

Fc N-glycosylation heterogeneity of anti-MDA5 IgG is associated with interstitial lung disease severity via regulating FcγR/C1q signaling in MDA5+ dermatomyositis—a cross-sectional study

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Abstract

Background

To characterize Fc N-glycosylation patterns of disease-specific IgG (DSIgG) in anti-MDA5-positive dermatomyositis (MDA5 + DM) and evaluate their associations with interstitial lung disease (ILD) severity.

Methods

Eighty-four patients with MDA5 + DM and 60 healthy controls were enrolled. Disease-specific anti-MDA5 IgG glycopeptides were analyzed by UPLC-MS/MS. Associations between glycoform features and ILD severity were assessed using correlation analysis, ROC analysis, and logistic regression. Surface plasmon resonance and in vitro functional assays were used to compare glycoform-dependent engagement of FcγRIIa, FcγRIIIa, and C1q, as well as downstream ADCP, ADCC, CDC, and endothelial inflammatory responses.

Results

Compared with controls, DSIgG1 from patients with MDA5 + DM showed higher fucosylation and lower sialylation. Severe ILD was associated with increased DSIgG1F and decreased DSIgG1S. In multivariable analysis, lower DSIgG1S remained independently associated with severe ILD (OR = 0.07, P = 0.0065), and DSIgG1S showed strong discriminatory performance for severe ILD (AUC = 0.9133). Functionally, G1S exhibited stronger FcγRIIa-related binding and phagocytic activity, whereas G1F displayed stronger FcγRIIIa and C1q binding, together with increased ADCC and CDC. In endothelial-cell experiments, the two glycoforms induced distinct inflammatory response patterns.

Conclusion

Anti-MDA5 DSIgG Fc glycosylation is associated with ILD severity in MDA5 + DM. Distinct glycoforms exhibit differential FcγR/C1q-related effector profiles, supporting a potential role for Fc glycosylation heterogeneity in disease stratification and pathogenesis.

1 Background

MDA5-positive dermatomyositis (MDA5 + DM) is a clinically distinctive subset of idiopathic inflammatory myopathy characterized by cutaneous manifestations and a high risk of interstitial lung disease (ILD), particularly rapidly progressive ILD (RP-ILD). Despite aggressive immunosuppressive treatment, pulmonary involvement remains a major cause of mortality, with reported mortality rates of 50–70% [1–

^{3]}. Identifying biomarkers that better reflect pathogenic immune activity and discriminate pulmonary severity remains an important unmet need.

Immunoglobulin G (IgG) is the major serum glycoprotein, and its Fc N-glycosylation critically modulates immune effector functions. The composition of Fc glycans influences Fcγ receptor engagement, complement activation, and the balance between pro-inflammatory and anti-inflammatory signaling. Aberrant IgG glycosylation has been reported in several autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus ^[4–7]. However, profiling total serum IgG may dilute disease-relevant changes because it captures both pathogenic and homeostatic antibody pools.

Disease-specific IgG (DSIgG) directed against key autoantigens may provide a more precise window into disease biology. In MDA5 + DM, anti-MDA5 antibodies are closely linked to pulmonary involvement and poor outcome. Our previous work showed that RNA-containing immune complexes formed by anti-MDA5 antibodies can induce plasmacytoid dendritic cell activation and type I interferon production ^[8], supporting a direct pathogenic role for anti-MDA5 humoral immunity.

On this basis, we hypothesized that Fc glycosylation heterogeneity of anti-MDA5 DSIgG may contribute to differences in downstream immune effector function and clinical severity. The present study therefore aimed to characterize the Fc N-glycosylation profile of anti-MDA5 DSIgG, determine its association with ILD severity, and examine how distinct glycoforms differentially engage FcγRIIa, FcγRIIIa, and C1q in vitro.

2 Method

2.1 Study population

A total of 84 serum samples were collected from consecutive patients with anti-MDA5-positive dermatomyositis who attended the Department of Rheumatology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, between January 2021 and December 2024. Sixty age- and sex-matched healthy donors served as controls. Residual serum samples were anonymized and stored at -80°C after collection. The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants. (Ethics Approval Number: 2025-74).

2.2 Definition and Severity Stratification of ILD

MDA5 + DM-associated ILD was defined using clinical and high-resolution computed tomography findings after exclusion of alternative causes such as infection, heart failure, drug-induced lung injury, and other interstitial lung disorders. To reduce treatment-related confounding, clinical data and biological samples were collected within 3 months of onset of respiratory symptoms whenever possible.

ILD severity was stratified according to PaO₂/FiO₂ ratio: severe ILD, ≤ 200 mmHg; moderate ILD, 200–300 mmHg; and mild ILD, > 300 mmHg ^[9].

2.3 Isolation of Total IgG and anti-MDA5 disease-specific IgG

Total IgG was isolated from serum using Protein G magnetic beads (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Anti-MDA5 DSIgG was isolated by immunoprecipitation using recombinant human MDA5 protein coupled to anti-Flag magnetic beads (BeyoMag™, Beyotime Biotechnology). After incubation and washing to remove non-specific binding, bound antibodies were eluted under acidic conditions and immediately neutralized. Protein concentrations were measured using a BCA assay (BeyoMag™, Beyotime Biotechnology).

2.4 Tryptic Digestion and Glycopeptide Enrichment

Purified total IgG and DSIgG were processed using a filter-aided sample preparation workflow. Samples were denatured, reduced, alkylated, and digested with sequencing-grade trypsin. Glycopeptides were enriched using amide-based solid-phase extraction and reconstituted in 0.1% formic acid before LC-MS/MS analysis ^[10, 11].

2.5 UPLC-MS/MS analysis

N-glycopeptide profiling was performed on a Nexera LC-30 UPLC system coupled to an LCMS-8050CL triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). Separation was achieved on a C18 analytical column using a water/acetonitrile gradient containing 0.1% formic acid. Data were acquired in positive-ion multiple reaction monitoring mode, and peak identification was based on retention time and fragment-ion features. Relative abundance was calculated from integrated peak areas. Quality-control samples were analyzed every 14 test samples, and assay precision was assessed by calculating relative standard deviations (RSDs) of representative glycoforms.

