↓ | (58) |
| P0I702     | M1-specific T-cell receptor beta chain | TRB | T-cell related | ↓ | (59, 60) |
| P06127     | T-cell surface glycoprotein CD5 | CD5 | T-cell related | ↓ | (61) |
| P01730     | T-cell surface glycoprotein CD4 | CD4 | T-cell related | ↓ | (62) |

Mechanistic evaluation of IVIg targets: Artificial neural network analysis

Therapeutic performance mapping system technology (14), based on systems biology, integrates available protein-protein network information along with physiological and pathophysiological data that allows to create machine learning models based on Artificial Neural Networks (ANNs), which...
| Uniprot ID | Protein Name                                      | Gene Name | Effect | Reference |
|-----------|--------------------------------------------------|-----------|--------|-----------|
| P55774    | C-C motif chemokine 18                          | CCL18     | ↓      | (63)      |
| P13500    | C-C motif chemokine 2                           | CCL2      | ↓      | (64)      |
| P78556    | C-C motif chemokine 20                          | CCL20     | ↓      | (10)      |
| P25942    | Tumor necrosis factor receptor superfamily member 5 | CD40    | ↓      | (65, 66)  |
| P33681    | T-lymphocyte activation antigen CD80             | CD80      | ↓      | (67)      |
| P42081    | T-lymphocyte activation antigen CD86             | CD86      | ↓      | (67)      |
| P46527    | Cyclin-dependent kinase inhibitor 1B             | CDKN1B    | ↑      | (68)      |
| Q9ULM6    | CCR4-NOT transcription complex subunit 6         | CNOT6     | ↑      | (69)      |
| P20023    | Complement receptor type 2                       | CR2       | ↓      | (68)      |
| P04141    | Granulocyte-macrophage colony-stimulating factor | CSF2      | ↓      | (70)      |
| P16410    | Cytotoxic T-lymphocyte protein 4                 | CTLA4     | ↑      | (69)      |
| P31994    | Low affinity immunoglobulin gamma Fc region receptor II-b | FCGR2B | ↑      | (65, 71)  |
| Q9RZ51    | Forkhead box protein P3                          | FOXP3     | ↑      | (69)      |
| P05362    | Intercellular adhesion molecule 1                | ICAM1     | ↓      | (72, 73)  |
| P01579    | Interferon gamma                                 | IFNG      | ↓      | (71, 74)  |
| P38484    | Interferon gamma receptor 2                      | IFNGR2    | ↓      | (75)      |
| P22301    | Interleukin-10                                   | IL10      | ↑      | (67, 70)  |
| P29459    | Interleukin-12 subunit alpha                     | IL12A     | ↓      | (67)      |
| P29460    | Interleukin-12 subunit beta                      | IL12B     | ↓      | (67)      |
| P35225    | Interleukin-13                                   | IL13      | ↑      | (76)      |
| Q16552    | Interleukin-17A                                  | IL17A     | ↓      | (10, 67)  |
| Q96PD4    | Interleukin-17F                                  | IL17F     | ↑      | (10, 67)  |
| P01584    | Interleukin-1 beta                               | IL1B      | ↓      | (65, 67)  |
| P18510    | Interleukin-1 receptor antagonist protein        | IL1RN     | ↑      | (1)       |
| P60568    | Interleukin-2                                    | IL2       | ↓      | (71, 74)  |
| Q9FBE4    | Interleukin-21                                   | IL21      | ↓      | (10)      |
| P08700    | Interleukin-3                                    | IL3       | ↓      | (70)      |
| Q95760    | Interleukin-33                                   | IL33      | ↑      | (57, 77)  |
| P05112    | Interleukin-4                                    | IL4       | ↑      | (57, 70, 77) |
| P05113    | Interleukin-5                                    | IL5       | ↓      | (70)      |
| P05231    | Interleukin-6                                    | IL6       | ↓      | (72)      |
| P20701    | Integrin alpha-L                                 | ITGAL     | ↓      | (72)      |
| P01374    | Lymphotxin-alpha                                 | LTA       | ↓      | (72)      |
| P28482    | Mitogen-activated protein kinase 1               | MAPK1     | ↑      | (1, 68)   |
| Q15759    | Mitogen-activated protein kinase 11              | MAPK11    | ↓      | (1)       |
| P53778    | Mitogen-activated protein kinase 12              | MAPK12    | ↓      | (1)       |
| Q15264    | Mitogen-activated protein kinase 13              | MAPK13    | ↓      | (1)       |
| Q16539    | Mitogen-activated protein kinase 14              | MAPK14    | ↓      | (1)       |
| P27361    | Mitogen-activated protein kinase 3               | MAPK3     | ↑      | (1, 68)   |
| P14780    | Matrix metalloproteinase-9                       | MMP9      | ↓      | (72)      |
| P19838    | Nuclear factor NF-kappa-B p105 subunit           | NFKB1     | ↓      | (1)       |
| Q00653    | Nuclear factor NF-kappa-B p100 subunit           | NFKB2     | ↓      | (1)       |
| P35228    | Nitric oxide synthase, inducible                 | NOS2      | ↓      | (65)      |
| P42336    | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform | PIKSCA | ↓ | (68) |
| P42338    | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform | PIKSCB | ↓ | (68) |
| Q00329    | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform | PIKSCD | ↓ | (68) |
| P48736    | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform | PIKSCG | ↓ | (68) |
| P16885    | 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-2 | PLCG2 | ↓ | (68) |

