

α -2,3-Sialyltransferase 1 and neuraminidase-3 from monocytes in patients with rheumatoid arthritis correlate with disease activity measures: A pilot study

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Abstract

Background: We decided to study the association of monocyte α -2,3-sialyltransferase 1 (ST3Gal-1), neuraminidase-3 (Neu3), α -2,6-sialyltransferase 1 (ST6Gal-1), and neuraminidase-1 (Neu1) levels with disease activity score 28 (DAS28) in human rheumatoid arthritis (RA), considering that mouse monocytes' sialic acid (SIA) levels relate to their phagocytosis and IgG binding ability.

Methods: ST3Gal-1, Neu3, ST6Gal-1, Neu1, α -2,3-SIA, and α -2,6-SIA levels on RA peripheral blood monocytes, T cells, and polymorphonuclear cells were determined by using fluorochrome-conjugated anti-cell-specific marker antibodies and fluorochrome-conjugated anti-enzyme antibodies. Simple correlation and linear regression were used to correlate enzyme levels with DAS28.

Results: RA monocyte ST3Gal-1 and Neu3 levels correlated with DAS28 in patients having DAS28 >5.1 ($r = 0.469$, $p = 0.002$; $r = 0.410$, $p = 0.006$, respectively). When multivariable analysis was performed for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and SIA-related enzyme levels in different cell types as independent variables with DAS28 as a dependent variable, monocyte ST3Gal-1 levels correlated with DAS28 ($p = 0.009$) but not ESR and CRP in patients having DAS28 >5.1 (both $p \geq 0.292$). RA monocyte ST3Gal-1 levels correlated with DAS28 ($p = 0.010$) and with ESR ($p < 0.001$) at month 0 when applied to all RA patients including both remission and nonremission groups in multivariable analysis. The latter findings persisted longitudinally at month 3.

Conclusion: Monocyte ST3Gal-1 and Neu3 levels correlated longitudinally with DAS28 by two different methods suggest that monocyte ST3Gal-1 and Neu3 levels may be used as biomarkers to monitor RA disease activity.

Keywords: Monocytes; Neuraminidase; Sialyltransferases

1. INTRODUCTION

A lot of different molecules have been implicated in inducing pathological changes and indicating clinical disease activity in rheumatoid arthritis (RA); however, the correlation between sialic acid (SIA) and SIA-related enzyme levels of monocytes and RA disease activity has not yet been studied. Guinea pig peritoneal macrophages exhibit increased immunoglobulin G (IgG) binding post-neuraminidase treatment, which abolishes cell surface SIA, and is caused by an increased affinity but not by a change in number of FcR for IgG (Fc γ) receptors.¹ Cell surface sialylated N-glycans repress phagocytosis and decreased sialylation triggers an acquired phagocytic ability in mouse monocytic cells.² Furthermore, tolerogenic, immature dendritic cells with high α -2,6-SIA levels are considerably downregulated after

dendritic cell maturation.³ These results suggest that phagocytes or antigen-presenting cells with high cell surface SIA levels are immunologically immature, exhibiting less phagocytosis or less IgG binding. Yet, SIA expression and enzymes that make or remove them (see below) on monocytes in RA have not been reported.

Sialyltransferases (STs) are enzymes that transfer SIA to a nascent glycolipid or glycoprotein.^{4,5} α -2,6-sialyltransferase 1 (ST6Gal-1, Galactosyl- α -D-(1 \rightarrow 4)-N-acetyl-D-glucosamine) mediates transfer of the α -2,6-SIA residue to a terminal galactose residue of a type 2 disaccharide (Gal- α 1-4GlcNAc). ST6Gal-1 localizes on the cell membrane, in the Golgi apparatus, and in the extracellular region.⁵ Similarly, α -2,3-sialyltransferase 1 (ST3Gal-1) catalyzes α -2,3 sialylation at sites similar to those of ST6Gal-1 and is engaged in T-cell apoptosis and tumorigenesis.⁶⁻⁸ Furthermore, ST levels are used as a biomarker to distinguish cancer patients from non-cancer patients.^{9,10} Interestingly, ST6Gal-1 modifies sialylation of IgG Fc through α 1-3 mannose.¹¹ Moreover, ST activity increases in B cells in primary Sjögren's syndrome.¹² Nonetheless, no report has investigated association of monocyte ST6Gal-1 levels with laboratory and clinical variables in RA patients. Consequently, we sought to examine ST6Gal-1 and ST3Gal-1 levels that modify IgG's pro- and anti-inflammatory capacity and that produce cell surface SIA levels which could affect monocyte's immune reaction to clarify if they could be markers of disease activity in RA patients.

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2019) 82: 179-185.

Received April 13, 2017; accepted November 21, 2018.

doi: 10.1097/JCMA.000000000000027.