2.6 Orthogonal detection of anti-MDA5 IgG glycoforms

A glycoform-directed ELISA was used as an orthogonal assay to evaluate anti-MDA5 IgG glycoform enrichment. Briefly, serum was incubated on MDA5-coated plates, followed by glycoform-directed detection reagents and HRP-based readout at 450 nm.

2.7 Surface Plasmon Resonance (SPR)

SPR analysis was performed on a Biacore T200 instrument (Cytiva, Marlborough, MA, USA) to compare the binding of G1S, G1F, and G1N anti-MDA5 antibody preparations to FcγRIIIa (UniProt: P12318), FcγRIIIa (V158, UniProt: P08637), and C1q (Complement Technology, Tyler, TX, USA). Ligands were immobilized on CM5 sensor chips (Cytiva) by amine coupling. Antibodies were injected at serial concentrations, and kinetic parameters (k_a , k_d , and KD) were fitted using a 1:1 Langmuir binding model ^[12, 13].

2.8 Cell models and functional assays

HEK293T cells overexpressing human MDA5 (Sangon Biotech (Shanghai) Co., Ltd., China) were used as target cells. NK cells were isolated from healthy donors and activated with IL-2 (PeproTech, USA). THP-1

cells (iCell Bioscience Inc, China) were differentiated into macrophage-like cells with PMA (Sigma-Aldrich, USA) and M-CSF (PeproTech, USA). FcγRIIIa-knockout THP-1 cells and FcγRIIIa-knockout NK cells (provided by Jiangsu University, Zhenjiang, China) were used to assess receptor dependence in selected assays. ADCP was quantified by confocal microscopy using fluorescently labeled MDA5-overexpressing target cells and THP-1-derived macrophages. ADCC was measured by LDH release in NK cell-target co-cultures. CDC was assessed in the presence of human complement, with additional C1q-blocking experiments to test pathway specificity.

2.9 Endothelial-cell experiments and signaling analysis

Human pulmonary microvascular endothelial cells (HPMECs) were used to investigate endothelial inflammatory responses. Co-culture systems with macrophages or NK cells were established using Transwell inserts. Western blotting was used to assess p38/JNK (Cell Signaling Technology, 1:1000 dilution) signaling in macrophages, PI3K-Akt/NF-κB (Cell Signaling Technology, 1:1000 dilution) signaling in NK cells, and pyroptosis-related proteins in HPMECs, including p-MLC2, NLRP3, caspase-1, and N-GSDMD (Cell Signaling Technology, 1:1000 dilution). Cytokines in culture supernatants were measured by ELISA (R&D Systems, USA).

2.10 Statistical analysis

Continuous variables were summarized as mean ± SD or median (interquartile range), as appropriate. Two-group comparisons were performed using Student's t test or the Mann-Whitney U test, and three-group comparisons were performed using one-way ANOVA or the Kruskal-Wallis test with appropriate post hoc correction. Categorical variables were compared using the chi-square test or Fisher's exact test. Correlations were evaluated using Pearson or Spearman analysis, as appropriate. ROC curves and AUCs were used to assess discriminatory performance. Univariable logistic regression was performed to screen variables associated with severe ILD; variables with $P < 0.05$ were entered into a multivariable logistic regression model using stepwise selection. For cell experiments, data from at least three independent experiments were analyzed using one-way or two-way ANOVA with Tukey's multiple-comparison test. A two-sided P value < 0.05 was considered statistically significant. Treatment status (treatment-naïve vs. prior therapy) was included as a covariate in multivariable logistic regression models.

3 Results

3.1 Anti-MDA5 DSIgG shows a distinct Fc glycosylation profile in MDA5 + DM

Compared with healthy donors, patients with MDA5 + DM exhibited significantly higher IgG fucosylation (5.53 ± 5.91 vs 0.49 ± 0.42 , $P = 0.0004$) and lower IgG sialylation (4.17 ± 3.27 vs 22.43 ± 3.16 , $P < 0.0001$) (Fig. 1A). Five major DSIgG glycoforms were identified in the anti-MDA5 antibody fraction, including three IgG1-associated forms (G1, G1F, and G1S) and two IgG2-associated forms (G2 and G2S) (Fig. 1B). These

findings support marked remodeling of the Fc glycosylation landscape in disease-specific anti-MDA5 IgG.

3.2 Glycoform features are associated with ILD severity

When patients were stratified according to ILD severity, DSIG1F was significantly higher ($P < 0.0001$) and DSIG1S significantly lower ($P < 0.0019$) in the severe ILD group than in the mild-to-moderate ILD groups (Table 1). Correlation analyses further linked DSIG1F and DSIG1S to pulmonary function indices and inflammatory biomarkers (Fig. 1C and Supplementary Fig. 1).

Table 1
Baseline characteristics of patients with MDA5 + DM combined with ILD of varying severity