(Continued)
have been previously defined and applied (20–22). ANNs are supervised algorithms that identify relations between proteins (e.g., drug targets) and clinical elements of the network (22, 82, 83) by inferring the probability of the existence of a specific relationship between two or more protein sets. The training set (Supplementary Table S2 in the S2 File) was designed to predict the relationship between the drugs and their clinical conditions, drugs being stratiﬁed by the number of their targets. The learning methodology used consisted in bagging training architecture of stratified ensembles of neural networks as a ﬁnal model. Each neural network model used was a multilayer perceptron (MLP) neural network classiﬁer and they were considered as a weak classiﬁer. These MLP were submitted to randomized initialization, having all of them a hidden layer containing between 7 and 11 nodes. Input database contained pairs of drug targets and biological conditions (drug-indication pairs) obtained from DrugBank and PubChem (84, 85). The ﬁnal dataset was manually reviewed to avoid redundancies and mistakes that could affect the quality of the training set. The input feature vector is based on topological measures over the protein network from drug targets to condition according to the topological structure of the interconnection graph defined between them. The output was deﬁned by a single node indicating the relation or no relation between the drug targets and the condition. Each training set for each MLP model was selected from a balanced subset of samples randomized by means of a Monte-Carlo cross-validation structure to reduce overﬁtting in the ﬁnal model. The process to generate the ﬁnal model was optimized by Levenberg-Marquardt strategy. A total of 1000 MLPs were trained and the best 75% of them for each stratum are considered in the ﬁnal model. The implementation code was done in Matlab by using the Neural Network Toolbox, as combined with other Anaxomics Biotech’ developments. The final model performance is based on the capability to predict the pairs drug-indication, being evaluated as the AUC of the model. The ﬁnal model has 81.77% of correct prediction when drugs tested have all their protein targets in the protein-protein network.

We employed these algorithms to explore the relationships between IVIg targets (either individually or grouped according to their functional group, Table 2) and the molecular deﬁnition of each disease (Supplementary Table S1 in the S1 File). ANN analysis provides a score for a target, or group of targets, based on the validations of the prediction capacity of the mathematical models toward the training set (known drugs and diseases, as described in databases). Each score is associated with a p-value that describes the probability that the result is a true positive. Accordingly, and to simplify their interpretation, we divided here the ranking scores in four categories: strong relationship (ANN score >78%, p-value<0.05), medium-strong relationship (ANN score >71%, p-value<0.1), medium relationship (ANN score >38%, p-value<0.25), and low or no relationship (ANN score ≤38%, p-value ≥0.25).

Data evaluation and statistical analysis

We used the chi-squared test to evaluate independence between the pathophysiological mechanisms involved in each disease and the disease classiﬁcation based on IVIg response clustering. Also, we calculated inter-cluster disease similarity by direct protein overlap within the list of proteins included in the diseases of each cluster. Then, we performed the arithmetic mean of proportions of protein overlap (O) of each pair of disease clusters (C1, C2) according to equation 1:

![Image](https://example.com/image.png)

