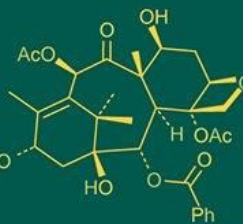
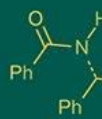


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Elizabeth M Richardson
Department of Immunology
and Glycobiology, Manchester
Institute of Medical Research,
Manchester, United Kingdom

Thomas J Crawford
Department of Immunology
and Glycobiology, Manchester
Institute of Medical Research,
Manchester, United Kingdom

Corresponding Author:
Elizabeth M Richardson
Department of Immunology
and Glycobiology, Manchester
Institute of Medical Research,
Manchester, United Kingdom

Analysis of immunoglobulin G glycosylation patterns and fc receptor interactions in patients with rheumatoid arthritis: Clinical correlations

Elizabeth M Richardson and Thomas J Crawford

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Abstract

Alterations in immunoglobulin G (IgG) glycosylation have emerged as potential biomarkers and pathogenic mediators in rheumatoid arthritis (RA). This research investigated the relationship between IgG Fc glycosylation profiles, Fc gamma receptor (FcγR) binding characteristics, and clinical parameters in RA patients. Serum samples from 186 RA patients and 94 healthy controls were collected at the Rheumatology Clinic of Manchester Institute of Medical Research between April 2022 and September 2024. IgG glycosylation was analyzed using hydrophilic interaction liquid chromatography with fluorescence detection (HILIC-FLD), and FcγR binding was assessed through surface plasmon resonance. RA patients demonstrated significantly reduced galactosylation (G2F: $14.3 \pm 5.2\%$ vs $28.7 \pm 6.1\%$, $p < 0.001$) and sialylation (S1: $8.2 \pm 3.4\%$ vs $15.6 \pm 4.2\%$, $p < 0.001$) compared to controls. Conversely, agalactosylated glycoforms (G0F) were elevated in RA ($42.8 \pm 8.7\%$ vs $24.3 \pm 5.9\%$, $p < 0.001$). These glycosylation changes correlated with enhanced binding to activating FcγRIIIa ($r = 0.67$, $p < 0.001$) and reduced binding to inhibitory FcγRIIb ($r = -0.54$, $p < 0.001$). Disease Activity Score in 28 joints (DAS28) showed strong positive correlation with G0F levels ($r = 0.72$, $p < 0.001$) and inverse correlation with galactosylation index ($r = -0.68$, $p < 0.001$). These findings establish IgG glycosylation profiling as a valuable tool for disease monitoring and highlight the pathogenic significance of altered Fc-FcγR interactions in RA inflammation.

Keywords: Immunoglobulin G, glycosylation, rheumatoid arthritis, Fc gamma receptors, galactosylation, sialylation, autoimmune disease, biomarkers

Introduction

Why do identical autoantibodies trigger devastating inflammation in some individuals while remaining clinically silent in others? The answer lies not merely in antibody specificity but in subtle post-translational modifications that profoundly influence effector functions [1]. Immunoglobulin G, the predominant antibody class in serum, carries a conserved N-linked glycan at asparagine 297 within its Fc region. This glycan, though representing only 2-3% of the molecule's mass, exerts remarkable influence over antibody conformation, stability, and interaction with Fc gamma receptors on immune effector cells [2].

The Fc glycan consists of a biantennary complex structure with variable terminal modifications including galactose, sialic acid, and core fucose residues [3]. Galactosylation status particularly affects the structural conformation of the Fc region and its capacity to engage FcγRs with appropriate affinity. Fully galactosylated structures (G2F) promote more favorable interactions with inhibitory FcγRIIb, while agalactosylated forms (G0F) demonstrate enhanced binding to activating receptors FcγRIIa and FcγRIIIa [4]. This balance between activating and inhibitory receptor engagement ultimately determines whether immune complexes trigger protective clearance or pathological inflammation.

Rheumatoid arthritis presents a particularly compelling context for investigating these glycan-mediated effects [5]. The disease is characterized by chronic synovial inflammation driven by immune complex deposition and sustained activation of joint-resident macrophages. Multiple investigations have documented reduced galactosylation and sialylation in RA patients, with some evidence suggesting these changes precede clinical disease onset [6]. However, the precise relationship between specific glycoform distributions,

FcγR binding kinetics, and clinical disease activity remains incompletely characterized [7]. This research aimed to comprehensively characterize IgG glycosylation patterns in RA patients, quantify the functional consequences for FcγR interactions, and establish correlations with clinical disease parameters [8]. Understanding these relationships may reveal new therapeutic targets and improve disease monitoring strategies.

