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Short communication

Sugars make the difference – Glycosylation of cardiodepressant antibodies regulates their activity in dilated cardiomyopathy☆

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ABSTRACT

Background: Cardiodepressant antibodies contribute to cardiac dysfunction in dilated cardiomyopathy (DCM). Changes in immunoglobulin G (IgG) glycosylation modulate the activity of various autoimmune diseases and influence disease activity as well as severity of various autoimmune diseases. We hypothesized that alterations in IgG glycosylation are involved in the disease course of DCM.

Methods and results: IgG glycosylation was analyzed in plasma samples of 50 DCM patients using a lectin-based ELISA. Negative inotropic (cardiodepressant) activity (NIA) of antibodies was assessed by measuring the effect of purified DCM-IgG on the shortening of isolated rat cardiomyocytes by means of a video-edge detection system. IgG obtained from plasma of healthy blood donors served as control.

DCM-IgG contained significantly less sialic acid (−25%) and galactose (−16%; both $P < 0.001$), but showed no significant differences in core-fucosylation compared to controls. Interestingly, IgG with NIA displayed a lower percentage of sialylation (−16%, $P < 0.001$) core-fucosylation (−15%, $P = 0.015$) and galactosylation (−10%, $P = 0.129$) than IgG without NIA. The extent of NIA was directly associated with IgG sialylation ($r = 0.68$; $P < 0.001$) and galactosylation ($r = 0.37$; $P = 0.001$).

Conclusion: Reduced sialylation and galactosylation of IgGs enhances their cardiodepressant activity in DCM indicating that changes in IgG glycosylation may be involved in the pathogenesis of DCM.

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1. Introduction

Dilated cardiomyopathy (DCM) is characterized by left ventricular or biventricular dilatation, and systolic dysfunction [1]. Disturbances in humoral immunity seem to be involved in the pathogenesis of DCM [2] where numerous cardiac-specific autoantibodies have been identified in patients and animal models of DCM [3,4].

IgG is the most abundant glycoprotein in human serum and plays a key role in the humoral adaptive immune response. They bear *N*-linked glycans attached to the IgG Fc-region and consist of a constant core region with variable terminal sugar residues [5]. While glycans in the Fab-fragment influence binding to antigens [6] and antibody clearance [7], glycans at the Fc-fragment assure optimal binding between Fc-portion and Fc-receptors [8]. Changes in glycan structures affect physical and biochemical properties of IgGs and consequently their effector functions and bioactivity [9] as seen in autoimmune diseases where agalactosylated and asialylated serum IgG is markedly increased in comparison to healthy individuals [10]. We therefore hypothesize that changes in IgG-glycosylation patterns modulate its biological activity in DCM.

We analyzed the levels of terminal sugar residues in IgG-glycans of DCM patients and compared with healthy blood donors. Since we had previously demonstrated that purified antibodies obtained from a subset of DCM patients induce cardiodepressant (negative inotropic) effects in isolated rat cardiomyocytes (iCMs) [11], we investigated whether negative inotropic activity (NIA) of DCM antibodies is associated with modification of IgG-glycosylation. We focused on IgG

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galactosylation, sialylation and fucosylation, as these glycosylation patterns are well described in other autoimmune diseases.

2. Methods

2.1. Patients and study design

Clinical data and plasma samples of 50 consecutive DCM patients (35 male/15 female, 53 ± 9 years) were included. Plasma from 25 healthy blood donors (17 male/8 female, 52 ± 8 years) served as controls. Details are available in Table 1 and Supplementary Material online, Methods.

2.2. Detection of terminal sugar residues in IgG-glycan structures

For the detection of terminal sialic acid (SA; sialylation), galactose (Gal; galactosylation) and core-fucose (Fuc; core-fucosylation) IgG-glycan structures, we established a lectin-based ELISA (enzyme-linked immunosorbent assay). For details see Supplementary Material online, Methods.

