Mechanistic Modeling of IgG4-related Disease Informs Disease Pathogenesis and Therapeutic Targets

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ABSTRACT

Objectives

Immunoglobulin G4-related disease (IgG4-RD) is a rare systemic inflammatory condition with limited treatment options. We aimed to develop a mechanistic model of the circulating immune system for IgG4-RD to inform therapeutic targets.

Methods

Peripheral blood was collected from eight IgG4-RD patients and nine controls, and analyzed by flow cytometry, serum cytokine proteomics, and single-cell RNA sequencing. Differential cells, cytokines and cell-cell interactions, along with literature knowledge were integrated to construct a model topology of IgG4-RD and derive ordinary differential equations. Using the maximum likelihood approach, the model was fitted to multi-omic data to infer interaction kinetics, yielding a calibrated model. *In silico* perturbations were then performed by varying the model parameters to identify immune components and interactions with the greatest impact on reversing disease hallmarks, potentially serving as therapeutic targets.

Results

Multi-omic measurements revealed characteristic changes of IgG4-RD, including elevated plasma cells, reduced naïve $CD4^+$ T cells, and enhanced expression and signaling of IL-4, IL-6, and TGF-β. Fitting our IgG4-RD disease model to the multi-omic measurements, we obtained 15 distinct parameter sets, each representing a possible set of interaction kinetics under healthy homeostasis and IgG4-RD. *In silico* perturbations indicated that therapies targeting B cells, IL-4, and IFN-γ could ameliorate the disease. Furthermore, our model supports potential therapeutic targeting of disease-associated genes discovered in other studies, as exemplified by KZF1 and UBR4.

Conclusions

Our mechanistic model of IgG4-RD highlights essential immune components for disease pathology and provides a quantitative framework to systematically assess the effects of potential therapeutic targets.

KEYWORDS

Immunoglobulin G4-related disease, IgG4-RD; multi-omics; mechanistic modeling; in silico perturbation; target identification

ABBREVIATION

Act CD4	active $CD4^+$ T cells
Act B	active B cells
BIC	Bayesian Information Criteria
CBC	complete blood count
CD16NKs	$CD16^+$ natural killer cells
CM	central memory
CRP	C-reactive protein
CTLs	$CD4^+$ cytotoxic T lymphocytes
DCs	dendritic cells
e-GFR	estimated glomerular filtration rate
EM	Effector memory
ESR	erythrocyte sedimentation rate
FYN	FYN Proto-Oncogene, Src Family Tyrosine Kinase
GM-CSF	granulocyte-macrophage colony-stimulating factor
HC	healthy controls
HSPC	hematopoietic stem progenitor cells.
IFN-γ	interferon gamma
Ig	serum immunoglobulin
IgE	immunoglobulin E
IgG4	immunoglobulin G4
IgG4-RD	immunoglobulin G4-related disease
IKAROS	effector memory
IL	interleukin
iT_{reg}	induced regulatory T cells
mDC	myeloid dendritic cells
mono	monocyte
MSD	Meso Scale Discovery
naïve NKs	naïve natural killer cells
naïve CD4	naïve $CD4^+$ T cells
nDC	naïve dendritic cells
nT_{reg}	natural regulatory T cells
NK	natural killer cells
NKT	natural killer T cells
ODE	ordinary differential equation
PBMC	peripheral blood mononuclear cells
PCA	principal component analysis

pDC	plasmacytoid dendritic cells
scRNA-seq	single-cell RNA sequencing
TCR	T Cell Receptor
TD-Plasma cell	T-dependent plasma cells
TEM	$CD8^+$ effector memory T cells
T_{FH}	T follicular helper
TGF-β	transforming growth factor beta
T_H2	T helper 2 cells
TI-Plasma cell	T-independent plasma cells
TRDV1	T cell receptor delta variable 1
TRDV2	T cell receptor delta variable 2
T_{reg}	regulatory T cells
TWEAK	TNF-related weak inducer of apoptosis
UBR4	E3 ubiquitin-protein ligase
UMAP	uniform manifold approximation and projection
VEGI	vascular endothelial growth inhibitor

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INTRODUCTION

Immunoglobulin G4-related disease (IgG4-RD) is a recently recognized rare systemic fibroinflammatory condition with an estimated prevalence rate of 0.78-1.39 per 100,000 person-years [1][2][3]. The afflicted patients exhibit elevated serum IgG4 concentrations [4] and the affected organs often display lymphoplasmacytic infiltrates, storiform fibrosis, obliterative phlebitis, and mild to moderate eosinophilia, which can lead to highly destructive conditions [5]. Its diagnosis requires combining histopathological analysis via biopsy, blood tests to assess IgG4 antibodies, and exclusion of other conditions.