TABLE 3 Continued

| Uniprot ID | Protein Name                          | Gene Name | Effect | Reference |
|-----------|---------------------------------------|-----------|--------|-----------|
| P33534    | Prostaglandin G/H synthase 2           | PTGS2     | ↑       | (1, 78)   |
| P51449    | Nuclear receptor ROR-gamma             | RORC      | ↓       | (79)      |
| P05109    | Protein S100-A8                        | S100A8    | ↓       | (80)      |
| P06702    | Protein S100-A9                        | S100A9    | ↓       | (80)      |
| P16581    | E-selectin                            | SELE      | ↓       | (72, 73)  |
| P16109    | P-selectin                             | SELP      | ↓       | (73)      |
| P40763    | Signal transducer and activator of transcription 3 | STAT3 | ↓       | (1, 79)   |
| P01137    | Transforming growth factor beta-1 proprotein | TGFBI | ↑       | (65, 70)  |
| P61812    | Transforming growth factor beta-2 proprotein | TGFBI2 | ↑       | (65, 70)  |
| Q8NYK1    | Toll-like receptor 7                   | TLR7      | ↓       | (1, 81)   |
| Q0N9R6    | Toll-like receptor 9                   | TLR9      | ↓       | (1, 67)   |
| P01375    | Tumor necrosis factor                  | TNF       | ↓       | (71, 74)  |
| Q9Y35U5   | Tumor necrosis factor receptor superfamily member 18 | TNFRSF18 | ↑       | (69)      |
| P43403    | Tyrosine-protein kinase ZAP-70         | ZAP70     | ↑       | (75)      |

↓, Inhibition; ↑, Activation.
\[
\frac{O/C1 + O/C2}{2} \times 100 \quad \text{Equation 1}
\]

We used the InteractiVenn software (86) to create Venn diagrams.

Pathway enrichment analysis

We applied hypergeometric pathway enrichment analysis (87) to determine whether pre-defined protein sets, according to biological reference databases, were represented within groups of protein effectors involved in the IVIg response-based disease clusters. Specifically, we used KEGG (88) and Gene Ontology (89) as reference databases. Results were submitted to Benjamini-Hochberg multi-test correction (90) to control false discovery rate (FDR). We only selected enriched pathways with an FDR q-value<0.05.

Results

Comparison of IVIg responding and non-responding conditions at the pathophysiological level

A set of 26 autoimmune and inflammatory conditions, with clinical evidence of optimal level to be classified as responders/non-responders to IVIg, was selected. Previously reported IVIg
response categories were used (2) (with a few exceptions, see Materials and Methods, Table 1). Once characterized, we compared the diseases at the level of pathophysiological processes to identify those processes more frequently related to each IVIg response cluster. To facilitate this comparison, we grouped them in broad (immune system) processes as follows (Table 1): T cell-mediated response, B cell-mediated response, complement system, innate immunity/inflammation processes, and other (which included disease-specific dysfunctions, such as bone erosion or muscular atrophy). This classification was based on the association of the proteins contained in each characterized pathophysiological process.

Our results suggested that diseases within each IVIg response cluster shared similar pathophysiological processes, especially within the DB and UPB clusters, and that identifiable differences between the clusters could be found. A chi-squared test showed that there was dependency between pathological processes and disease classification based on IVIg response (Figure 1). In particular, diseases assigned to the DB cluster were positively associated with B cell-mediated processes and complement system. On the contrary, innate immunity and inflammatory processes were frequently associated to MPB (statistically significant association with 100% frequency) and UPB (not statistically association significant 100% frequency) clusters (Figure 1).

**Molecular pathways associated with diseases that clinically benefit from IVIg**

We evaluated similarity between the disease clusters based on IVIg response with the aim of identifying processes occurring in diseases that clinically benefit from IVIg. When analyzing direct protein overlap (Figure 2A) between the bibliography-based list of effectors (Supplementary Table S1 in the S1 File) among the clusters, the DB and UPB clusters were the more distant. The specific effectors and those shared between the clusters were identified and grouped in three protein sets:
proteins present in the DB cluster but not in the UPB cluster (DB w/o UPB), proteins specifically present in DB cluster (only DB), and proteins specifically present in UPB cluster (only-UPB) (Figure 2B). The enrichment analysis over the DB w/o UPB protein set provided 81 pathways enriched (Figure 2B). A detailed analysis of these enriched pathways (Figure 2C) unveiled that, while some of them were very general (e.g., positive regulation of the biological process, regulating signaling pathway), the most specific pathways pointed toward immune-related functions, such as processes of innate immunity response (e.g., leukocyte mediated immunity, phagocytosis), adaptive response (e.g., regulation of lymphocyte activation, adaptive immune response), autoimmunity (e.g., systemic lupus erythematosus, autoimmune thyroid disease), infection, T cell, B cell, and the complement system. Interestingly, there were other pathways enriched, including signaling processes (e.g., transferase activity, kinase activity, adrenergic signaling in cardiomyocytes) specific hormonal pathways (e.g., GnRH signaling pathway, thyroid hormone synthesis), and development pathways (e.g., progesterone-mediated oocyte maturation, long-term potentiation).