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In contrast, neuraminidases desialylate SIA in cells. Neuraminidase-1 (Neu1) could be involved in the course of the immune response. For example, Neu1 is required in the regulation of macrophage phagocytosis,¹³ production of IgG1 and IgE by B cells,¹⁴ and early interleukin-4 (IL-4) production in T cells during contact with antigen-presenting cells.¹⁵ Neu1 is present on the cell surface and in the lysosomal compartment, whereas neuraminidase-3 (Neu3) exists only on the cell surface.¹⁶ Neu3, a ganglioside-specific sialidase, may be important in cell surface events through modulation of gangliosides.¹⁶ Interestingly, lupus B cell ST3Gal-1/Neu3 ratios correlate positively with systemic lupus erythematosus (SLE) disease activity index. In contrast, both ST3Gal-1 and Neu3 levels in RA B cells correlate positively with RA disease activity (Disease Activity Score 28 [DAS28]).¹⁷ Nonetheless, no report has investigated whether Neu1 and Neu3 levels on RA monocytes relate to RA disease activity, considering that these enzymes remove cell surface SIA levels which might affect monocyte's function in immune reactions.

In summary, the ST3Gal-1/Neu3 pair acts in the α -2,3-SIA pathway, and the ST6Gal-1/Neu1 pair acts in the α -2,6-SIA pathway. We hypothesize that immune cell neuraminidases desialylate cell SIA to promote inflammation. STs might have an opposite effect on monocytes from that by neuraminidases. Thus, a tentative hypothesis may be that ST6Gal-1/ST3Gal-1 levels in phagocytes decrease and Neu1/Neu3 levels increase disease activity in human autoimmune diseases, because of chronic antigen exposure. Because RA patients often undergo long-term exposure to autoantigens, the correlation between cell surface ST and neuraminidase expression on peripheral blood monocytes and laboratory and clinical variables in RA patients is an interesting topic to pursue. Hence, we explored the correlation between ST and neuraminidase expression on monocytes and disease activity parameters in RA patients.

2. METHODS

2.1. Enrollment of patients with recording of clinical and laboratory data

This study was approved by Chang Gung Memorial Hospital Institutional Review Board, Taoyuan City, Taiwan. Written informed consent was obtained from 157 randomly selected 115 patients who fulfilled the 1987 American College of Rheumatology criteria for RA and the 1997 American College of Rheumatology criteria for SLE. Twenty milliliters of blood was collected from each patient at baseline and after 3 months. DAS28 was calculated for RA patients. Forty-seven hospital personnel and author's friends were included as healthy controls after obtaining signed informed consent.

2.2. Cell separation and plasma/serum collection

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid, and cells were centrifuged and collected. These cells were separated further as described previously to obtain peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells (PMN).¹⁸

2.3. Examination of ST3Gal-1, Neu3, ST6Gal-1, Neu1, and cell surface SIA on monocytes, PMNs, and B and T lymphocytes

PBMCs were prepared in 100 μ L of phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) (Sigma, St. Louis, MO, USA) and stained with phycoerythrin (PE)-mouse anti-human CD14 (clone: M5E2) for monocytes, PE-mouse anti-human CD3 (clone: HIT3a) for T cells, or PE-mouse anti-human CD19 (clone: HIB19) for B cells (used at manufacturer suggested dilutions) (BD Pharmingen, Mountain View, CA, USA) and protected from light for 1 hour at room temperature (20-25°C). The isotype control used was PE-mouse IgG1 κ isotype control (clone: MOPC-21) (for anti-CD14 and anti-CD19) or PE-mouse IgG2a isotype

control (clone: G155-178) (for anti-CD3) (used at manufacturer suggested dilutions) (BD Pharmingen, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated *Maaackia amurensis* lectin (40 μ g/mL), which specifically binds α -2,3-SIA, and FITC-conjugated *Sambucus nigra* lectin (20 μ g/mL), which specifically binds α -2,6-SIA, were diluted in 100 μ L of PBS with 1% BSA (both from EY Laboratories, Inc, San Mateo, CA, USA) and used to stain cells as previously described.³ FITC-mouse IgG1 κ isotype was used as the control (used at manufacturer suggested dilutions) (BD Pharmingen). In addition, rabbit polyclonal IgG anti-ST3Gal-1 (0.6 μ g/mL), rabbit polyclonal IgG anti-Neu3 (0.4 μ g/mL), mouse monoclonal IgM anti-ST6Gal-1 (anti-CD75, clone: B-L5) (7.5 μ L from a 2-mL vial; the manufacturer recommendation is 10 μ L), or rabbit polyclonal IgG anti-Neu1 (51 μ g/mL) (Abcam, Cambridge, MA, USA) was used to stain cells. Rabbit polyclonal IgG (Jackson ImmunoResearch, West Grove, PA, USA) (2 μ g/mL) and mouse IgM isotype control (clone: MOPC1041) (4 μ g/mL) (Sigma, St. Louis, MO, USA) were used as the controls.

After washing and centrifugation at 644g (Eppendorf centrifuge 5810R) for 5 minutes, cells suspended in 100 μ L of PBS were subsequently stained with either allophycocyanin (APC)-goat polyclonal antibody to rabbit IgG (2 μ g/mL) or APC-rat monoclonal antibody (clone: 1B4B1) to mouse IgM (2 μ g/mL) (Abcam, Cambridge, MA, USA) protected from light at room temperature (20-25°C) for 1 hour. Using similar washing and centrifugation techniques, cells were resuspended in 500 μ L of PBS at 4°C for flow cytometric analysis using the BD FACSCalibur System (BD Biosciences, San Jose, CA, USA). The minimum number of cells collected was 20 000. FlowJo 7.6.1 program (FlowJo, LLC, Ashland, OR, USA) was used to analyze flow cytometry data.