The hallmarks of IgG4-RD include plasma cells, $CD4^+$ cytotoxic T lymphocytes (CTLs), T follicular helper (T_{FH}) cells, regulatory T cells (T_{reg}), M2 macrophages, upregulation of Th2 cytokines, as well as elevated serum levels of IgG4 and IgE [5-8]. Current treatments for IgG4-RD rely on steroids to suppress immune responses, which often lead to side effects and relapses [9]. The discovery that B cells play a central role in IgG4-RD has encouraged monoclonal antibody trials to target B cell surface proteins, such as rituximab for CD20 and inebilizumab for CD19 [10-13]. Recently, inebilizumab became the first approved drug for IgG4-RD by the U.S. Food and Drug Administration.

Numerous recent studies utilized single-cell RNA sequencing (scRNA-seq) technologies to investigate IgG4-RD, confirming a significant increase in IgG4-secreting plasma cells [14-18]. In the tissue lesions, specific lymphocyte subsets were discovered, such as TOP2A-expressing T and B cells; furthermore, communications between myeloid and lymphoid cells were enhanced [15]. While these studies unveiled disease features, key questions remain, such as what additional cell types are involved and how cells and cytokines interact to drive disease progression. Understanding the disease mechanism is essential for developing targeted therapies, a crucial step towards improved patient outcomes.

Mechanistic modeling refers to the construction of mathematical representations of biological systems grounded in underlying biological processes. These models facilitate dynamic simulation of pathological processes, enabling prediction of disease progression, identification of key drivers, and rational design of therapeutic interventions [19]. For example, mechanistic modeling has been instrumental in predicting treatment response and optimizing therapeutic interventions for rheumatoid arthritis and inflammatory bowel disease [20, 21]. Here, we employ multi-omic measurements, disease biology, and ordinary differential equation (ODE)-based modeling techniques to develop a mechanistic model of IgG4-RD (Figure 1). Our model delineates a disease network that captures complex interactions among essential immune cell populations, cytokines, and cell-cell communications. It recapitulates the

effects of existing therapies, such as rituximab and inebilizumab, and provides a quantitative framework for discovering novel targets with therapeutic potential.

METHODS

Study ethics

This study was performed in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee at Peking University International Hospital (ethics approval number: 2023-KY-0035-02). All patients provided written informed consent.

Patient recruitment and sample collection

Eight treatment-naïve IgG4-RD patients (4 males, 4 females, aged 39-72 years) were enrolled from Peking University International Hospital, Peking University People's Hospital, and Peking Union Medical College Hospital. They were surveyed for medical history, physical examination, as well as imaging, laboratory, and pathological evaluations (Table 1, Supplementary Table 1). Nine healthy individuals (3 males, 7 females, aged 51-62 years) were also recruited. Peripheral blood was collected for further measurements (Supplementary Table 2).

Immune profiling by flow cytometry

Eleven immune cell populations were measured by flow cytometry (Beckman DxFLEX instrument), namely granulocytes, monocytes, dendritic cells (DCs), B cells, natural killer cells (NKs), natural killer T cells (NKTs), and the following T cell subsets, $CD4^+$ T cells, $CD8^+$ T cells, regulatory T cells (T_{reg}), naïve $CD8^+$ T cells, and naïve $CD4^+$ T cells. The antibody markers are provided in Supplementary Table 3, and gating strategies are displayed in Supplementary Figure 1. Software Flowjo (V.10.8.1) was used for analysis. Concurrently, the total count of white blood cells (number/ml) in the peripheral blood was measured by Dymind 5-Part Auto Hematology Analyzer DH56CRP.