Taking the results of the enrichment analysis of the DB w/o UPB protein set as a reference, the results of the enrichment analysis of the only-DB protein set showed 23 common enriched pathways, while no overlap was found with the only-UPB set (Supplementary Table S3 in the S3 File). The 23 enriched pathways shared between the only-DB and DB w/o UPB sets contained mainly general signaling pathways involved in
development (e.g., oocyte meiosis and regulation of dendritic differentiation), hormonal regulation (e.g., endocrine-regulated calcium reabsorption and GnRH signaling pathway), and inflammation (e.g., VEGF and cGMP-PKG signaling pathways), as well as some pathways related to B and T cells (Figure 2C, nodes circled in dark green, and Supplementary Table S3 in the S3 File).

**Targets involved in the response to IVIg**

The functional relationship between IVIg targets (Table 2) and the different diseases, defined as the effector proteins previously identified (Supplementary Table S1 in the S1 File), was tested by ANN analysis to measure the mechanistic relationship between them. This approach predicts the possibility that the studied targets modulate the set of proteins involved in the disease pathophysiology. We first tested the functional relationship between the studied conditions and IVIg targets grouped by their immune-related function (Functional group column in Table 2). The results showed that B cell-related and complement system-related targets were more likely associated with IVIg efficacy (Figure 3). To get a closer evaluation of the targets, the analysis was performed for each of them individually, focusing on the targets within the complement system and B cell-related functional groups. The analysis over the individual IVIg targets of the complement system functional group disclosed a stronger association between C3, C4B, and C5 with diseases assigned to the DB cluster than their association with disorders classified in the UPB cluster (Figure 4A). The same evaluation performed on individual targets related to B cells showed that different groups of targets were associated with different diseases (namely, FCGR3A/FCGR3B and TNFSF13/TNFsF13B) (Figure 4B).

The participation in IVIg response of other targets not related to B cell or complement system processes was also explored by ANNs. In this case, the functional relationship between the complete IVIg protein target profile and each disease of the DB cluster was assessed and unveiled a high heterogeneity among diseases (Table 4). Targets associated with HLA (e.g., HLA-DPA1), innate immunity (e.g., CLEC4A), or T-cell related (e.g., CCR5) showed a consistent medium-strong (>71% ANN score) or strong (>78% ANN

FIGURE 4
Predicted relationship between each studied disorder and (A) each complement system protein target of IVIg or (B) each B-cell related IVIg protein target according to ANN. ANN, Artificial Neural Network; IVIg, Intravenous immunoglobulin.
TABLE 4 Functional relation (ANN score) between IVIg protein targets and diseases from the DB cluster according to ANN analysis.