Characteristics (n = 84)	Types of ILD			p value
	Severe (n = 29)	Moderate(n = 37)	Mild(n = 18)	
Age, years	49.79 ± 12.59	52.27 ± 8.33	48.94 ± 12.35	0.5648
Male, n (%)	13 (44.83)	13 (35.14)	6 (33.33)	0.7054
ILD course (months)	1.57 ± 0.96	1.70 ± 0.94	1.51 ± 1.32	0.7868
FVC, %	60.70 ± 20.43	78.17 ± 16.57	95.22 ± 19.91	< 0.0001
FEV1, %	63.94 ± 21.13	80.20 ± 14.99	96.69 ± 20.41	< 0.0001
DLCO SB, %	43.72 ± 16.32	59.48 ± 19.46	65.88 ± 17.33	0.0007
PaO ₂ /FiO ₂ (mmHg)	153.37 ± 34.52	251.57 ± 35.74	410.92 ± 38.99	< 0.0001
WBC (×10 ⁹ /L)	6.52 ± 2.59	7.96 ± 5.60	6.73 ± 2.21	0.9145
Lymphocyte (×10 ⁹ /L)	0.79 ± 0.29	0.76 ± 0.45	1.06 ± 0.39	0.6261
CRP (mg/L)	4.76 ± 5.45	7.04 ± 9.36	2.57 ± 2.22	0.5977
ESR (mm/h)	37.61 ± 22.25	31.78 ± 17.18	34.40 ± 29.49	0.9940
SF (ng/ml)	1325.03 ± 958.16	755.86 ± 554.09	570.71 ± 376.89	0.4815
ALT (U/L)	90.83 ± 134.58	81.83 ± 121.48	69.67 ± 102.06	0.3686
AST (U/L)	83.56 ± 75.21	85.68 ± 121.48	69.67 ± 102.06	0.4655
LDH (U/L)	342.17 ± 63.28	350.35 ± 134.95	254.55 ± 107.60	0.0033
CK (U/L)	89.38 ± 92.93	163.94 ± 471.46	37.67 ± 19.91	0.4387
KL-6 (U/mL)	1242.30 ± 1488.95	1120.08 ± 908.40	806.63 ± 357.49	0.2891
Anti-MDA5 antibody (RU/ml)	152.42 ± 50.47	149.38 ± 46.08	141.60 ± 39.38	0.7662
ILD: Interstitial Lung Disease, FVC: Forced Vital Capacity, FEV: Forced Expiratory Volume, DLCO SB: Diffusing Capacity of the Lung for Carbon Monoxide (Supplemental Breath), PaO ₂ /FiO ₂ : Arterial Oxygen Partial Pressure to Fractional Inspired Oxygen Ratio, WBC: White Blood Cell, ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein, SF: Serum Ferritin, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, LDH: Lactic Dehydrogenase, CK: Creatine Kinase, KL-6: Krebs von den Lungen-6, G1F: IgG1 fucose, G2S: IgG2 sialic acid. MP: methylprednisolone. Statistical significance was achieved at the p < 0.05 level.				

Characteristics (n = 84)	Types of ILD			p value
	Severe (n = 29)	Moderate(n = 37)	Mild(n = 18)	
DSIgG1, Relative Abundance	57.78 ± 33.20	51.05 ± 33.72	43.62 ± 17.22	0.3085
DSIgG1F	3.35 ± 2.63	2.02 ± 0.86	0.96 ± 0.41	< 0.0001
DSIgG1S	0.73 ± 1.29	1.95 ± 1.61	4.31 ± 6.49	0.0019
DSIgG2, Relative Abundance	35.24 ± 10.02	32.27 ± 16.42	35.74 ± 13.71	0.6033
DSIgG2S	1.51 ± 0.81	1.60 ± 0.85	1.50 ± 0.68	0.8675
Maximum MP dosage (mg/day)	158.62 ± 226.40	85.03 ± 32.55	60.00 ± 30.49	0.0064
Mortality, n (%)	13 (44.83)	8 (21.62)	0 (0.00)	0.0011
ILD: Interstitial Lung Disease, FVC: Forced Vital Capacity, FEV: Forced Expiratory Volume, DLCO SB: Diffusing Capacity of the Lung for Carbon Monoxide (Supplemental Breath), PaO ₂ /FiO ₂ : Arterial Oxygen Partial Pressure to Fractional Inspired Oxygen Ratio, WBC: White Blood Cell, ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein, SF: Serum Ferritin, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, LDH: Lactic Dehydrogenase, CK: Creatine Kinase, KL-6: Krebs von den Lungen-6, G1F: IgG1 fucose, G2S: IgG2 sialic acid. MP: methylprednisolone. Statistical significance was achieved at the <i>p</i> < 0.05 level.				

In univariable analysis, several clinical and serological variables were associated with severe ILD. After multivariable adjustment, lower DSIgG1S remained independently associated with severe ILD (OR = 0.07, 95% CI 0.01–0.36, *P* = 0.0065), supporting DSIgG1S as a potential protective marker or inverse risk indicator rather than a conventional risk factor (Table 2).

Table 2
Univariable and Multivariable analysis factors associated with severe ILD

Variable	Univariable Analysis		Multivariable Analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Demographics				
Male	1.04 (0.55–2.01)	0.8845		
Age	0.98 (0.93–1.03)	0.3727		
Clinical Indicators				
ILD course	0.88 (0.52–1.47)	0.6318		
PaO ₂ /FiO ₂	1.29 (0.97–1.81)	0.0996		
Immunological Indicators				
WBC	0.90 (0.73–1.06)	0.2615		
Lymphocyte	1.08 (0.99–1.12)	0.0860		
CRP	0.97 (0.86–1.04)	0.4579		
ESR	1.00 (0.98–1.03)	0.9261		
SF	1.00 (1.00–1.00)	0.1260		
ALT	0.99 (0.97–1.00)	0.1057		
AST	1.00 (0.99–1.00)	0.3326		
LDH	1.56 (1.13–2.01)	0.0021		
CK	0.99 (0.97–1.00)	0.2020		
KL-6	1.00 (1.00–1.00)	0.0905		
Anti-MDA5 antibody	1.00 (0.98–1.01)	0.4769		
DSIgG1F	1.47 (1.14–2.00)	0.0057		
DSIgG1S	0.08 (0.02–0.23)	< 0.0001	0.07 (0.01–0.36)	0.0065
DSIgG2S	0.91 (0.46–1.77)	0.7906		

3.3 DSIgG1S shows strong discriminatory performance for severe ILD

ROC analysis showed that DSIgG1S had the strongest discriminatory performance for severe ILD (AUC = 0.9133), exceeding conventional biomarkers such as LDH (AUC = 0.7821), KL-6 (AUC = 0.6900), ferritin

(AUC = 0.6014), CK (AUC = 0.5491), and anti-MDA5 antibody titer (AUC = 0.5863). DSIgG1F also showed moderate discriminatory value (AUC = 0.7799) (Fig. 2).