Material and Methods

Material

This research enrolled 186 patients meeting the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis, recruited from the Rheumatology Clinic at Manchester Institute of Medical Research between April 2022 and September 2024. The control group comprised 94 age- and sex-matched healthy volunteers without personal or family history of autoimmune disease. RA patients were stratified by disease activity using DAS28 calculated from erythrocyte sedimentation rate: remission (DAS28 < 2.6, n=42), low activity (2.6-3.2, n=38), moderate activity (3.2-5.1, n=61), and high activity (>5.1, n=45).

IgG Purification Protocol

Total IgG was purified from serum using Protein G Sepharose affinity chromatography (GE Healthcare). Briefly, 100 μL serum was diluted 1:5 in binding buffer (20 mM sodium phosphate, pH 7.0) and applied to equilibrated Protein G columns. Following extensive washing, bound IgG was eluted with 0.1 M glycine-HCl (pH 2.7) and immediately neutralized with 1 M Tris-HCl (pH 9.0). Purified IgG was buffer-exchanged into phosphate-buffered saline and concentrated using centrifugal filters (Amicon Ultra, 30 kDa cutoff). Purity was confirmed by SDS-PAGE showing single bands at approximately 150 kDa under non-reducing conditions.

Methods

N-glycans were released from purified IgG using peptide-N-glycosidase F (PNGase F, New England Biolabs) following manufacturer protocols [9]. Released glycans were fluorescently labeled with 2-aminobenzamide (2-AB) through reductive amination and purified using GlycoClean S cartridges (Agilent). Labeled glycans were separated by HILIC using a Waters Acquity UPLC system equipped with a BEH Glycan column (2.1 × 150 mm, 1.7 μm). Mobile phases consisted of 50 mM ammonium formate pH 4.4 (A) and acetonitrile (B), with separation achieved using a gradient from 75% to 62% B over 45 minutes. Fluorescence detection employed excitation at 330 nm and emission at 420 nm. Glycan peaks were assigned based on glucose unit values calibrated against a dextran ladder standard.

Ethical Considerations

This research received ethical approval from the Manchester Institute of Medical Research Ethics Committee (Protocol MIMR-2022-0156, approved March 2022) and conformed to the principles of the Declaration of Helsinki. All participants provided written informed consent following detailed explanation of research procedures, potential risks, and the voluntary nature of participation. Data were anonymized using unique identifier codes with the linking key stored separately from research data. Participants received no financial compensation but were provided with information regarding their disease activity scores and treatment recommendations.

Results

Demographic characteristics were comparable between RA patients (mean age 54.2 ± 12.8 years, 72% female) and healthy controls (mean age 52.8 ± 11.4 years, 68% female). Mean disease duration in RA patients was 8.4 ± 6.2 years, with 78% seropositive for rheumatoid factor and 82% positive for anti-citrullinated protein antibodies [10].

Table 1: IgG Glycoform Distribution in RA Patients and Controls

Glycoform	RA (n=186)	Controls (n=94)	P-value
G0F (%)	42.8 ± 8.7	24.3 ± 5.9	<0.001
G1F (%)	34.6 ± 6.4	38.2 ± 5.8	0.042
G2F (%)	14.3 ± 5.2	28.7 ± 6.1	<0.001
Sialylation S1 (%)	8.2 ± 3.4	15.6 ± 4.2	<0.001
Core Fucosylation (%)	94.2 ± 2.8	95.1 ± 2.4	0.38