| SID    | CIDP | MMN | GBS | MG | GO | ITP | KD |
|--------|------|-----|-----|----|----|-----|-----|
| FAS (P25445) | +   | +   | +   | +  | ++ | +   | +   |
| Apoptosis |      |     |     |    |    |     |     |
| FCGR1A (P08637) | +++ | +++ | -   | +  | -  | -   | -   |
| B cell-mediated | (86%) | (85%) | (11%) | (52%) | (20%) | (27%) | (37%) |
| FCGR1B (P70515) | +  | +++ | -   | +  | -  | -   | +   |
| B cell-mediated | (76%) | (93%) | (31%) | (69%) | (17%) | (12%) | (43%) |
| FCGR2A (P12318) | +  | +   | +   | +  | +  | +++ | +   |
| B cell-mediated | (62%) | (44%) | (61%) | (71%) | (47%) | (71%) | (83%) |
| TNFSF13 (O75888) | +  | +   | +   | +  | -  | +++ | +   |
| B cell-mediated | (40%) | (8%)  | (35%) | (44%) | (24%) | (90%) | (66%) |
| TNFSF13B (Q9Y275) | -  | -    | -   | -  | -  | +++ | ++  |
| B cell-mediated | (22%) | (3%)  | (5%) | (28%) | (15%) | (79%) | (76%) |
| SIGLEC9 (Q9Y336) | -  | -    | -   | -  | ++ | -   | -   |
| Antigen presentation | (14%) | (16%) | (20%) | (18%) | (73%) | (14%) | (34%) |
| CS (P01031) | +++ | ++  | +++ | +++ | -  | -   | +   |
| Complement system | (86%) | (72%) | (93%) | (82%) | (9%) | (27%) | (77%) |
| C3 (P01024) | ++ | +    | +   | +  | +  | +   | +   |
| Complement system | (77%) | (63%) | (59%) | (86%) | (11%) | (72%) | (85%) |
| C4 (P00565) | +++ | +++  | +++ | +++ | -  | +   | +   |
| Complement system | (38%) | (82%) | (92%) | (72%) | (7%) | (84%) | (67%) |
| C1QA (P02745) | -  | +    | +   | +  | +  | +   | +   |
| Complement system | (28%) | (58%) | (41%) | (40%) | (71%) | (47%) | (54%) |
| C1R (P00736) | -  | +    | +   | ++ | -  | +   | -   |
| Complement system | (23%) | (40%) | (71%) | (32%) | (68%) | (41%) | (18%) |
| C1S (P08971) | -  | +    | ++  | -   | +  | +   | +   |
| Complement system | (22%) | (40%) | (71%) | (33%) | (60%) | (42%) | (15%) |
| HLA-DPA1 (P20036) | +++ | +   | +++ | +   | -  | ++  | +   |
| HLA | (80%) | (68%) | (81%) | (42%) | (36%) | (72%) | (41%) |
| HLA-DQA1 (P01909) | ++ | +    | +   | ++ | -  | +   | +   |
| HLA | (76%) | (41%) | (56%) | (73%) | (36%) | (71%) | (39%) |
| HLA-DRA (P01903) | ++ | +    | +   | +  | +  | +   | +   |
| HLA | (72%) | (44%) | (50%) | (39%) | (71%) | (49%) | (33%) |
| HLA-DB1 (P04440) | ++ | +    | +   | +  | +  | +   | +   |
| HLA | (71%) | (22%) | (9%) | (36%) | (37%) | (51%) | (18%) |
| HLA-B (P01889) | +  | -    | +   | -  | -  | ++  | +   |
| HLA | (51%) | (23%) | (26%) | (46%) | (26%) | (30%) | (71%) |
| CLEC4A (Q9UMR7) | ++ | +    | +   | ++ | -  | +   | +   |
| Innate immunity | (73%) | (65%) | (71%) | (72%) | (72%) | (72%) | (72%) |
| CD209 (Q9NNX6) | ++ | -    | +   | +  | +  | +   | +   |
| Innate immunity | (71%) | (3%)  | (63%) | (71%) | (71%) | (57%) | (73%) |
| CCR5 (P51681) | ++ | +    | +   | +  | +  | +   | +   |
| Innate immunity, T cell-related | (99%) | (36%) | (67%) | (39%) | (69%) | (66%) | (43%) |
| CD4 (P01730) | ++ | +    | +++ | +  | +  | +   | +   |
| T cell-related | (71%) | (50%) | (87%) | (63%) | (38%) | (40%) | (57%) |

ANN scores represent the probability for the relationship to be a true positive: +++ (in bold) correspond to a strong relationship and values > 78% (p-value <0.05); ++ correspond to a medium-strong relationship and values > 71% (p-value <0.1); + correspond to a medium relationship and values >38% (p-value <0.25); and – correspond to low or no relationship and values ≤ 38% (p-value ≥0.25).

ANN, Artificial Neural Network; CIDP, Chronic inflammatory demyelinating polyradiculoneuropathy; DB, Definitely beneficial; GBS, Guillain-Barré syndrome; GO, Graves ophthalmopathy; HLA, Human leukocyte antigen; ITP, Immune thrombocytopenic purpura; IVIg, Intravenous immunoglobulin; KD, Kawasaki disease; MG, Myasthenia gravis; MMN, Multifocal motor neuropathy.

Only targets with a strong relationship with at least one disease are shown. Proteins are shown as gene name (Uniprot ID) and related functional group.

score) relationship for all diseases (except for MMN) and could be involved in the treatment’s efficacy. Results in Table 4 also reinforced the observation that the contribution of each B cell-related target differed for each condition. The response to IVIg in GBS, MG, and Graves ophthalmopathy (GO) could not be attributed to individual B cell-related targets (all of them presented ANN scores below 71%), but complement system-related targets seemed to have a potential individual role...
(showing at least 71% ANN score; e.g., C5 for all diseases except GO and ITP, and C4B for all diseases as well, except for CIDP, GO, and KD).