3.4 Distinct glycoforms differentially engage FcγRIIa, FcγRIIIa, and C1q

SPR experiments demonstrated clear glycoform-dependent binding differences. G1S showed the strongest binding to FcγRIIa, with a K_D of 3.15 ± 0.27 nM, compared with 13.50 ± 1.05 nM for G1F and 12.58 ± 0.93 nM for G1N (Fig. 3A; Supplementary Tables 1 and 4). In contrast, G1F showed the strongest binding to FcγRIIIa, with a K_D of 4.65 nM, whereas G1N and G1S bound less strongly (Fig. 3B; Supplementary Tables 2 and 5). C1q binding followed a similar pattern, with G1F showing the highest affinity ($K_D = 0.85$ μM), followed by G1S (1.89 μM) and G1N (4.00 μM) (Fig. 3C; Supplementary Tables 3 and 6). Together, these data support distinct FcγR/C1q engagement profiles for the major anti-MDA5 glycoforms.

3.5 Glycoforms confer distinct immune effector functions

In functional assays, G1S consistently induced the strongest antibody-dependent cellular phagocytosis. At 100 nM, G1S achieved a phagocytosis rate of $54.82\% \pm 1.44\%$, compared with $33.54\% \pm 1.14\%$ for G1F and $38.63\% \pm 0.75\%$ for G1N. This effect was largely lost in FCGR2A-knockout macrophages, indicating dependence on FcγRIIa (Fig. 4A-C).

By contrast, G1F drove the strongest ADCC response. At 100 nM, G1F induced $78.3\% \pm 3.2\%$ specific lysis, compared with $58.2\% \pm 2.1\%$ for G1N and $35.6\% \pm 1.8\%$ for G1S. G1F-mediated cytotoxicity was abrogated in FCGR3A-knockout NK cells, supporting a dominant role for FcγRIIIa in this phenotype (Fig. 4D-E).

G1F also induced the strongest CDC response. At 100 nM, G1F produced $45.33\% \pm 0.46\%$ specific lysis, compared with $33.06\% \pm 0.66\%$ for G1N and $23.42\% \pm 0.70\%$ for G1S. C1q-neutralizing antibody markedly reduced the cytotoxic effect of G1F, confirming C1q dependence (Fig. 4F-G).

3.6 G1S antibody specifically activates p38-MAPK/JNK signaling via FcγRIIa

To investigate the specificity of G1S antibody in activating FcγRIIa signaling, we assessed p38-MAPK pathway phosphorylation in wild-type (WT) and FcγRIIa-knockout (FcγRIIa-KO) macrophages. As shown in Fig. 5A, treatment with G1S antibody (10 nM) significantly upregulated p-p38 (4.39-fold, $P < 0.001$) and p-JNK (4.32-fold, $P < 0.001$) in WT macrophages compared with control. G1N antibody induced moderate activation (p-p38: 0.60-fold; p-JNK: 0.54-fold), whereas G1F showed minimal effect (p-p38: 0.40-fold; p-JNK: 0.36-fold). No notable changes were observed in the negative IgG control group. Importantly, these activating effects were completely abolished in FcγRIIa-KO macrophages, confirming that G1S specifically activates the p38-MAPK pathway through FcγRIIa.

3.7 G1S antibody induces endothelial cell pyroptosis and inflammatory cytokine release via FcγRIIa

We next examined pyroptosis-related pathways in human pulmonary microvascular endothelial cells (HPMECs). G1S antibody (10 nM) markedly upregulated p-MLC2 (3.88-fold), NLRP3 (3.32-fold), Caspase-1 (3.25-fold), and N-GSDMD (7.12-fold) in WT HPMECs (all $P < 0.001$, Fig. 5B). These effects were eliminated in FcγRIIa-KO HPMECs, indicating that G1S specifically activates pyroptosis through FcγRIIa.

Cytokine analysis further revealed that G1S significantly promoted the release of IL-1 β (4.56-fold), IL-6 (3.45-fold), and TNF- α (3.10-fold) in WT HPMECs (all $P < 0.001$, Fig. 5C). G1N and G1F had minimal effects. These pro-inflammatory responses were abrogated in FcγRIIa-KO HPMECs, confirming that G1S mediates endothelial inflammation through FcγRIIa.

3.8 G1F antibody activates the PI3K-Akt/NF- κ B pathway in NK cells via FcγRIIIa

We evaluated PI3K-Akt/NF- κ B pathway activation in WT and FcγRIIIa-KO NK cells (Fig. 6A-B). G1F antibody (100 nM) significantly upregulated p-Akt (6.35-fold, $P < 0.001$) and p-NF- κ B p65 (8.94-fold, $P < 0.001$) in WT NK cells compared with control. G1N and G1S showed no significant effect. In FcγRIIIa-KO NK cells, the activating effects of G1F were completely absent, confirming that G1F specifically activates this pathway through FcγRIIIa.

3.9 G1F antibody induces complement-dependent endothelial inflammation via C1q

We further investigated complement pathway activation. As shown in Fig. 6C, G1F antibody (10 nM) combined with C1q significantly increased C3a (67.60 ± 14.96 vs. 14.96 ± 0.41 pg/mL) and C5a (37.37 ± 2.21 vs. 9.40 ± 0.89 pg/mL) in HPMECs (both $P < 0.001$). G1N induced moderate activation, whereas G1S showed minimal effects. Preincubation with a C1q-neutralizing antibody completely blocked G1F-induced complement activation, restoring C3a and C5a to baseline.

Regarding endothelial inflammation, G1F + C1q significantly promoted IL-1 β (105.96 ± 7.6 vs. 26.90 ± 2.07 pg/mL), IL-6 (223.83 ± 3.77 vs. 63.86 ± 3.36 pg/mL), and TNF- α (124.78 ± 2.92 vs. 40.19 ± 1.52 pg/mL) release (all $P < 0.001$). C1q neutralization completely abolished these effects, demonstrating that G1F induces endothelial inflammation through C1q-mediated complement activation.