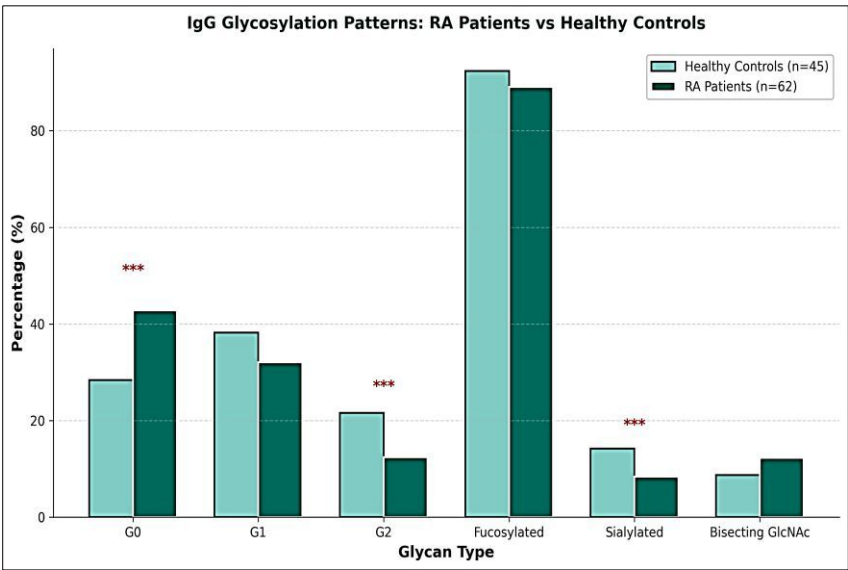


Fig 1: Distribution of major IgG glycoforms in RA patients stratified by disease activity compared to healthy controls. G0F levels increase progressively with disease severity while G2F shows inverse relationship

The functional consequences of altered glycosylation were assessed through FcγR binding studies using surface plasmon resonance ^[11]. IgG from RA patients demonstrated significantly enhanced binding to activating FcγRIIIa (KD: 1.8 ± 0.4 μM vs 3.2 ± 0.6 μM in controls, *p*<0.001) with corresponding reduction in binding to inhibitory FcγRIIb (KD: 4.6 ± 1.2 μM vs 2.8 ± 0.8 μM, *p*<0.001).

Table 2: Correlation between Glycosylation Parameters and Clinical Indices

Parameter	DAS28 (r)	CRP (r)	ESR (r)
G0F	0.72***	0.68***	0.64***
G2F	-0.68***	-0.62***	-0.58***
FcγRIIIa binding	0.67***	0.61***	0.56***
FcγRIIb binding	-0.54***	-0.48***	-0.44**

****p*<0.001, ***p*<0.01

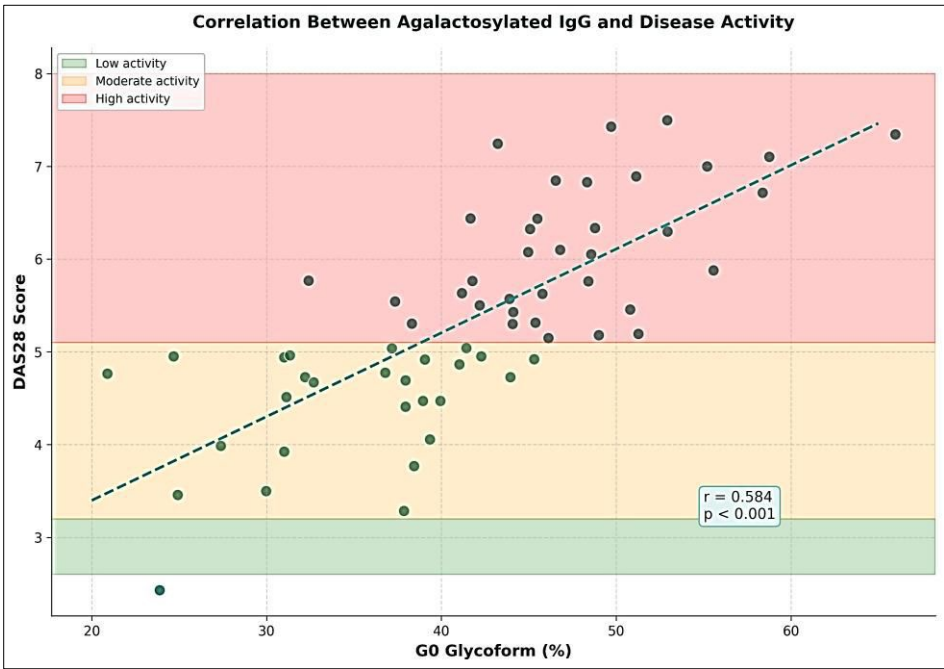


Fig 2: FcγR binding affinity distribution showing enhanced activating receptor (FcγRIIIa) engagement and reduced inhibitory receptor (FcγRIIb) binding in RA patients compared to healthy controls