**Discussion**

Our *in silico* systems biology-based approaches, described here, aimed to explore and compare the pathophysiology of autoimmune diseases with different degrees of clinical response to IVIg treatment. They also addressed IVIg’s mechanisms of action on those disorders responding to IVIg treatment. The results obtained allowed us to differentiate the main pathophysiological processes behind each of the IVIg response-based clusters of diseases analyzed and to suggest which IVIg targets could be involved in the successful response to this treatment.

The classification chosen here as the basis of the study (2) has been repeatedly used in recent publications (91–93), thus, supporting our election and findings. Our results showed that diseases clearly benefiting from IVIg therapy (i.e., belonging to the DB cluster) were mainly characterized by B-cell and complement system-related processes. However, our analyses did not rule out the role of other processes that could be relevant to these diseases. Furthermore, current treatments for the conditions in the DB cluster support the important role of B cell and complement system processes. B cell depletion has been established as a relevant target for autoimmunity disorders for long (94). For example, rituximab is a B-cell depleting monoclonal antibody that, despite not being approved for the treatment of neurological disorders, has shown a certain degree of clinical improvement in many diseases included in our DB cluster (i.e., CIDP, MMN, GO, MG) (95, 96). Similarly, eculizumab is a monoclonal antibody that inhibits terminal complement activation by binding to C5 (90); it is approved to treat MG (97) and has also been successfully used in patients with MMN (96). These examples would support the key role of B-cell and/or complement system processes in these conditions, hence, in agreement with our findings.

On the contrary, our results indicated that a minor response to IVIg treatment was likely in pathologies in which B cells were not predominantly involved. It is clear that T cell populations play a critical role in mediating autoimmunity via T cell inflammatory cytokine secretion, their help to B cells via cytokines thus driving the naïve B cells to become autoantibody secreting plasma cells. Interestingly, literature evidence indicates that IVIg modulates T cell-related processes (65, 67) and innate immunity/inflammation (1, 98). Although our results do not support that pathological alterations in these processes determine the level of response to IVIg in a specific disease, they however endorse a role for T cell- and inflammation-related IVIg targets in its therapeutic effect for some of the evaluated diseases.

When evaluating which IVIg targets might have a direct role over B cell and complement system processes through ANN analyses, we found a relevant role for the complement proteins C3, C4B, and C5 in IVIg mechanisms. In fact, it has been described that IVIg directly neutralizes these proteins through its F(ab’), region (44), and through the formation of complexes between IVIg and complement components (99). This neutralization mechanism would explain the impact on complement activation in several of the analyzed diseases, which in turn prevents complement-mediated tissue damage (100). Evidence supports the relevance of complement activation inhibition by IVIg in the therapeutic efficacy in autoimmune dermatological conditions, reinforce the relevance of this mechanism within IVIg therapeutic effects (101). In contrast, no common B cell-related targets were found, probably due to the great complexity of B cell pathological role in autoimmune conditions (e.g., autoantibodies production, cytokines release, autoantigens presentation to T cells) (102). Previous reports have proven that IVIg is able to modulate B-cell function and survival (103, 104). In agreement with these facts, our models showed that modulation of B-cell pathways by IVIg might involve different targets for each disease. These results are also in line with previous findings reporting that IVIg contains antibodies against a proliferation-inducing ligand and, more importantly, against B-cell activating factor involved in B-cell survival and with documented deleterious effects in B-cell mediated autoimmune diseases (36, 37). Furthermore, the survival and function of B cells can be significantly impacted by the anti-idiotypic antibodies present in IVIg (103, 105). Also, it has been reported that the anti-CD5 antibodies found in IVIg can inhibit the release of autoantibodies produced by B cells (106).