4 Discussion

In this study, we identified substantial Fc glycosylation heterogeneity in anti-MDA5 disease-specific IgG from patients with MDA5 + DM and found that these features were associated with ILD severity. In particular, increased DSIgG1F and reduced DSIgG1S were linked to more severe pulmonary involvement.

Functional assays further showed that distinct glycoforms differed in their engagement of FcγRIIa, FcγRIIIa, and C1q, with corresponding differences in ADCP, ADCC, CDC, and endothelial inflammatory responses. Together, these findings support the concept that anti-MDA5 IgG glycosylation may influence immune effector functions relevant to lung injury in MDA5 + DM.

One of the clinically important observations is the association between reduced DSIgG1S and severe ILD. In the present dataset, DSIgG1S remained independently associated with severe ILD after multivariable adjustment and outperformed several conventional serum biomarkers in ROC analysis. This finding aligns with emerging evidence that sialylated IgG generally exerts anti-inflammatory effects^[14]. In the context of autoimmune disease, sialylated Fc glycans have been shown to promote anti-inflammatory signaling through engagement of type I and II Fc receptors^[15]. The most appropriate interpretation of these data is that reduced DSIgG1S is inversely associated with severe ILD and may have value for risk stratification. Because the study is cross-sectional and single-center, however, DSIgG1S should be described as a candidate biomarker requiring independent validation rather than a definitive predictor. Broader reviews of antibody glycosylation in autoimmune diseases further support the concept that specific glycan modifications can shape disease phenotypes^[16].

The in vitro data provide biologic plausibility for these clinical associations. G1S favored FcγRIIa engagement and phagocytic activity, whereas G1F favored FcγRIIIa and C1q engagement together with stronger cytotoxic and complement-mediated effects. This divergence is consistent with known differences in how IgG allotypes and glycoforms interact with Fcγ receptors^[17–18]. Specifically, afucosylated IgG has been shown to enhance FcγRIIIa binding and ADCC activity, whereas sialylation can attenuate effector functions and reduce toxicity^[19]. Our data extend these observations to disease-specific autoantibodies in MDA5 + DM, suggesting that Fc glycosylation may shape the balance between immune clearance and tissue-damaging effector programs. Importantly, the endothelial-cell experiments indicate that glycoform-specific effects are not restricted to classical immune effector cells and may extend to structural lung cells involved in ILD pathogenesis. This is consistent with reports that endothelial cells are active participants in lung injury, including through AKT signaling pathways^[20] and broader cellular and molecular determinants of pulmonary fibrosis^[21].

This revised interpretation also requires restraint. The current experiments support differential receptor engagement and downstream signaling in vitro, but they do not establish that Fc glycosylation alone determines clinical severity in vivo. Likewise, the HPMEC experiments support FcγRIIa-dependent and C1q-related inflammatory responses, but they do not by themselves prove direct receptor binding on endothelial cells at the level implied in the original abstract. For this reason, causal language such as "dictates," "defines," or "mechanistic driver" should be avoided in favor of formulations such as "is associated with," "supports a role for," or "may contribute to." Recent work on B-cell-depleting strategies in autoimmune disease underscores the complexity of translating in vitro findings to in vivo outcomes^[22].

Several limitations should be acknowledged. First, the study used a cross-sectional design and therefore cannot address temporal changes in glycosylation during treatment or disease progression. Second, the cohort was derived from a single center, and external validation is needed. Although treatment status was adjusted for in multivariable models and no significant glycoform differences were observed between treatment-naïve and treated patients, a modifying effect of prior immunomodulatory therapy on Fc glycosylation cannot be completely excluded.

Given the cross-sectional and single-center design of the present study, our findings require further validation. Future studies should employ a multicenter, prospective, longitudinal cohort design to enroll treatment-naïve patients with MDA5 + DM-ILD. Serial serum samples should be collected at baseline and during follow-up to dynamically monitor Fc glycosylation profiles of anti-MDA5 IgG. The primary objectives will be to externally validate the predictive performance of DSIgG1S for severe ILD, evaluate correlations between glycosylation dynamics and clinical improvement or lung function decline, and establish a combined risk prediction model incorporating glycan features and conventional biomarkers. Ultimately, these efforts may facilitate glycoform-guided risk stratification and precision therapy for patients with MDA5 + DM-associated ILD.

5 Conclusions

Anti-MDA5 DSIgG in MDA5 + DM displays a remodeled Fc glycosylation profile associated with ILD severity. Reduced DSIgG1S is a promising candidate biomarker for pulmonary risk stratification, while G1S and G1F show distinct FcγR/C1q-related effector profiles in vitro. These findings support further investigation of anti-MDA5 IgG glycosylation as both a mechanistic axis and a translational biomarker in MDA5 + DM-associated ILD.

Abbreviations

Abbreviation	Full Form
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CDC	Complement-dependent cytotoxicity
CI	Confidence interval
CK	Creatine kinase
CRP	C-reactive protein
DLCO SB	Diffusing capacity of the lung for carbon monoxide (single breath)
DM	Dermatomyositis
DSIgG	Disease-specific immunoglobulin G
ESR	Erythrocyte sedimentation rate
FcγR	Fc gamma receptor
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
G1F	IgG1 fucosylated glycoform
G1N	IgG1 native (non-modified) glycoform
G1S	IgG1 sialylated glycoform
G2S	IgG2 sialylated glycoform
HPMEC	Human pulmonary microvascular endothelial cell
HRP	Horseradish peroxidase
IL	Interleukin
ILD	Interstitial lung disease
KL-6	Krebs von den Lungen-6
LDH	Lactate dehydrogenase
MDA5	Melanoma differentiation-associated protein 5
MDA5+DM	Anti-MDA5-positive dermatomyositis
MP	Methylprednisolone

NK cell	Natural killer cell
OR	Odds ratio
PaO ₂ /FiO ₂	Arterial oxygen partial pressure to fractional inspired oxygen ratio
PBS	Phosphate-buffered saline
PMA	Phorbol 12-myristate 13-acetate
ROC	Receiver operating characteristic
RP-ILD	Rapidly progressive interstitial lung disease
RSD	Relative standard deviation
SD	Standard deviation
SEM	Standard error of the mean
SF	Serum ferritin
SLE	Systemic lupus erythematosus
SPR	Surface plasmon resonance
TNF- α	Tumor necrosis factor-alpha
UPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrometry
WBC	White blood cell
WT	Wild-type

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University (Ethics number: 2025-74).