2.3. Measurement of cell shortening in isolated adult rat cardiomyocytes

Measurement of systolic cell shortening was performed in isolated ventricular cardiomyocytes of healthy adult wild type female Wistar rats (age 10–12 weeks). According to the potential of IgGs to induce cardiodepressant effects, they were differentiated into IgGs with negative inotropic activity (NIA(+)) and without negative inotropic activity (NIA(−)). The threshold for NIA was defined as the mean effect induced by control-IgG minus twice the standard deviation (change by −3.04 ± 2.33% from baseline) and was calculated to be −7.70% in our study. Details are available in the Supplementary Material online, Methods.

2.4. Statistical analysis

For baseline characteristics of DCM patients in Table 1, differences in continuous variables were tested using a *t*-test with Welsh correction for inhomogeneous variances. Differences in categorical variables were tested using the Chi^2-test (Pearson) or Fisher's exact test. Linear associations were analyzed using Pearson's correlation or linear regression models adjusted for sex and age. The data obtained from measurements of cell shortening are expressed as percentage change from baseline and those for terminal glycosylation, as relative fluorescence units (RFU). All values are given as mean ± standard error of the mean (SEM), as mean ± standard deviation (SD) or as total number (n) followed by percentage values, unless otherwise indicated. All calculations were performed with Stata 14.1 (StataCorpLP, College Station, USA), with a significance level of $\alpha = 0.05$. For details see Supplementary Material online, Methods.

Table 1
Characteristics of all DCM patients and DCM patients classified into NIA(−) and NIA(+).

	DCM	NIA(+)	NIA(−)	*P*-value
	(n = 50)	(n = 20)	(n = 30)	NIA(+) vs NIA(−)
Age (years ± SD)	53 ± 9	52 ± 9	54 ± 10	0.60
Gender (♂/♀)	35/15	12/8	23/7	0.21
LVEF (%) ± SD	31 ± 7	29 ± 6	31 ± 8	0.27
LVEDD (mm) ± SD	71 ± 9	70 ± 8	72 ± 10	0.35
NYHA classification	2.5 ± 0.6	2.6 ± 0.6	2.4 ± 0.7	0.46
Disease duration (month) ± SD	40 ± 67	25 ± 36	50 ± 82	0.17
Body mass index (kg/m^2) ± SD	28 ± 5	28 ± 5	29 ± 6	0.52
Diabetes (n/%)	11/22	2/10	9/30	0.16
Smoking (n/%)	15/30	3/15	12/40	0.07
Endomyocardial biopsy				
Inflammation positive (n/n) biopsies[a]	28/45	12/17	16/28	0.37
Medication (n/%)				
β-Blocker	45/90	17/85	28/93	0.34
ACE-inhibitor	41/82	16/80	25/83	0.76
AT1-antagonist	15/30	5/25	10/33	0.75
Diuretics	41/82	15/75	26/87	0.29
Digitalis	13/26	4/20	9/30	0.52
MR-antagonist	31/62	12/60	19/63	0.47
Clinical chemistry				
NT-proBNP (pmol/l) ± SD	1633 ± 1873	1589 ± 2273	1657 ± 1666	0.92
Total IgG (g/l) ± SD	10.6 ± 2.7	11.3 ± 2.6	10.1 ± 2.6	0.13

Values are mean ± standard deviation (SD) or n (%). DCM, dilated cardiomyopathy; NIA, negative inotropic activity; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme; AT1, angiotensin-II-receptor-subtype-1; MR, mineralocorticoid-receptor; NT-proBNP, N-terminal, pro brain natriuretic peptide; IgG, immunoglobulin G.

[a] Inflammation of myocardial biopsies: >14 leucocytes/mm^2 (CD^{3+} T-lymphocytes and/or $CD68^+$ macrophages), in addition to enhanced expression of HLA class II molecules,

3. Results

3.1. Characteristics of patients and NIA classification

Contractility measurements on iCMs were performed and used to classify DCM patients according to the NIA status of their IgGs. Compared to control-IgG, purified DCM-IgG caused a significantly stronger reduction in cell shortening (−6.9 ± 0.7% vs. −3.0 ± 0.5%, $P < 0.001$, Fig. 1iA) and calcium transients (Supplementary Material online, Fig. S1A). IgGs purified from twenty DCM patients showed NIA on iCMs and were therefore considered as NIA(+) (mean reduction: −12.3 ± 0.8%) while the IgGs of thirty DCM patients were classified as NIA(−) as they did not affect iCM shortening (mean reduction: −3.3 ± 0.4%; Fig. 1iB). The effect on cell shortening of NIA(+) IgGs was significantly stronger when compared to the effects of IgGs from NIA(−) and controls ($P < 0.001$; Fig. 1iB). Comparable effects were found for the calcium transients (Supplementary Material online, Fig. S1B).