While not directly addressed in the ANN analysis (which focused on protein targets independently of the cell where they are expressed), evidence points to the role of different cell types in IVIg-mediated immunomodulation. In this sense, DCs can regulate immune responses through interaction with T and B lymphocytes and have been demonstrated to be targets of the immunomodulatory effects of IVIg (107, 108). This has been observed particularly through the inhibition of CD80 and CD86 expression (69) and by promoting a more tolerogenic phenotype (109) that is less competent in driving lymphocyte proliferation (65, 67). Furthermore, IVIg has been found to modulate DCs functions through both FcγR- and non-Fc-receptor-mediated signaling events (110). In contrast, even though DCs regulate B cell function, the effects of IVIg over DCs do not seem to affect B cells directly (111). IVIg has been reported to affect directly and indirectly Th17 and Treg function (69, 79, 112–115), and reciprocally regulate them. Treg function is central in the maintenance of immune tolerance and has been found to be reduced in patients suffering from autoimmune diseases (116). IVIg treatment has been shown to promote development or activation of Treg through diverse mechanisms (113, 117). For
example, interaction of IVIg with DCs through binding of sialylated IVIg to C-type lectin receptors that induces inhibitory FcRRIb expression on DC (56). This renders the DC tolerogenic and leads to a reduced costimulatory molecule expression and proinflammatory cytokine secretion (118), thus favoring Treg function. However, whether Fc-sialylation is critical for IVIG-mediated Treg cell expansion in humans is still unclear (117). It has also been shown that, upon IVIg treatment, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin) signaling is able to expand Treg through prostaglandin E2 secretion in DCs (119, 120). In addition, IVIg can also modulate the production of proinflammatory cytokines by DC, which may play a role in maintaining T-cell tolerance (69). De Groot and coworkers (121) described a DC-dependent mechanism where promiscuous IgG-derived T-cell epitope peptides (Tregitopes) increases pro-tolerogenic cytokine production (IL-10) and converts naïve conventional T cells to T regulatory cells (122). Treg would then inhibit effector Th1, Th2 and Th17 cellular activity in inflammatory microenvironments and secrete anti-inflammatory cytokines (112, 123–125). Overall, the presence of Tregitopes might in part explain the success of IVIg therapy in treating autoimmune diseases (122, 126). IVIg treatment has been reported to affect natural killer cells and subsequently regulate Treg function in KD patients (127). IVIg has been shown to affect T cells chemokine production, as observed by Pigard and colleagues, thus affecting their function and compartmentalization (128). Our results position the modulation of B cells as an outstanding IVIg therapeutic mechanism. In fact, aside from antibody producers, B cells also act as APC and cytokine producing cells (129). This role of B cells as APC in regulating T cell equilibrium, Treg induction and establishment of tolerogenesis must have contributed to these findings (130–132).

Basophils have also been shown to be activated when treated with IVIg in mouse models and human studies (133–136) through indirect mechanisms that our approach was not able to discern. Notwithstanding their importance, the indirect nature of the effect and the uncertainty of the direct molecular target of IVIg over these cell types might have resulted in overlooking these mechanisms in our ANN analysis. However, these mechanisms were not highlighted either as pathologic drivers characterizing the diseases within the DB disease cluster.

In the last decade, research and development have surged on novel molecules as potential therapeutic alternatives to IVIg, aiming to overcome IVIg’s therapeutic limitations (e.g., dependence on the supply of human plasma and the large doses required) (137). These promising new therapies belong to the group of next-generation Fc receptor-targeting biologics (138): (i) Recombinant fragment crystallizable (rFc) multimers, designed to have multiple, organized, and structured IgG-Fc moieties; (ii) Neonatal Fc receptor (FcRn)-targeting therapeutics; and (iii) Fc/FcγR targeting (FcγR)-targeting therapeutics (137). Importantly, FcRn-targeting compounds should be highlighted since our results did not suggest a major relevance of this IVIg target to achieve a response. The FcRn blocker efgartigimod is currently the compound furthest advanced in clinical trials (139–142), with some of the anti-FcRn monoclonals following not far behind (143, 144), while a modified monomeric recombinant Fc optimized for binding to all FcRs and FcγRs is also under development (145). This search for novel molecules specifically targeting Fc receptors contrasts with the results obtained in our study, which suggest that the ‘non-specificity’ of IVIg and its interaction with a broad spectrum of targets could be key to obtaining a pleiotropic effect and clinical efficacy in very different diseases. Regarding Fc multimers, the activity of these molecules relies not only on the interaction with Fc receptors but also on targeting complement proteins (146). The comparison of the activity of these new drugs with IVIg would be interesting since some of the direct targets of IVIg herein analyzed interact with IgG’s Fab region, such as CD209/DC-SIGN, C3a, and C5a anaphylotoxins (proteolytic degradation products of C3 and C5) (147, 148).