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form (including individual details, images or videos).

Availability of data and materials

The original contributions presented in this study are included in the article and its supplementary materials. Additional information may be obtained from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

J.Z. and Z.Y. contributed equally to this work. J.Z. and H.Z. designed the study. K.W. performed data analysis. Z.C., Y.F., L.G., H.Y., and S.Y. collected experimental data. All authors read and approved the final manuscript.

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Figures

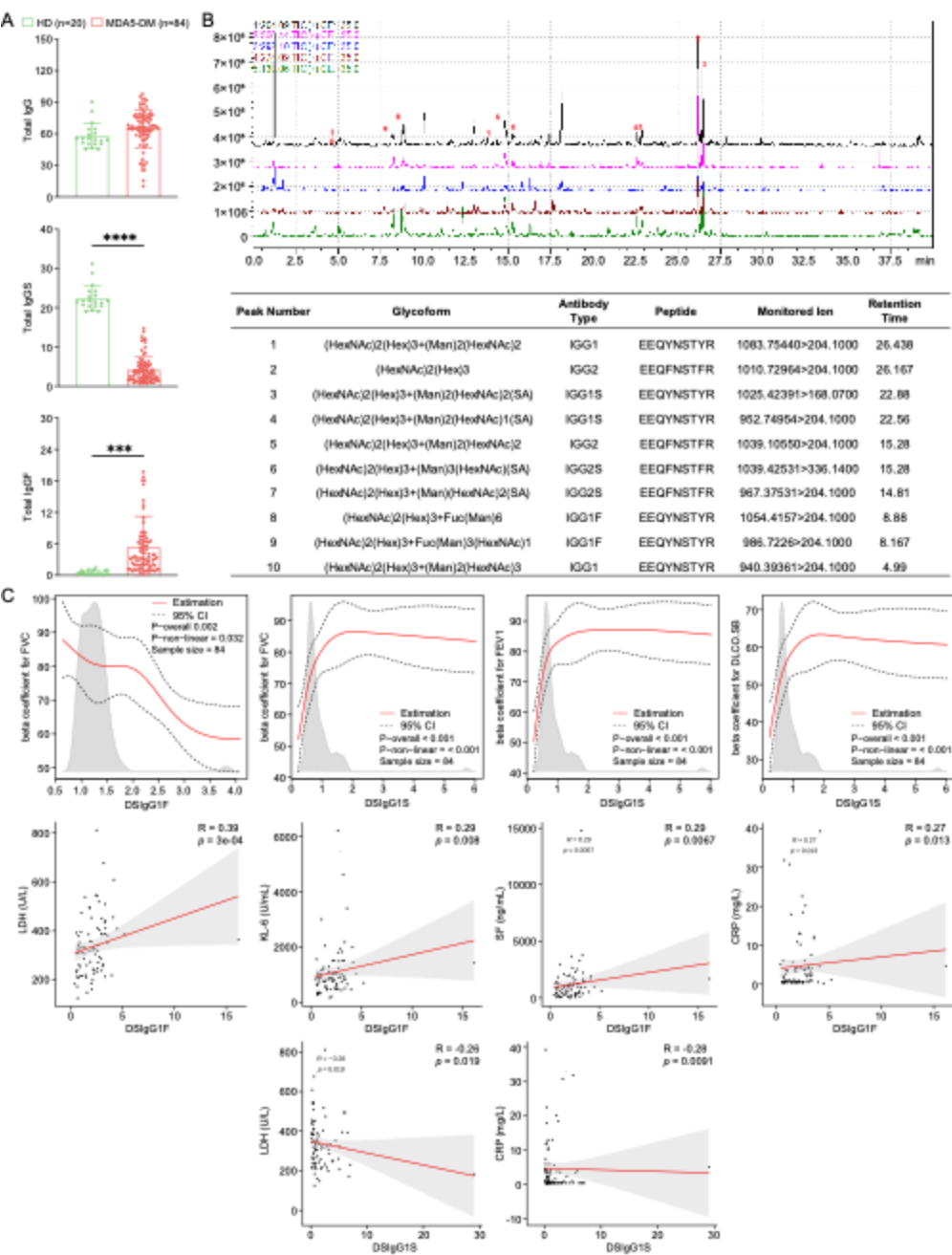


Figure 1

Glycosylation profiling of anti-MDA5 disease-specific IgG (DSIgG) and its clinical correlations in MDA5+DM. (A) Comparison of total IgG fucosylation and sialylation between MDA5+DM patients and healthy controls. (B) Representative mass spectra of tryptic DSIgG glycopeptides showing five major glycoforms including three IgG1-associated (G1, G1F, G1S) and two IgG2-associated (G2, G2S) species. (C) Correlations between DSIgG1F / DSIgG1S levels and clinical parameters related to ILD severity.