2.4. Expression of ST3Gal-1 and α -2,3-SIA levels after in vitro stimulation of monocytes by different cytokines

Beyond some PBMCs were stained for cell surface molecules, other PBMCs from RA and SLE patients, and healthy controls were cultured in vitro with different cytokines (IL-1 β , IL-6, and tumor necrosis factor- α (TNF- α), from R&D Systems, Minneapolis, MN, USA) for 4 hours. Then cells were obtained and stained again for monocyte's α -2,3-SIA, Neu3, and ST3Gal-1 levels.

2.5. Statistical analyses

We used the SPSS 16.0 (IBM, Armonk, NY, USA) software package to analyze the data. We assessed correlations between cell surface levels of ST3Gal-1, Neu3, ST6Gal-1, Neu1, α -2,3-SIA, α -2,6-SIA, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) as independent variables and clinical outcome variables (DAS28) in RA patients. DAS28 (containing ESR, designated as DAS28 throughout this study) was calculated based on the universally used equation (including ESR, tender joint count (TJC), swollen joint count (SJC), and global health (GH) as described.¹⁹ Pearson correlation coefficients (r) were used when outcome variables were normally distributed, whereas Spearman correlation coefficients (ρ) were used when the outcome variable (DAS28) was not normally distributed. Linear regression was used for multivariable analysis. A p value of <0.05 was considered to indicate statistical significance. Bonferroni's correction was used for multiple analyses in particular sets of data.

3. RESULTS

3.1. Correlation of ST3Gal-1 and Neu3 on RA monocytes with measures of disease activity

The demographic data from the enrolled patients are shown in Table 1.

RA monocyte ST3Gal-1 and Neu3 levels were correlated with DAS28 in patients having a DAS28 >5.1 (Fig. 1E, F). Similarly, low-level correlations were found between monocyte ST3Gal-1 levels and DAS28 in RA patients with nonremission and with moderate and high disease activity (Fig. 1A, C), based on the criteria of statistical significance cutoffs as described.²⁰ The

Table 1**Demographic data of enrolled subjects**

	RA	Controls	SLE
No. of subjects (M:F)	157 (4.2:1)	47 (4.9:1)	115 (10.5:1)
Mean age (range)	53.0 ± 11.7 (20-80)	47.4 ± 17.9 (23-78)	41.9 ± 11.6 (21-69)
Disease duration (mo)	96.6 ± 85.4 (2-417)	...	109.3 ± 63.8 (1-336)
CRP (mg/L)	9.3 ± 17.2 (0.2-133.2)
ESR (mm/h)	24.6 ± 20.9 (2-101)
Rheumatoid factor (IU/mL)	137.8 ± 246.2 (1.2-1200)
Anti-dsDNA (mg/dL)	171.3 ± 128.9 (35.3-546.9)
No. of arthritic joints	6.7 ± 5.6 (0-26) ^a
DAS28	4.3 ± 1.3 (1.9-7.3)
SLEDAI	3.4 ± 2.6 (0-12.0)
Prednisolone ^b (mg/d)	6.0 ± 3.1 (2.5-15): 34%	...	11.7 ± 8.4 (2.5-50.0): 76.5%
1 DMARD ^b	19.7%	...	24.4%
≥2 DMARDs ^b	72.0%	...	73.0%
Biologicals ^b	24.8%

^aTwo RA patients had no arthritic joints. Normal ranges: CRP: <3.0; ESR: <15; rheumatoid factor: <15.0; anti-dsDNA: <92.7.

^bData are expressed as mean ± SD (ranges); percentage of patients administered a particular medicine for >3 months (those durations not fulfilled were not counted).

Adapted from Liou and Huang.¹⁷

CRP = C-reactive protein; DAS28 = Disease Activity Score 28; DMARD = disease-modifying antirheumatic drugs; dsDNA = double-stranded deoxyribonucleic acid; ESR = erythrocyte sedimentation rate; M:F ratio = male:female ratio; RA = rheumatoid arthritis; Controls = healthy controls; SLE = systemic lupus erythematosus; SLEDAI = systemic lupus erythematosus disease activity index.

correlation coefficients were still significant after Bonferroni's correction. These results were supported by multivariable analysis of ESR, CRP, and SIA-related enzyme molecule levels of the four different cell types (B and T cells, monocytes, and PMNs) as independent variables, with DAS28 as a dependent variable (Table 2). In particular, ESR and CRP did not correlate with DAS28 in RA patients with high disease activity scores (Table 2). Hence, similar results for monocyte ST3Gal-1 were obtained by two different statistical methods (Fig. 1 and Table 2).

In addition, both ST3Gal-1 and α -2,3-SIA (made by ST3Gal-1) levels of RA patients were significantly higher than those of healthy controls and SLE patients (Fig. 2).