3.2. Terminal sugar residue patterns of IgG-glycosylation

Glycans attached to DCM-IgG exhibited significantly lower levels of sialylation and galactosylation than control-IgG (sialylation: 1.43 ± 0.03 RFU vs. 1.90 ± 0.03 RFU; galactosylation: 1.61 ± 0.05 RFU vs. 1.91 ± 0.05 RFU; both $P < 0.001$; Fig. 1ii). In contrast, core-fucosylation remained unchanged in DCM-IgG compared to controls (controls: 0.34 ± 0.02 RFU vs. DCM: 0.38 ± 0.01 RFU; $P = 0.05$; Fig. 1ii).

3.3. Association of glycosylation pattern of IgG with negative inotropic activity (NIA)

Sialylation of DCM-IgG was significantly reduced in both NIA(−) and NIA(+) groups compared to controls (controls: 0.34 ± 0.02 RFU; NIA(+): 1.28 ± 0.04 RFU; NIA(−): 1.53 ± 0.02 RFU; controls vs NIA(+), $P < 0.001$; controls vs NIA(−), $P < 0.001$). Sialylation of IgGs was furthermore significantly reduced in NIA(+) group when compared to NIA(−) group ($P < 0.001$) (Fig. 1iiiA). A significant lower level of core-fucosylation could be observed in both control-IgG (0.34 ± 0.02 RFU, $P < 0.01$) and NIA(+) IgG (0.34 ± 0.01 RFU, $P < 0.05$) when compared to NIA(−) (0.40 ± 0.02 RFU). Galactosylation was also significantly reduced in both NIA(−) and NIA(+) groups compared to controls DCM-IgG (controls: 1.91 ± 0.05 RFU; NIA(+): 1.51 ± 0.08 RFU; NIA(−): 1.67 ± 0.07 RFU; controls vs NIA(+), $P < 0.001$; controls vs NIA(−), $P < 0.01$); no difference was observed between NIA(+) and NIA(−) groups (Fig. 1iiiA).

In addition, comparison of the IgG-glycosylation data with our findings from iCM contractility measurements (Fig. 1iiiB) demonstrated a significant inverse association between the extent of negative inotropic effect of IgGs and their levels of sialylation ($r = 0.68$; $P < 0.001$) and galactosylation ($r = 0.37$; $P < 0.001$), but not with core-fucosylation ($r = 0.15$; $P =$ n.s.).

4. Discussion

Variations in IgG sialylation and galactosylation of total serum IgGs [10,12] and/or disease-specific autoantibodies [13,14] were shown to modulate the activity and severity of various autoimmune diseases. In addition to these data we now report that IgG-glycan structures are altered in DCM patients and terminal glycosylation levels are associated with NIA of IgG, which may reflect a functional relevance in DCM. More specifically, by analyzing total IgG-glycosylation in a cohort of 50