Cytokine profiling by MSD proteomics

A total of 40 serum cytokines were quantified by Meso Scale Discovery (MSD) enzyme-linked immunoassays, according to manufacturer's guidelines (MSD SQ120MM instrument). The premixed antibodies listed in Supplementary Table 4. The plates were immediately analyzed on an. The MSD Discovery Workbench software was used to establish a standard curve and conduct data analysis. Non-detectable values were imputed by 0.1*lower limit of detection (LLOD).

Single-cell RNA sequencing and quality control

scRNA-seq was performed on peripheral blood mononuclear cells (PBMCs) derived from the IgG4-RD patients. Briefly, 5 ml of peripheral blood was drawn and PBMCs were collected. After determining their concentration and viability, cells were loaded onto the 10X Genomics Chromium for on-machine and library construction. Sequencing was performed on the Illumina NovaSeq X Plus platform. Raw sequences were processed using the Cell Ranger software (V.6.0.2, 10x Genomics) and aligned to the reference genome GRCh38-2020-A. For healthy controls, single-cell transcriptome of PBMCs derived from healthy individuals with matching age, sex, and ethnicity as the case group were obtained from public datasets, with the GEO accession numbers listed in Supplementary Table 5.

Cells detected with < 200 genes, and genes expressed in < 3 cells were filtered out. For each sample, cells with > 10% transcripts mapping to the mitochondrial genes, or cells with a unique feature count distributed in < 2.5% or > 97.5% in all cell populations were removed. All mitochondrial genes were removed. Finally, 18,884 genes expressed in both the patient group and the healthy control group were preserved.

Clustering and annotation of single cells

The single-cell transcriptome from all samples were merged in Seurat [22] (V.4.1.1) into a combined dataset. Log-normalization was applied to the expression values. The top 2,000 highly variable genes were identified, scaled and centered, followed by dimensionality reduction via principal component analysis (PCA). Harmony [23] package (V.0.1.0) was then applied to the PCA embeddings for batch correction. The top 30 Harmony-corrected principal components were selected for cell clustering using Seurat's shared nearest neighbor (SNN) graph construction and the Louvain algorithm. For cell type annotation, reference labels from a publicly available PBMC single-cell transcriptome dataset [24] were transferred using Seurat's FindTransferAnchors and TransferData functions. Annotation results were validated with the automatic annotation tool SingleR (V.1.8.1) and cross-checked with canonical marker genes.

Detection of cell-cell communications

CellChat [23] (V.1.5.0) was used to assess the global communications among cells and quantify the intercellular communication networks. The normalized expression matrix was loaded into CellChat. The CellchatDB.human reference database was used for setting the secreted signaling pathways.

Construction of the disease model topology

The topology of the IgG4-RD disease model was informed by essential cell types, cytokines, and inter-cellular interactions detected by our multi-omic measurements. Additionally, critical cell-cell and cell-cytokine interactions were sourced from the

IgG4-RD literature and the ImmunoGlobe database were incorporated into the model. Based on this topology, an ODE model was developed using the dMod package [24] to describe the interaction dynamics in IgG4-RD. Inter-cellular interactions were modeled by mass action kinetics and Hill kinetics [25]. Mass action kinetics described processes such as cellular differentiation and cytokine production, while the Hill kinetics characterized the activating or inhibiting roles of cytokines in cellular interactions. Detailed mathematical formulas are provided in the Supplementary Note.

Estimation of model parameters

Model parameters were estimated by fitting the ODE model to the multi-omic data obtained from both patients and healthy controls. A maximum-likelihood approach implemented through a multi-start gradient-based optimization algorithm in the dMod package was employed. Specifically, cell abundances and cytokine concentrations simulated by the model were fitted to the population counts and ratios obtained from flow cytometry and scRNA-seq, as well as cytokine levels measured by MSD proteomics.

The multi-omic measurements from the patients were assumed to represent the disease steady state, whereas those from healthy controls reflected the healthy steady state. The model structure and parameters were held constant across both states, except for specific interactions listed in Supplementary Table 6, which, as informed by disease biology, represented alterations in immune interactions associated with IgG4-RD. A key distinction between the two states was the recognition of a self-antigen, which reflects the autoimmune nature of IgG4-RD. Furthermore, the healthy state was treated as the initial condition for the disease model.