Moreover, the above findings were supported by a multivariable analysis containing ESR, CRP, and monocyte ST3Gal-1, Neu3, ST6Gal-1, and Neu1 levels as independent variables with DAS28 as a dependent variable at month 0 for all RA patients (Table 3). Next, we investigated whether the correlation was maintained at different time points. The correlation of RA monocyte ST3Gal-1 levels with DAS28 was confirmed in RA patients at month 3 (in multivariable analysis containing 4 cell types' ST3Gal-1 or Neu3 in 122 RA patients: monocyte ST3Gal-1 levels correlated with DAS28 at $p = 0.021$ and monocyte Neu3 levels correlated with DAS28 at $p = 0.016$). A correlation was also found for ESR (correlated with monocyte ST6Gal-1, Neu1, ST3Gal-1, and Neu3 at $p < 0.001$ for all). Hence, RA monocyte ST3Gal-1 levels were correlated longitudinally with DAS28 in RA patients.

3.2. Correlation of ST6Gal-1 and Neu1 on RA monocytes with measures of disease activity

There was no significant correlation between monocyte ST6Gal-1 levels and DAS28 in nonremission RA patients having DAS28 ≥ 2.6 ($n = 146$) ($r = -0.039$, $p = 0.643$), in RA patients having DAS28 > 3.2 ($n = 123$) ($r = 0.012$, $p = 0.892$), and in RA patients having DAS28 > 5.1 ($n = 43$) ($r = 0.281$, $p = 0.068$). Moreover, monocyte Neu1 levels did not correlate with DAS28 in all RA patients, unlike those for ESR and CRP (in all RA patients with $n = 157$: monocyte Neu1 with DAS28 gave $r = 0.083$, $p = 0.301$; ESR with DAS28 gave $r = 0.586$, $p < 0.001$; CRP with DAS28 gave $r = 0.356$, $p < 0.001$). Nevertheless, monocyte Neu1 levels correlated with DAS28 in RA patients having DAS28 > 5.1 , that is, with high disease activity. In this subset of RA patients ($n = 43$), monocyte Neu1 with DAS28 gave $r = 0.402$, $p = 0.007$; ESR with DAS28 gave $r = -0.014$, $p = 0.930$; CRP with DAS28 gave $r = -0.115$, $p = 0.462$. The P value (0.007) for monocyte Neu1 with DAS28 was still significant after Bonferroni's correction was

applied for correlation of the four different cell types Neu1, ESR, and CRP levels with DAS28, yielding a corrected significant $p = 0.008$ (that is, 0.05 divided by six correlations for four cell types Neu3, ESR, and CRP). This latter finding was similar to those of monocyte Neu3 in RA patients with high DAS28 (Fig. 1F).

3.3. Correlation of STs and neuraminidases among different blood leucocytes

Monocyte ST3Gal-1 levels correlated significantly with B cells and T cells but not with PMNs ($\rho/p = 0.414/<0.001$, $0.398/<0.001$, and $0.185/0.021$, respectively). Similar results were seen for monocyte Neu3 level associated with other cells ($\rho/p = 0.382/<0.001$, $0.273/0.001$, and $0.184/0.021$, respectively). Intriguingly, cell surface α -2,3-SIA levels of monocytes were correlated with those of B and T cells and PMNs ($\rho/p = 0.455/<0.001$, $0.710/<0.001$, and $0.470/<0.001$, respectively).

Similarly, with other three peripheral blood leucocytes, monocyte ST6Gal-1 ($\rho/p = 0.648/<0.001$, $0.578/<0.001$, and $0.297/<0.001$, with B and T cells, and PMNs, respectively), Neu1 (not shown), and α -2,6-SIA (not shown) levels were correlated with those of other blood cells.

3.4. Cytokine IL-1 β able to elevate ST3Gal-1 and α -2,3-SIA levels similarly on RA monocytes

Besides some of fresh PBMCs were stained for cell surface molecules, other PBMCs were cultured with different cytokines for 4 hours and obtained/stained again. As seen (Fig. 3), monocyte's α -2,3-SIA levels were much increased after stimulation with IL-1 β (the cytokine concentration was selected as in Liou²¹) over baseline fresh levels in some control and RA patients than in all SLE patients (Fig. 3A, D, and G). Monocyte α -2,3-SIA level changes of control and RA patients correlated significantly with their ST3Gal-1 level changes, but not of SLE patients (Fig. 3A, B, D, E, G, and H).