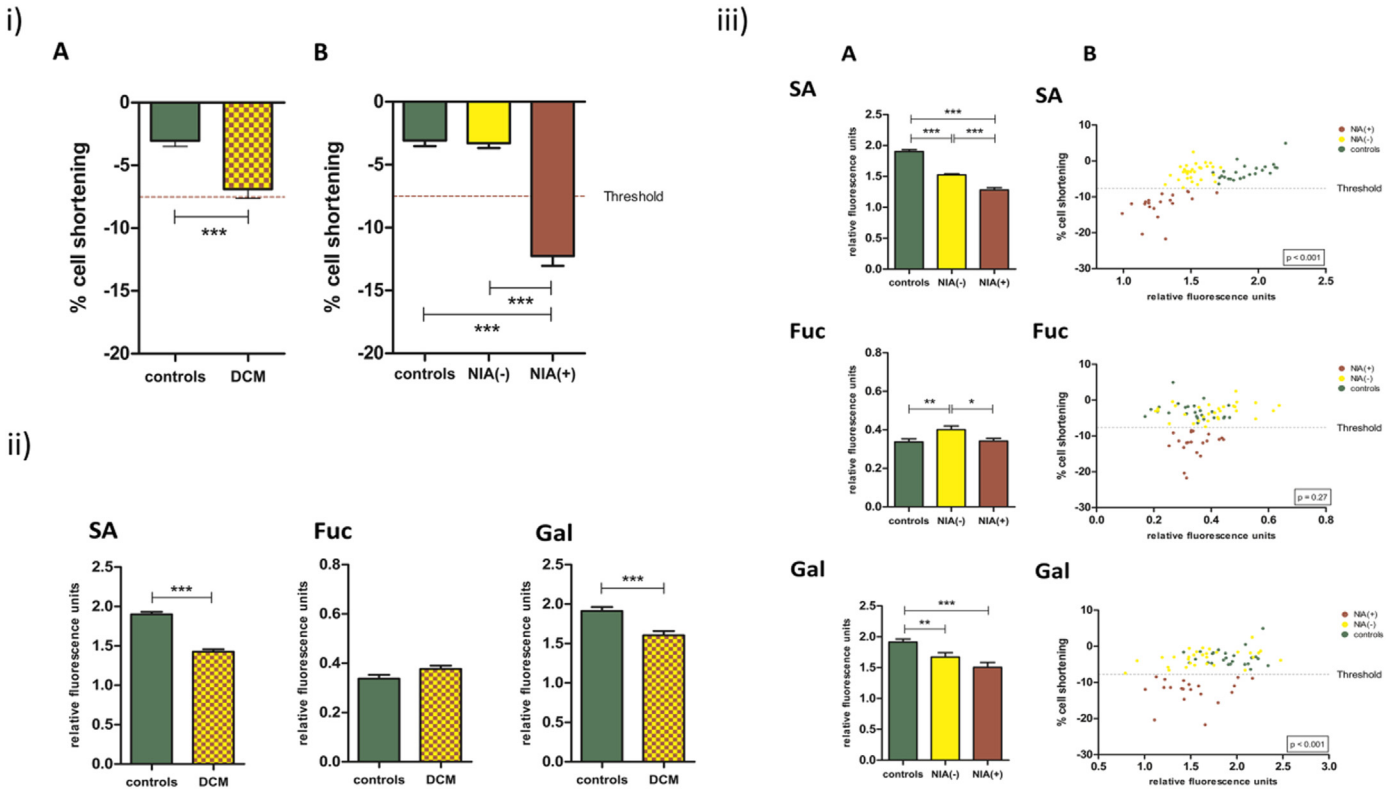

Fig. 1. i) Effects of IgG on cell shortening of isolated adult rat ventricular cardiomyocytes (iCM). (A) The graph depicts the changes in iCM shortening induced by control IgG (n = 25, green) and DCM-IgG (n = 50, red and yellow checked). (B) DCM-IgG were classified according to their negative inotropic activity (NIA) using data from iCM shortening. The dashed red lines represent the threshold which is defined as a reduction of cell shortening by −7.7%. NIA(+) represents DCM-IgG that causes a reduction in iCMs shortening below the threshold (n = 20, red) while DCM-IgG that causes a reduction in iCM shortening above the threshold is considered as NIA(−) (n = 30, yellow). Values are given as mean percentage change from baseline ± SEM and are adjusted for sex and age. ***$P < 0.001$. ii) Levels of terminal sugar residues are altered in DCM-IgG. A lectin-based ELISA was used to detect terminal sugar residues in IgG of DCM patients and controls. The graphs show the mean fluorescence intensities detected for sialylation (SA), core-fucosylation (Fuc) and galactosylation (Gal) in glycans associated with IgG obtained from DCM patients (n = 50, red and yellow checked) and controls (n = 25, green). Values are mean ± SEM and are adjusted for sex and age. ***$P < 0.001$. iii) Association of IgG glycoforms and IgG's negative inotropic activity (NIA). (A) The fluorescence intensities detected for sialylation (SA), core-fucosylation (Fuc) and galactosylation (Gal) in glycans of DCM-IgG were classified according to IgG NIA. (B) The scatter plots display the results of the linear association between the fluorescence intensities for SA, Fuc, Gal and the data obtained from the measurements of cell shortening. Each dot represents the mean values of one individual. Controls are displayed in green, DCM-IgG NIA (−) in yellow (n = 30) and DCM-IgG NIA(+) in red (n = 20). Values are mean ± SEM and were adjusted for sex and age. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