Given the high number of model parameters relative to the multi-omic data, uncertainty in the estimated parameter values was anticipated. To address this, an ensemble approach was employed to infer multiple parameter sets that provided an acceptable fit to the multi-omic data. Using a threshold of 10 in Bayesian Information Criteria (BIC) differences, 15 unique parameter sets were accepted for the ODE model, resulting in 15 instances of the calibrated model.

Sensitivity analysis

The sensitivity analysis was performed by systematically altering each model parameter across all 15 parameter sets with several orders of magnitude. For each hallmark, the parameters associated with largest changes were identified, indicating the impactful cells and cytokines as potential therapeutic targets.

***In silico* perturbation**

In silico perturbation was performed on the calibrated disease model. Model parameters corresponding to each target were altered by several magnitudes of fold-changes (with log10 fold-change ranging from -1.1 to 9) to ensure robustness. Changes in disease hallmarks, in response to various target perturbations, were quantified as the relative post-treatment variation from baseline disease-state levels.

Statistical analysis

Mann Whitney U test was used to compare quantitative measurements in the patients against the healthy controls, e.g., cell populations derived from flow cytometry or cytokine levels derived from MSD proteomics. $P < 0.05$ was considered statistically significant. Data were analyzed with GraphPad Prism 10.0.2.

Data availability statement

Expression matrix of the scRNA-seq have been uploaded to GSA (GSA ID: PRJCA042846). Other data are available upon reasonable request.

RESULTS

Circulating immune cells in IgG4-RD patients

We utilized flow cytometry to measure populations and states of circulating immune cells in five IgG4-RD patients and nine healthy controls (Supplementary Figure 1, Supplementary Tables 2 & 3). Among the 11 measured immune cell types, modest changes were observed for three cell types in the patients: DCs and naïve $CD4^+$ T cells were decreased, and monocytes were increased (Figure 2A). The reduction of naïve $CD4^+$ T cells confirms the findings of a previous study [26].

Circulating cytokines in IgG4-RD patients

A total of 40 cytokines were measured by MSD proteomics platform in seven IgG4-RD patients and eight healthy donors (Supplementary Tables 2 & 4). In the patients, the concentration of interleukin (IL)-7 was significantly decreased, whereas those for IL-1, IL-4, IL-6, IL-10, IL-12p70, and IL-15 were increased (Figure 2B). Therein, the increase of IL-4 was the most significant (p=0.002). While some of these cytokines (IL-4, IL-6, and IL-10) were known to change in IgG4-RD [27], to our knowledge, others (IL-7, IL-1, IL-12p70 and IL-15) are reported for the first time.

Altered cell types and cell-cell communications in IgG4-RD

We performed scRNA-seq on PBMCs from eight IgG4-RD patients. For comparison, single-cell transcriptome of PBMCs from six healthy controls were obtained from public datasets, matching our IgG4-RD patients on age, sex, and ethnicity [28-30] (Supplementary Table 5). Following stringent batch correction, quality control and

data integration, 24 cell clusters were annotated and compared [31] (Figure 3A-B, Supplementary Figure 2).

The key metrics informed by the single-cell transcriptome for mechanistic modeling were cell proportions and cell-cell communications. Consistent with previous reports, the proportions of $CD16^+$ NK cells and plasma cells were increased in the patients [17, 32] (Figure 3C). Furthermore, naïve $CD4^+$ T cells and TRDV1 γδT cells were reduced, with the former also captured in our flow cytometry measurement. Leveraging these measurements, we computed ratios of several essential cell types, including pDC/mDC, $CD56^+$NK/$CD16^+$NK, memory-B/naïve-B, and plasma-cells/naïve-B (Figure 3D). Meanwhile, a dramatic shift of cell-cell communications was detected under IgG4-RD. Both incoming and outgoing signals were strengthened in $CD16^+$ NK cells, $CD14^+$ monocytes, $CD8^+$ effector memory T cells (T_{EM}), and TRDV2 γδT cells (Figure 3E). IL-4, IL-6, and IFN-γ were enhanced in the patients compared to the controls (Figure 3F), correlating to our MSD proteomic measurements. Unique signaling molecules were identified, such as the disease-specific TWEAK/TNFSF12 signaling in $CD14^+$ monocytes and the healthy control-specific VEGI/TNFSF15 signaling in $CD4^+$ T_{EM} cells (Supplementary Figure 3). Several other immune factors displayed notable changes in IgG4-RD, e.g., galectin that mediates both innate and adaptive immune responses [34], annexin that modulates stress and inflammatory responses [35], the C-C motif chemokine ligand (CCL), and IL-2 that regulates $CD4^+$ T cell differentiation, T_{reg} functions, and $CD8^+$ T cell functions [36] (Supplementary Figure 4).