4. DISCUSSION

DAS28 and DAS28(CRP) have been validated and used extensively for evaluating RA disease activity.²² However, the ESR and CRP used in calculating DAS28 are within normal ranges in up to 40% of RA patients.^{23,24} Thus, other biomarkers relating to DAS28 are sought. Biomarkers could provide objective assessments of the RA disease course beyond evaluating TJC, SJC, and GH. For example, serum CXCL 13 levels differed in the remission group compared with the active group.²⁵ Moreover, serum

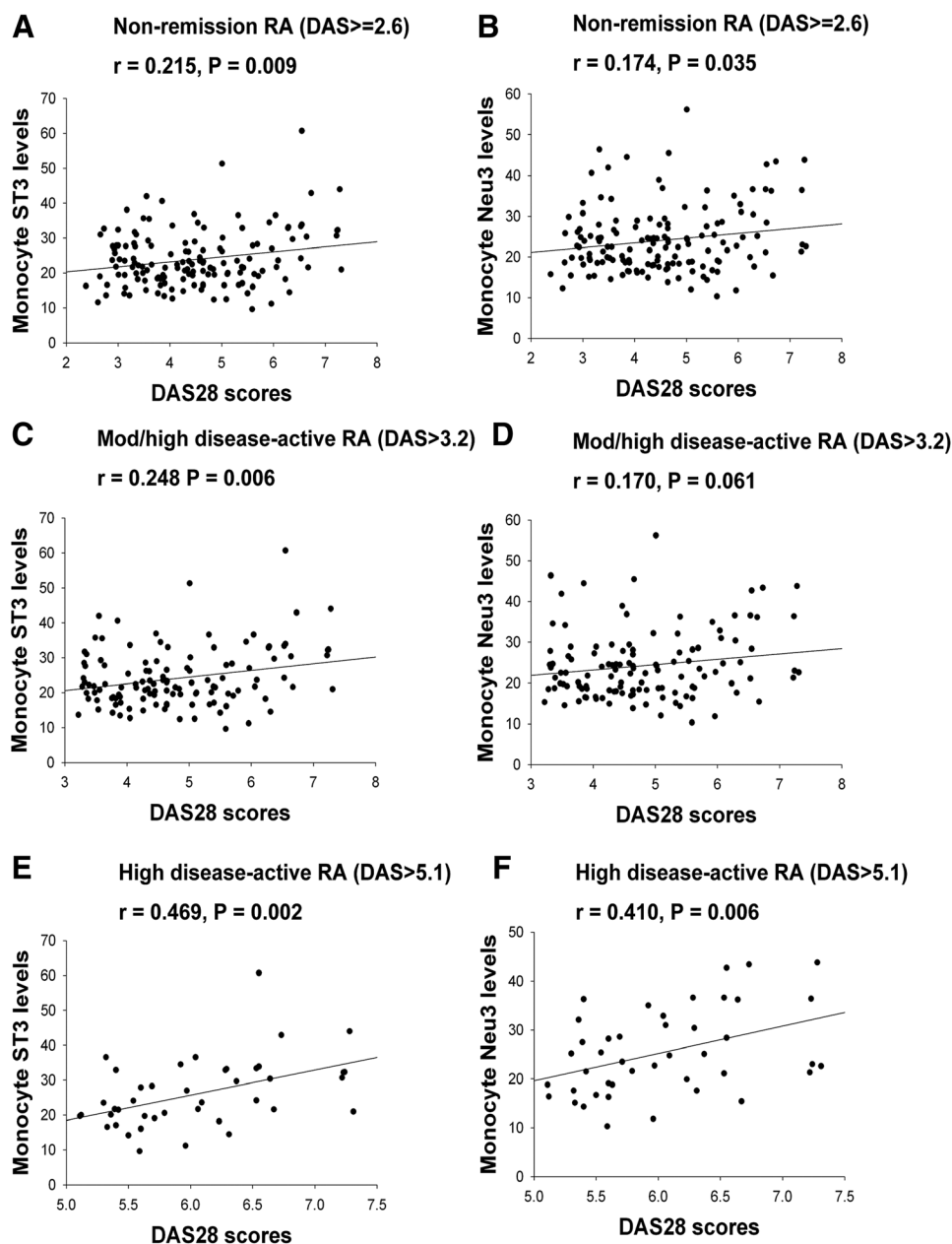


Fig. 1 Correlation of monocyte α -2,3-sialyltransferase 1 (ST3Gal-1) and neuraminidase-3 (Neu3) levels with Disease Activity Score 28 (DAS28) in rheumatoid arthritis (RA) patients. Monocyte ST3 (A) and Neu3 (B) levels indicate the mean fluorescence intensity in monocytes. Nonremission RA patients indicate those patients having DAS28 scores \geq 2.6; moderate and high disease activity designates those RA patients having DAS28 $>$ 3.2; high disease activity indicates those RA patients having DAS28 $>$ 5.1. A, B, Nonremission RA patients (n = 146). C, D, RA patients with moderate and high disease activity (n = 123). E, F, RA patients with high disease activity (n = 43). *p* values for A, C, E, and F were significant, after Bonferroni's correction was applied for ST3Gal-1 and Neu3 levels of the four different cell type correlations with DAS28, resulting in a corrected significant *p* = 0.013 (that is, 0.05 divided by 4 correlations for ST3Gal-1 or Neu3).