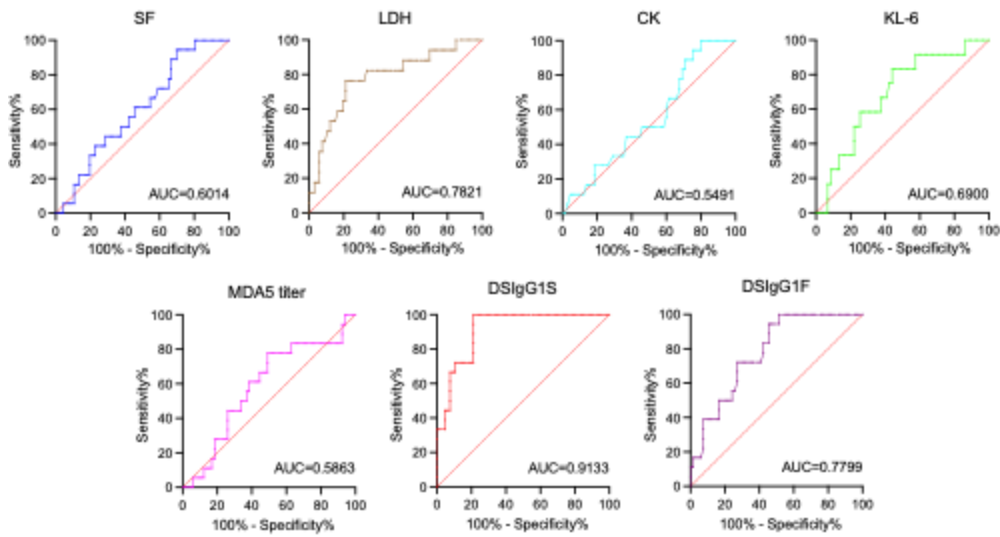


Figure 2

Diagnostic performance of DSIG glycoforms and conventional biomarkers for severe ILD in MDA5+DM. Receiver operating characteristic (ROC) curves and AUC values for DSIGG1S, DSIGG1F, LDH, KL-6, ferritin, CK, and anti-MDA5 antibody titer in discriminating severe from non-severe ILD.

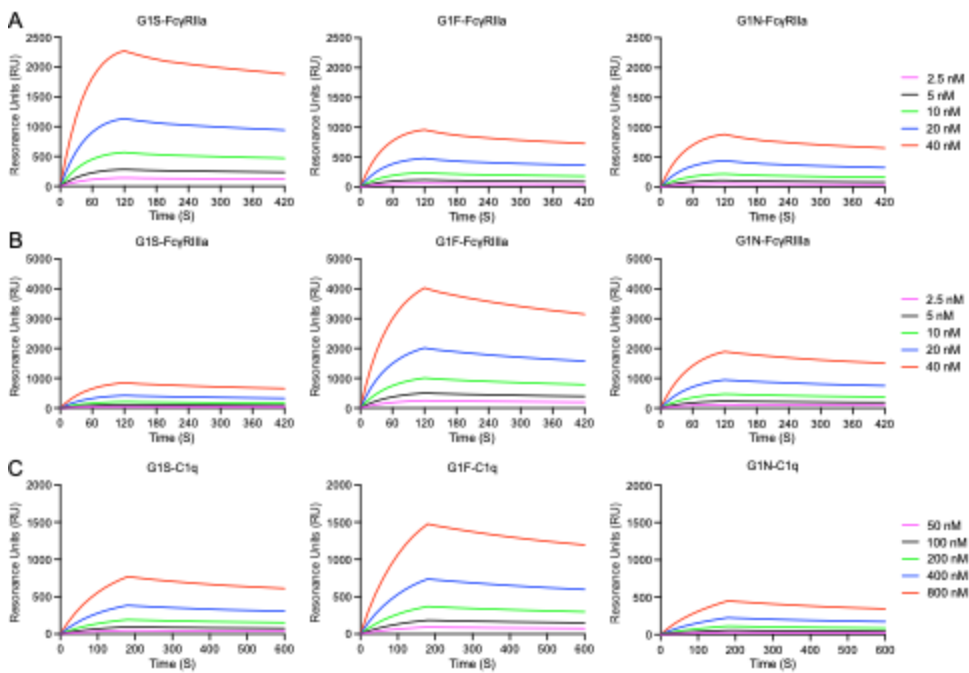


Figure 3

Glycoform-dependent binding of anti-MDA5 IgG to FcγRIIa, FcγRIIIa, and C1q measured by surface plasmon resonance (SPR). Representative sensorgrams and equilibrium dissociation constants (KD) of G1S, G1F, and G1N binding to (A) FcγRIIa, (B) FcγRIIIa, and (C) C1q. Data are mean ± SD from three independent experiments.