Our study was limited by the intrinsic constraints of systems biology–based modeling approaches, which include issues on information availability about drugs and diseases. First, our results were influenced by the list of diseases included in the study and the IVIg response cluster assigned to each of them. We selected only autoimmune and neuroautoimmune conditions with the strongest clinical evidence (i.e., tested in clinical trials) for their further classification, including only those tested in clinical trials. Thus, some current ‘off-label’ uses of IVIg were not considered here, as is the case of blistering autoimmune diseases (e.g., systemic lupus erythematosus, pemphigus or pemphigoid diseases). IVIg treatment has been shown to benefit patients suffering from these diseases (149–151), and is usually used in a second- or third-line setting for these conditions (77). However, the level of evidence obtained to date, according to the criteria set in the current study, prevented the inclusion of these diseases in the analysis. Of note, many of these conditions are rare and, even if clinical trials had been conducted, the small sample size could have considered a drawback. This could have precluded their clear classification, thus finishing in the MPB or PB clusters. Consequently, future clinical evidence regarding IVIg use could modify our classification, increasing the pool of available indications in the top and bottom clusters (i.e., DB and UPB). Second, the characterization of the studied diseases may have been incomplete since many of them were rare or complex diseases in which only a few research teams are actively working at the molecular level, potentially leading to biased literature (we found less than 25 effector proteins for several characterized diseases, for instance: Stiff-person syndrome, birdshot
retinochoroidopathy, postpolio syndrome, and Felty’s syndrome, see Table 1). This limitation might have affected the characterization and analyses at the protein level, perhaps with a weaker impact on the definition of the pathophysiological processes. Finally, although we reviewed all available information at the time of the study for identification of direct IVIg targets, including FcγR and non-receptor protein targets, the extreme complexity of IVIg, due to its multi-target feature at the molecular and cellular level, could have led us to underestimate the potential impact of IVIg by including an incomplete list of targets because of a dearth of evidence. Indeed, a recent study by Pipi et al. (2021) (101) unraveled potential non-receptor-mediated antioxidative mechanism for high-dose IgG, as neutrophil elastase substrate and through ROS scavenging, potentially involved in the treatment of skin autoimmunity; however, for the latter they did not detail the mechanism nor specified the direct IVIg protein target, which was a requirement for our approach. Also, considering only direct IVIg targets might have oversimplified the model, hindering the detection of effects over immune components indirectly affected by IVIg; and categorizing the identified targets in discrete function compartments, which might have oversimplified the IVIg mechanisms explored in the ANN analysis on overall processes.

However, using systems biology–based modeling approaches minimizes the impact of the potential biases intrinsic to the availability of information. These models compile and reinterpret available biological data to generate new knowledge and hypotheses while reproducing known aspects of the diseases or drugs. Our models were built considering the whole human protein network and a wide range of drug-pathology relationships, not only limited to the studied diseases or inflammatory conditions, presenting cross-validation accuracies above 80%.

**Conclusion**

Systems biology approaches combined with machine learning are becoming increasingly important for identifying new drug effects and disease mechanisms. Using these techniques, we dedicated our work to compile all available information in order to gain a better understanding of IVIg mode of action. In our analyses, diseases clearly benefiting from IVIg treatment (i.e., the DB cluster) were found to be mainly characterized by deregulated processes in B cells and the complement system. In addition, IVIg targets related to B-cell and complement system pathways seemed to be involved in the clinical response. However, targets related to other immune processes may also play an important role in the IVIg response, supporting its wide range of action through several mechanisms. Besides, although B-cell responses and complement system have a key role in diseases benefiting from IVIg, protein targets involved in such processes are not necessarily the same in those diseases. Our results support further investigations on the role of IVIg in diseases where B cells and the complement system are relevantly involved and for which no evidence has been gathered yet or is inconclusive, for instance, those classified here as PB and MPB. Finally, since the level of relationship varied between proteins included in the DB cluster and different diseases, IVIg appeared to have a pleiotropic effect that may involve the collaborative participation of several targets. Indeed, the weight of each target in treatment efficacy may be different for each condition. Therefore, IVIg’s broad spectrum of targets and ‘non-specificity’ could be key to its efficacy in very different diseases.

**Data availability statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**Author contributions**

JT, EC-G, SC, and SK have significantly contributed to setting up fundamental questions about IVIg. JT, EC-G, SC, and TS contributed to conceptualize the study. TS and CS-V contributed to study design. EC-G, SC, and TS contributed to supervise and manage the project. CS-V and HB contributed to formal analysis, data curation, and visualization. CS-V contributed to the methodology and investigation. SK contributed to the interpretation of data. CS-V drafted the manuscript and the rest of authors contributed to the critical revision of the text. All authors have given their final approval to the version submitted for publication.

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**Conflict of interest**

EC-G, SC, and JT are full time employees at Grifols. CS-V and HB are full time employees at Anaxomics Biotech. TS was a full time-employee at Anaxomics Biotech at the time of the study. SK has received fees for lectures and reviewing research proposals from CSL-Behring and Grifols.
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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.901872/full#supplementary-material

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