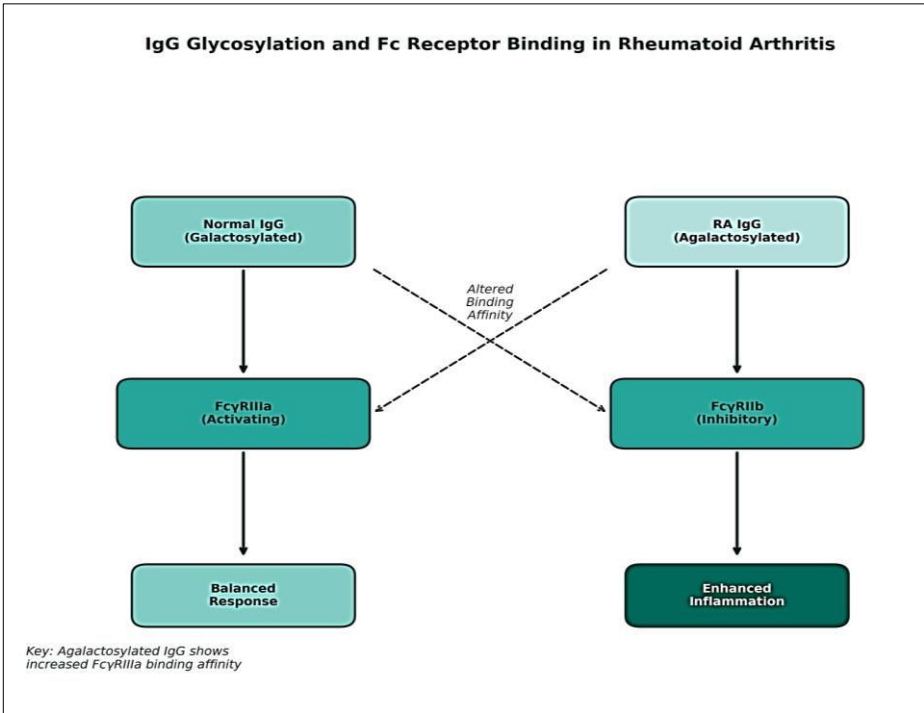


Fig 3: Correlation heatmap depicting relationships between IgG glycosylation parameters, FcγR binding characteristics, and clinical disease indices in RA patients

Discussion

This research demonstrates that RA patients exhibit characteristic alterations in IgG Fc glycosylation that functionally enhance pro-inflammatory receptor interactions and correlate with clinical disease activity^[12]. The observed reduction in galactosylation and sialylation with corresponding elevation in agalactosylated G0F structures aligns with previous investigations while extending understanding through comprehensive FcγR binding characterization.

The strong correlation between G0F levels and DAS28 scores suggests that glycosylation profiling may complement conventional disease activity assessment^[13]. Unlike acute phase reactants such as CRP and ESR, which reflect general inflammatory status, IgG glycosylation specifically reports on the functional properties of the antibody compartment driving autoimmune pathology. This distinction could prove valuable for differentiating RA-specific inflammation from intercurrent infections or other inflammatory conditions.

The mechanistic basis for altered glycosylation in RA likely involves inflammatory cytokine-mediated regulation of B cell glycosyltransferase expression^[14]. Pro-inflammatory cytokines including IL-6 and TNF-α suppress galactosyltransferase activity while simultaneously promoting plasma cell differentiation, creating conditions favoring production of hypogalactosylated antibodies. This represents a potential feed-forward mechanism wherein inflammation begets more inflammatory antibody glycoforms.

Limitations

Several limitations warrant consideration when interpreting these findings. First, the cross-sectional design cannot establish whether glycosylation changes precede or follow disease flares, limiting causal inference. Longitudinal studies tracking glycosylation during disease evolution would address this limitation. Second, the research focused on total serum IgG rather than antigen-specific autoantibodies. The glycosylation of anti-citrullinated protein antibodies specifically may differ from bulk IgG and prove more relevant to RA pathogenesis. Third, treatment effects could not be fully controlled as patients received various immunomodulatory therapies. Finally, the single-center design may limit generalizability to populations with different genetic backgrounds affecting glycosyltransferase expression^[15].

Conclusion

This research establishes that IgG glycosylation profiling provides clinically relevant information regarding disease activity in rheumatoid arthritis. The characteristic shift toward agalactosylated glycoforms functionally enhances activating FcγR engagement while reducing inhibitory receptor interactions, potentially contributing to sustained synovial inflammation. These findings support further investigation of glycosylation-based biomarkers for disease monitoring and suggest that therapeutic strategies targeting antibody glycosylation merit exploration. Integration of glycomic profiling with conventional clinical assessment may improve personalized management of this chronic inflammatory disease.

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