Taken together, the single-cell measurements suggest that under IgG4-RD, $CD16^+$ NK cells were increased in both proportion and inter-cellular signaling; $CD4^+$ naïve cells were decreased; numerous other cell types, including $CD14^+$ monocytes, $CD4^+$ T_{EM}, $CD8^+$ T_{EM}, and γδT cells, were not changed in proportion but their inter-cellular signaling were enhanced; and the hallmark cytokines for inflammation including IL-4, IL-6, and IFN-γ, were increased in both expression and inter-cellular signaling.

Modeling the immune regulation for IgG4-RD

We leveraged these multi-omic measurements, along with established IgG4-RD pathophysiology and the canonical immunological reactions, to construct a disease model for IgG4-RD (Methods). Our model is a complex network comprised over 30 essential immune cells and cytokines, as well as more than 100 parameters modeled by ODE to describe the interaction kinetics (Figure 4, Supplementary Note). The key immune cells include **NK cells** ($CD56^+$ NK and $CD16^+$ NK), **DC cells** (naïve DC, myeloid DC and plasmacytoid DC), **B cells** (naïve and active B, T cell-dependent plasma and T cell-independent plasma), and **T cells** (naïve $CD4^+$ T, active $CD4^+$ T, natural T_{reg}, induced T_{reg}, cytotoxic $CD4^+$ T, T_{FH}, and T_H2 cells). Essential cytokines include **interleukins** (IL-1,

IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-33), **TGF-β**, **IFN-γ**, and **GM-CSF**. Notably, the ODE representations were fit with our multi-osmic measurements to derive calibrated model parameters. After assessing thousands of parameter sets, 15 top-performing parameter sets that best aligned with multi-omic measurements were identified, forming 15 calibrated instances of the disease model for IgG4-RD (Supplementary Figure 5, Supplementary Table 7). Essentially, these model instances depict how self-antigen triggers a cascade of outcomes in IgG4-RD, including activation, proliferation and differentiation of immune cells, as well as secretion of cytokines, resulting in a shift of the immune system away from homeostasis.

Biological insights derived from the mechanistic model

We verified that all 15 instances of the model recapitulated the measured changes in cells and cytokines (Figure 5A). Furthermore, for cell types detected by scRNA-seq in percentages, the model was applied to infer their population counts. As such, $CD16^+$ NK cells, myeloid or plasmacytoid DCs, $CD4^+$ CTLs, T-dependent plasma B cells were found elevated in the diseased state (Supplementary Figure 6), in line with various other reports [32, 39].

Next, we investigated the changes in interaction kinetics, which were arguably more subtle compared to the cell type changes. Some processes were inferred as differentially regulated in IgG4-RD, e.g., increased production of IL-4 and GMC-SF by T_H2 cells, enhanced differentiation of active $CD4^+$ T cells into $CD4^+$ CTLs, as well as heightened activation of naïve B cells. Conversely, some processes appeared as unchanged in IgG4-RD, including IL-2 production by active $CD4^+$ T cells and the differentiation of these cells into T_{FH} cells. These results suggest specific mechanistic differences in immune regulation in IgG4-RD (Figure 5B).

Therapeutic targets informed by the IgG4-RD mechanistic model

The quantitative nature of our disease model facilitates discovery of potential therapeutic targets through *in silico* perturbation experiments. To that end, we performed a sensitivity analysis to systematically interrogate the impact of each model parameter on IgG4-RD hallmarks, aiming to pinpoint those capable of reversing the hallmarks from the diseased state to the healthy state (Supplementary Figures 8-16). Each parameter was changed by several magnitudes of changes to ensure robustness of the results. As such, three most effective cytokines were identified: IL-4, IL-6, and IFN-γ. In addition, motivated by monoclonal antibody therapies targeting B cells, we assessed the effects of depleting $CD20^+$ B cells (including naïve and active B cells) by rituximab [40] and depleting $CD19^+$ B cells (including all B cells and plasma cells) by inebilizumab [13].