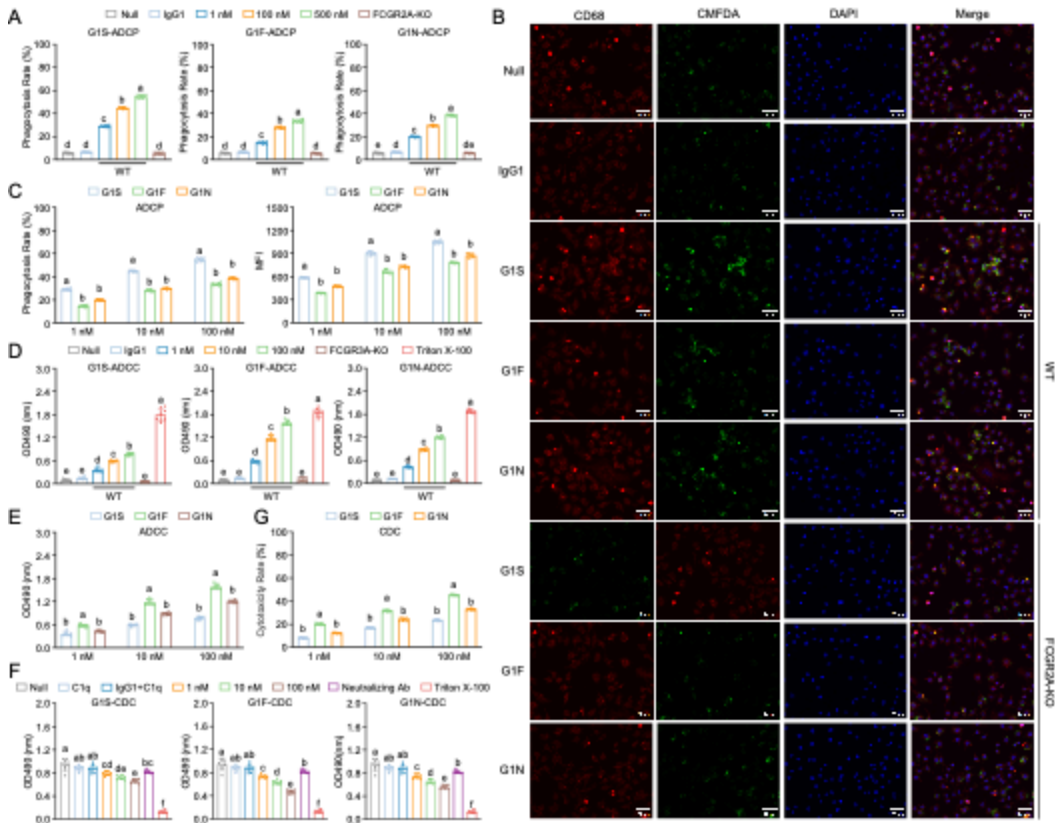


Figure 4

Distinct immune effector functions mediated by anti-MDA5 IgG glycoforms. (A–C) Antibody-dependent cellular phagocytosis (ADCP) by THP-1-derived macrophages. (D–E) Antibody-dependent cellular cytotoxicity (ADCC) by NK cells. (F–G) Complement-dependent cytotoxicity (CDC) in MDA5-overexpressing HEK293T cells. Data are mean $\pm$ SD ($n = 3$). Groups labeled with different letters are significantly different ($P < 0.05$; one-way ANOVA with Tukey's test).

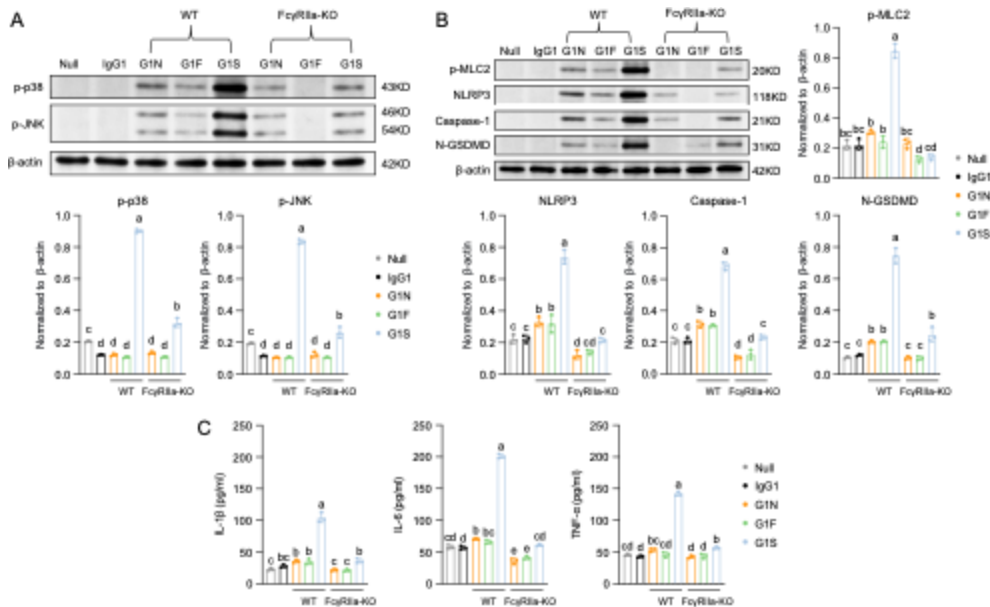


Figure 5

G1S drives FcγRIIIa-dependent signaling and endothelial inflammatory responses. (A) Phosphorylation of p38 and JNK in wild-type (WT) and FcγRIIIa-knockout (KO) macrophages. (B) Expression of pyroptosis-related proteins (p-MLC2, NLRP3, caspase-1, N-GSDMD) in HPMECs. (C) Release of IL-1β, IL-6, and TNF-α in HPMEC supernatants. Data are mean ± SEM (n = 3). Groups with different letters differ significantly (P < 0.05; one-way ANOVA with Tukey's test).

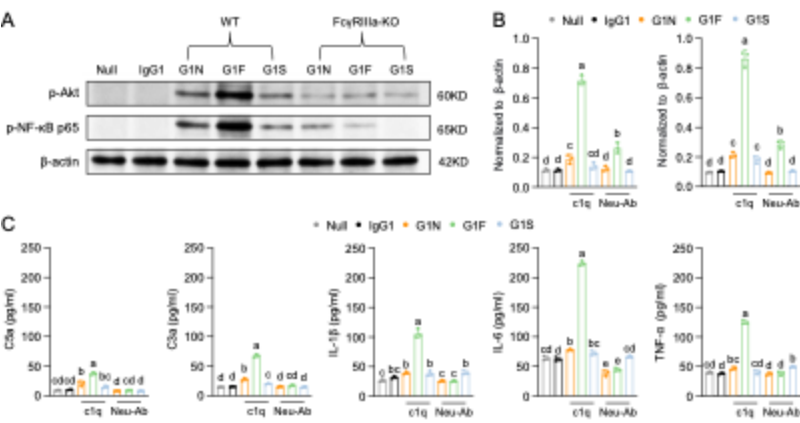


Figure 6

G1F induces FcγRIIIa-mediated signaling and C1q-dependent endothelial inflammation. (A) Phosphorylation of Akt and NF-κB p65 in WT and FcγRIIIa-KO NK cells. (B) Production of C3a and C5a in HPMECs. (C) Secretion of IL-1β, IL-6, and TNF-α in HPMECs. Data are mean ± SEM (n = 3). Groups labeled with different letters are statistically different (P < 0.05; one-way ANOVA with Tukey's test).

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