TNF- α , IL-6, soluble E-selectin, and asymmetric dimethyl-L-arginine levels were higher in the high DAS28 group than in the moderate and low DAS28 groups.²⁶ Neither report analyzed linear correlation or area under the receiver operating characteristic curve. In addition, serum levels of leucocyte complement receptor 1 transcript, leucine-rich α -2 glycoprotein, microRNA-223, and progranulin correlate with DAS28, though these results have not been validated.²⁷⁻³⁰ Specifically targeting normalization of matrix metalloproteinase 3 levels and DAS28 to $<$ 2.6 have achieved better clinical outcome than targeting either one alone.³¹ The latter report stresses the importance of a combination of different biomarkers in monitoring RA disease activity. Another report examining blood monocyte chemotactic protein-1 (MCP-1) and its adapted DAS28-MCP-1 have displayed the high correlation with DAS28 and DAS28-CRP.¹⁹ Nevertheless, it has not been

validated through functional assessment of patient daily activities and roentgen confirmation. Overall, except for DAS28, no single test has been validated and utilized worldwide, most likely because of the inconvenience and inherent drawbacks found in the individual tests. Nevertheless, ESR and CRP have not been shown to confidently predict high disease activity scores in multivariable analysis in RA patients in this study (Table 2). Therefore, new RA activity biomarkers need to be incorporated as part of a panel to predict disease activity in RA patients.

Deficiency of ganglioside monosialodihexosylganglioside (GM3) induced severe arthritis in a collagen-induced arthritis model of RA.³² Interestingly, Neu3 is a ganglioside-specific sialidase.¹⁶ Whether ganglioside GM3 belongs to a substrate of Neu3 is for future studies, considering that Neu3 levels of RA monocytes correlate significantly or show a trend with DAS28 (Tables 2

Table 2

Correlation between different variables and DAS28 >5.1 at month 0 in multivariable analysis

	ESR	CRP	T Cells	Monocytes	PMNs	Cells' variables
DAS28 scores	0.916	0.701	0.069	0.047	0.672	ST6
	0.568	0.650	0.032	0.044	0.719	Neu1
	0.943	0.707	0.073	0.183	0.495	α -2,6-SIA
	0.787	0.788	0.399	0.009	0.862	ST3
	0.467	0.292	0.979	0.077	0.746	Neu3
	0.950	0.066	0.035	0.070	0.360	α -2,3-SIA

p values for correlation (n = 43) by linear regressions for multivariable analysis with independent variables including ESR, CRP, and the four blood cell types ST6 levels (B, and T cells, monocytes, and PMNs; B cell's data not shown here) and the dependent variable of DAS28. Similarly, Neu1, α -2,6-SIA, ST3, Neu3, and α -2,3-SIA from the four blood cell types plus ESR and CRP were analyzed against DAS28 by linear regression. Among multivariable analysis, monocyte ST3 correlated with DAS28 at *t* = 2.751 (95% CI, 0.008-0.053) and monocyte Neu3 correlated with DAS28 at *t* = 1.819 (95% CI, -0.003 to 0.050). Similarly, monocyte ST6 correlated with DAS28 at *t* = 2.057 (95% CI, 0.000-0.040) and monocyte Neu1 correlated with DAS28 at *t* = 2.091 (95% CI, 0.001-0.062). CRP = C-reactive protein; DAS28 = Disease Activity Score 28; ESR = erythrocyte sedimentation rate; Neu1 = neuraminidase-1; Neu3 = neuraminidase-3; PMN = polymorphonuclear; SIA = sialic acid; ST3 = α -2,3-sialyltransferase 1; ST6 = α -2,6-sialyltransferase 1.