The effects of perturbing these five selected targets on disease hallmarks were assessed (Figure 6A, Supplementary Figures 18, 22). Four of the interventions, namely depleting B cells by both rituximab and inebilizumab, reducing IL-4, and increasing IFN-γ, would bring down plasma cells and consequently reduce the IgG4 production. Meanwhile, anti-IL-6 treatment did not seem to offer this benefit. We further scrutinized IL-6 related processes, and found that IL-6-induced T_{FH} differentiation impacted T_{FH}, T_{reg}, and plasma cells, all of which are essential cell types for IgG4-RD (Supplementary Figure 20,21). Since regulating cellular differentiation is inherently more complex than depleting specific cells or cytokines, IL-6 unlikely represents a viable therapeutic target. Taken together, therapies for B-cell depletion, anti-IL-4, and activating IFN-γ harbor the potential to correct for immune dysregulation in IgG4-RD.

Lastly, we assessed whether disease markers not directly included in our model could be evaluated. Liu et al. proposed IKAROS and UBR4 as drivers of autoimmunity in IgG4-RD [41]. They discovered that genomic variants IKAROS-p.(Arg183His) and UBR4-p.(Cys4179Ter) led to hyperactivation of the Src kinase FYN, which in turn augmented T Cell Receptor (TCR) signaling and T_H2 polarization in IgG4-RD. As such, restoring these hyperactive pathways could ameliorate the disease. To evaluate their translational potential, we simulated the effects of weakened TCR signaling through reducing the activation rate of $CD4^+$ T cells or depleting them. We also simulated the effects of weakened T_H2 polarization through reducing the differentiation rate of T_H2 cells or increasing their death rate. Our results suggested that these two approaches affected the disease hallmarks differently (Figure 6B). Attenuating T_H2 polarization primarily reduced T_H2, T_{reg}, plasma cells, and IL-4. Suppressing TCR signaling reduced T_{reg}, $CD4^+$ T cells, IL-2, TGF-β, IFN-γ, IL-6 and IL-10, while paradoxically increasing plasma cells and IL-4 levels. Overall, our simulations demonstrated the potential of targeting T_H2 polarization for treating IgG4-RD, and a mixed response from suppressing TCR signaling.

DISCUSSION

In this study we comprehensively characterized the circulating immune landscape of IgG4-RD through multi-omic profiling (flow cytometry, proteomics, and single-cell transcriptomics), revealing essential cellular and molecular signatures that defined the disease pathophysiology. By integrating these datasets with established IgG4-RD mechanisms, we constructed a mechanistic model that unifies individual discoveries into a cohesive framework, revealing feedforward and feedback loops that sustain immune dysregulation in IgG4-RD.

A notable finding was the significant reduction of IL-7 in the IgG4-RD patients, a cytokine critical for naïve T-cell homeostasis [42]. Indeed, the circulating naïve $CD4^+$ T cells were reduced in our patients. Reduced IL-7 likely contributes to impaired adaptive immune regulation, creating a permissive environment for pathogenic B-cell responses, leading to expansion of antibody-secreting plasma cells and elevation of IgG4 levels. Notably, our data also revealed hyperactivation of innate immunity, exemplified by increased $CD16^+$ NK cells and monocytes, alongside enhanced intercellular communications in these populations. The rise in NK cells paralleled elevated IFN-γ expression, suggesting heightened tissue inflammation. With these pathophysiological features, we observed a synergistic cytokine milieu: while pro-inflammatory cytokines (IL-1, IL-6, IL-12, IL-15) promoted chronic inflammation, anti-inflammatory cytokines (IL-4, IL-10) were upregulated for counterbalance. Paradoxically, the increase of IL-4 and IL-10 can exacerbate the disease by promoting B-cell differentiation [43] and fibrosis [44] under failed compensatory response. Besides, we also detected reduced γδT cells, a feature shared with other autoimmune diseases such as Rheumatoid Arthritis and Systemic Lupus Erythematosus [45], suggesting enhanced inflammation due to the loss of regulatory function. Collectively, these findings depict a coordinated breakdown of immune homeostasis in IgG4-RD characteristic of chronic inflammation, autoimmunity, and fibrosis.