DCM patients, we found sialylation and galactosylation levels were significantly less than those of controls. Similar findings have been reported in RA patients [15] underscoring the pathophysiological relevance of antibody glycosylation.

We observed that cardiodepressant IgGs were lower glycosylated in comparison to non-cardiodepressant IgGs. This was further supported by a significant inverse association between the degree of IgG sialylation and galactosylation and the extent of its cardiodepressive effects. In accordance with this finding, it was shown that activity of rheumatoid factors increases with decreased sialylation and galactosylation in RA [14].

Since terminal sialylation of IgG reduces its binding to FcγRs [9], its absence in IgG-G0 results in enhanced FcγR binding and their pathogenicity. This is in line with our observation that lower levels of IgG sialylation were associated with a more pronounced cardiodepressant activity of DCM-IgG on iCMs which are known to express the activating FcγRIIa [11].

In conclusion, in this study we show for the first time that the glycosylation patterns of IgG derived from DCM patients differ significantly from that of controls. The association between IgG sialylation and galactosylation and their cardiodepressant activity suggests that variations in IgG-glycoforms modulate their bioactivity in DCM patients. However, from our study we cannot answer the question whether IgG-glycosylation is a causal factor rather than a surrogate marker of the cardiodepressive IgG-effects. Based on previously published work regarding the role of IgG-glycosylation in the development, regulation and progression of several autoimmune diseases, our findings indicate that in addition to antigen-mediated effects of cardiac autoantibodies, changes in glycan structures of total IgG may play a functional role in DCM. We found reduced glycosylation patterns in NIA(+) and NIA (−) samples. Sialic acid was the only glycan which was significantly lower in NIA(+) compared to NIA(−) IgGs and control-IgG. It should, however, be mentioned that cardiodepressive activities of IgGs could also be associated with their activity against specific cardiac antigens. Therefore, it is possible that sialylation is more affected in cardiac specific antibodies than galactosylation or fucosylation. However, from our data we cannot deduce any information regarding the influence of glycosylation on the effects of specific cardiac antibodies. It may be possible that reduced glycosylation per se is responsible for the cardiodepressant IgG-effects. Further investigations are needed to elucidate the pathophysiological relevance of altered IgG-glycosylation in DCM.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.04.025.

Study limitations

Our study included a limited number of patients and due to the monocentric study design, a selection bias cannot be excluded. Therefore, our data cannot be generalized for the entire DCM entity and require confirmation by a larger study. Furthermore, the primary aim of our pilot study was to investigate whether IgG-glycosylation in general may have an impact on cardiac function in DCM. Hence, the major focus

of our study was to perform an association analysis rather than mechanistic experiments.

Authors' contributions

Y.R. planned the work, carried out the experiments and wrote the manuscript with input from all authors, S.K. and B.C. were involved in planning the work, aided in interpreting the results and worked on the manuscript, S.G. performed statistical analyses, K.W. and W.H. acquired qualitative data for secondary analysis and contributed to data management, K.K. performed the histopathological analyses of endomyocardial biopsies, M.N. performed routine clinical laboratory diagnostics, J.F. and M.D. discussed data and edited the manuscript, S.B.F. provided the conceptual ideas and supervised the work. All authors discussed the results, and contributed to and approved the final manuscript.

Disclosures

SBF received research grants from Fresenius Medical Care. The other authors declare that they have no conflict of interest.

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