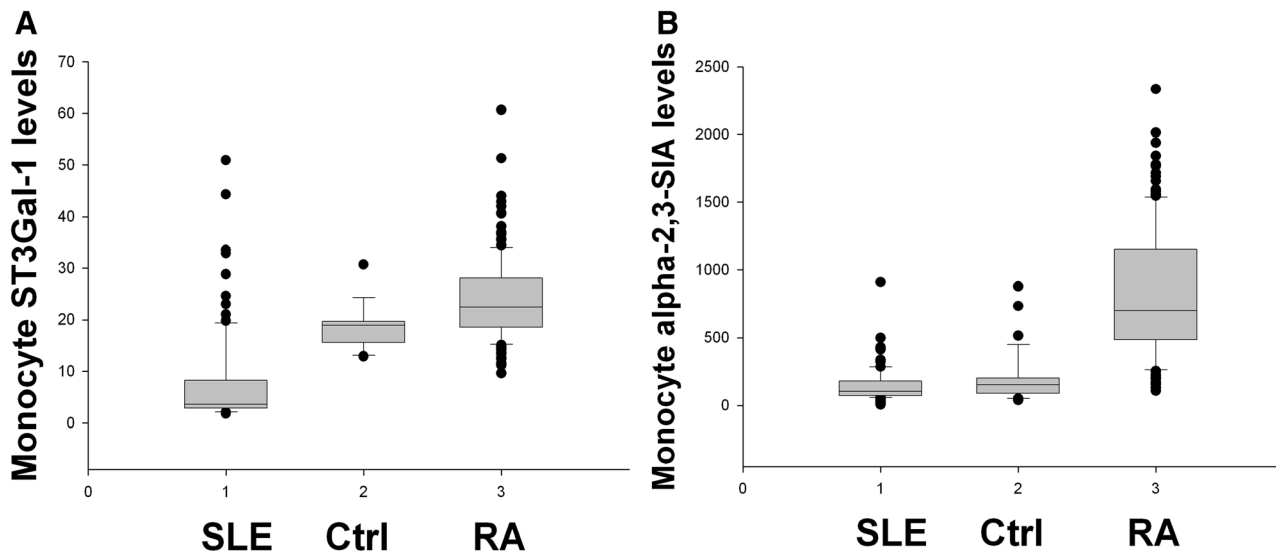


Fig. 2 Comparison of monocyte α -2,3-sialyltransferase 1 (ST3Gal-1) and α -2,3-sialic acid (SIA) levels among rheumatoid arthritis (RA) patients, controls, and systemic lupus erythematosus (SLE) patients. Monocyte ST3 and α -2,3-SIA levels indicated the mean fluorescence intensity of monocyte's ST3 and α -2,3-SIA staining results. *p* values calculated by Mann-Whitney U test for (A) ST3Gal-1 levels differed among groups. RA vs Ctrl gave *p* = 0.002; RA vs SLE rendered *p* < 0.001. B, α -2,3-SIA levels differed among groups. RA vs Ctrl gave *p* < 0.001; RA vs SLE rendered *p* < 0.001. The *p* values were still significant after Bonferroni's correction was applied for comparison of α -2,3-SIA levels among three groups to give corrected significant *p* at 0.017 (that is, 0.05 divided by three comparisons). Ctrl = healthy controls.

Table 3

Correlation between different variables and DAS28 in all RA patients at month 0 in multivariable analysis

	ESR	CRP	T cells	Monocytes	PMNs	Cells' variables
DAS28 scores	<0.001	0.753	0.514	0.668	0.962	ST6
	<0.001	0.731	0.787	0.220	0.412	Neu1
	<0.001	0.802	0.099	0.010	0.291	ST3
	<0.001	0.711	0.064	0.054	0.351	Neu3

p values for correlation (n = 157) by linear regressions for multivariable analysis with independent variables including ESR, CRP, and the four blood cell types' ST6 levels (B cell's data not shown here) and the dependent variable DAS28 as in Table 2. Similarly, Neu1, α -2,6-SIA, ST3, Neu3, and α -2,3-SIA from the four blood cell types plus ESR and CRP were analyzed against DAS28 by linear regression. Among multivariable analysis, monocyte ST3 correlated with DAS28 at *t* = 2.603 (95% CI, 0.008-0.056) and monocyte Neu3 correlated with DAS28 at *t* = 1.945 (95% CI, 0.000-0.048). In contrast, monocyte ST6 correlated with DAS28 at *t* = -0.429 (95% CI, -0.017 to 0.011) and monocyte Neu1 correlated with DAS28 at *t* = 1.232 (95% CI, -0.010 to 0.043). CRP = C-reactive protein; DAS28 = Disease Activity Score 28; ESR = erythrocyte sedimentation rate; Neu1 = neuraminidase-1; Neu3 = neuraminidase-3; PMN = polymorphonuclear; RA = rheumatoid arthritis; SIA = sialic acid; ST = α -2,6-sialyltransferase 1; ST3 = α -2,3-sialyltransferase 1.

and 3, and Results). Monocyte Neu3 levels increase along with arthritic activity, which could be because of their ability to deplete ganglioside GM3. Nonetheless, this hypothesis needs further studies. Thus, it opens up a new direction for bedside-to-bench studies. Sialylated phagocytes or antigen-presenting cells have been shown to confer less IgG binding, decreased phagocytosis, and less maturity.¹⁻³ Our study on monocytes revealed that ST3Gal-1 and cell surface α -2,3-SIA levels correlated positively with increasing

RA disease activity (Table 2 and Fig. 1). Interestingly, monocyte ST3Gal-1, Neu3, and cell surface α -2,3-SIA levels were significantly positively correlated with B cell ST3Gal-1, Neu3, and cell surface α -2,3-SIA (Results). In view of such correlations and similar results in RA B cells,¹⁷ the positive correlation of monocyte ST3Gal-1 and cell surface α -2,3-SIA levels with DAS28 is strengthened. Moreover, the positive correlation between monocyte ST3Gal-1 or Neu3 levels and DAS28 was consistent