Importantly, we leveraged ODEs and model fitting to obtain a quantitative model that captured immune interaction kinetics in IgG4-RD. This quantitative framework allows *in silico* perturbation to pinpoint most critical disease factors and predict intervention effects. Our simulations proposed that anti-IL-4 treatment, increasing IFN-γ, or depleting B cells could potentially reverse IgG4-RD. Notably, all three strategies would reduce naïve T_{reg} cells and plasma cells. Furthermore, intricate regulation lies in each strategy: neutralizing IL-4 suppresses pathogenic T_H2 response, enhancing IFN-γ restores T_H1 anti-fibrotic balance [46], and depleting B cells eliminates autoreactive plasma cells. Thus, each strategy targets a different aspect of the immune dysregulation, which all lead to reduced IgG4 production.

It is noteworthy that our model is generalizable to evaluate potential targets not directly included in our network. We simulated the impact of two recently identified driver genes (IKAROS and UBR4) for IgG4-RD [41] by disrupting the immune pathways they regulate. Although these interventions shifted several disease hallmarks toward a healthy state, our *in silico* perturbations highlighted additional cell types and cytokines that would require complementary approaches to target. Thus, our model can inform the rational design of combination therapies, such as multi-target “cocktail” regiments, to address the complexity of IgG4RD pathogenies.

As a rare fibro-inflammatory disease, IgG4-RD suffers from limited treatment options. With the recent approval of inebilizumab and other promising clinical trials [47], the therapeutic landscape is rapidly evolving. By integrating pharmacodynamic data from clinical trials, our disease modeling framework can further evolve into quantitative systems pharmacology modeling, which is hopeful for establishing robust, biology-informed relationships between drug exposures and clinical responses, improving success rates of late-stage drug development for IgG4-RD.

We acknowledge several limitations of this study. First, the modest sample size, though characteristic of rare disease research, may limit the generalizability of our findings. Second, as our cohort was comprised of Chinese patients, validation in diverse ethnic populations will be highly valuable. Third, our mechanistic model of IgG4-RD is a simplified representation of the disease biology. For example, circulating IL-15 was increased in our patients, and although anti-IL-15 trials are being tested for other auto-immune diseases [49], we did not explicitly model it in IgG4-RD due to limited information. Inclusion of additional cell types and disease markers in the future would allow for more comprehensive examinations. Lastly and importantly, therapeutic targets predicted by our *in silico* analyses require rigorous wet-lab experiments and clinical validation to establish their biological and therapeutic relevance. Future studies incorporating larger, multi-ethnic cohorts and experimental validation will further refine our model and accelerate the development of targeted therapies for this complex disorder.

ACKNOWLEDGEMENT

We thank members of the Intelligent Computing in Biomedicine Lab for insightful discussions. We thank the patients who donated their samples for this study.

CONTRIBUTIONS

HG, SJ, RBC, AK, and CP designed the study and drafted the manuscript. HG, HY, YF, SL and LZ assembled the study cohort, consented the patients, and collected patient information and blood samples. ZL[3] and RBC performed flow cytometry, MSD proteomics, and single-cell RNA sequencing. AK, MR, MG, AB and AZ[2] performed disease modelling and *in silico* perturbations. SJ, ZY, AZ[3], ZL[3] and CP performed bioinformatic analysis and statistical analysis. ZL[4,5], WZ, UW, AL and AZ[2] queried literature and curated information. All authors contributed to result interpretation and discussions. CP and HG are guarantors of this work.

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FUNDING

This work was supported by the Capital's Funds for Health Improvement and Research [CFH 2024-2-8022].

COMPETING INTERESTS

Simon Junankar, Marcus Rosenblatt, Aidin Biibosunov, Milad Ghomlaghi, Anqi Zhang, and Atefeh Kazeroonian are employed by Differentia Bio. Yan Ge, Zhou Lin, Ziyue Yan, Ava Zhao, and Robin B Chan were employed by AliveX Biotech. The remaining authors declare no competing interests.

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