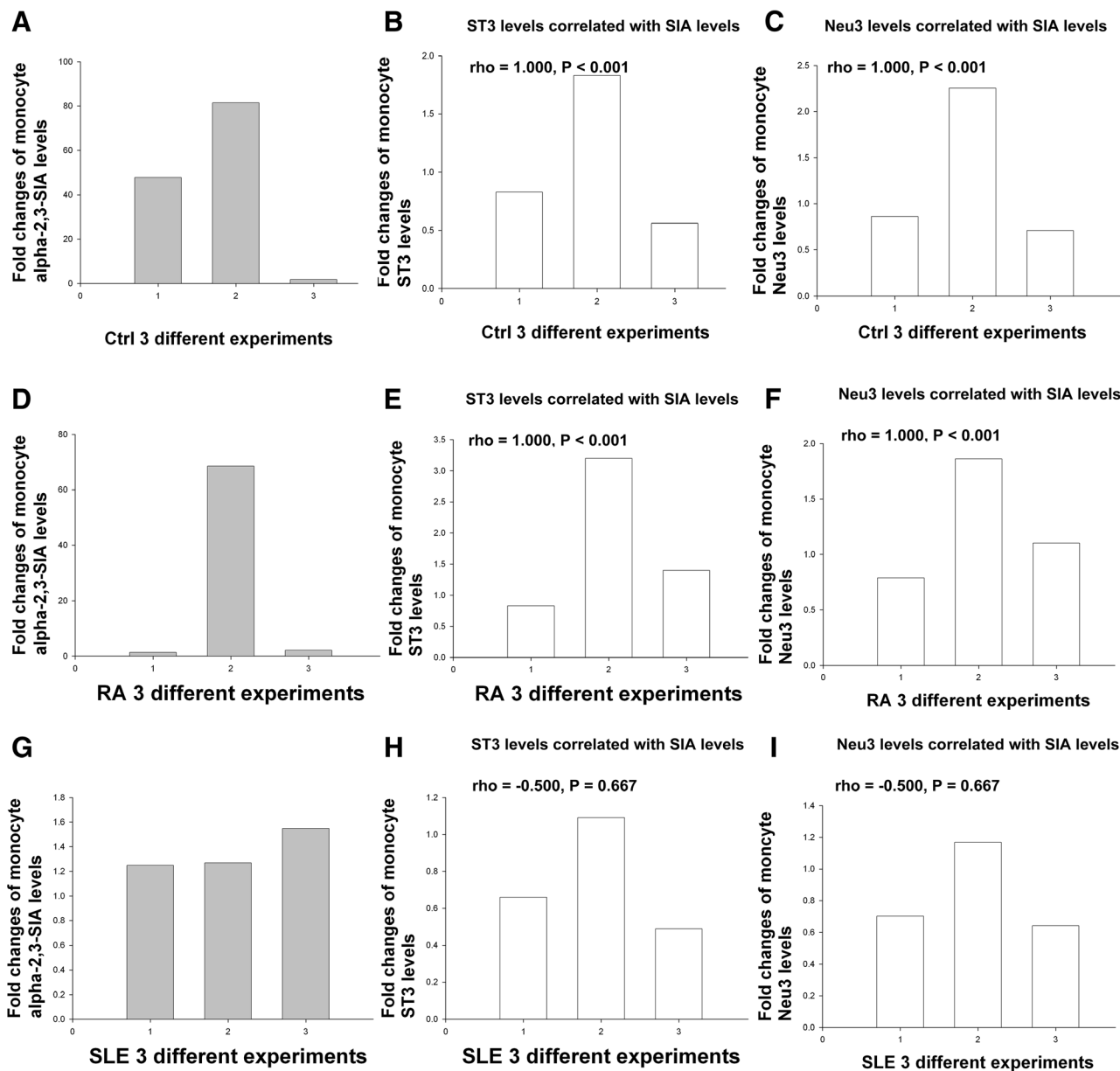


Fig. 3 Cytokine effects on α -2,3-sialic acid (SIA), α -2,3-sialyltransferase 1 (ST3Gal-1), and neuraminidase-3 (Neu3) levels of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) monocytes. Peripheral blood monocytes obtained were stimulated with either medium or IL-1 β (final concentration at 100 pg/mL) for 4 h. Then, these cells were rendered staining for ST3Gal-1, Neu3, α -2,3-SIA, and CD14. Monocyte α -2,3-SIA (A, D, G), ST3Gal-1 (ST3; B, E, H), and Neu3 (C, F, I) levels indicated the mean fluorescence intensity of monocyte's 2,3-SIA, ST3Gal-1, and Neu3 staining results. The sub-group of fresh monocytes (the baseline, not shown) of three different subjects was assigned as 1 for each subject group; experimental results were expressed as how many times of changes over the individual baseline (fresh monocytes) inside each subject group. Ctrl = healthy controls.

at different time points in multivariable analysis (Table 3 and Results). However, whether the contrary findings represent a biological difference between humans and mice¹⁻³ is currently unknown. Nevertheless, the positive correlation between monocyte Neu3 levels and DAS28 scores was further supported by following three findings. First, monocyte ST3Gal-1/Neu3 ratios did not correlate with DAS28 scores for all RA patients ($\rho = 0.022$ and $p = 0.781$) and for RA patients having DAS28 scores above 5.1 ($r = 0.125$ and $p = 0.424$). These results indicate that monocyte ST3Gal-1 and Neu3 levels did not change in an opposite direction on relating to DAS28 scores in RA patients. Second, the finding revealing that change of monocyte Neu3 levels after IL-1 β stimulation went in parallel to that of monocyte 2,3-SIA levels after IL-1 β stimulation (Fig. 3D and F) (similar to change of monocyte ST3 levels versus change of 2,3-SIA levels in Fig. 3D and E) in RA patients further supports the above results. Third, in

multivariable analysis, both monocyte ST3 and monocyte Neu3 levels correlated positively with DAS28 scores (Tables 2 and 3). That is, both changed in a same paralleled direction. Hence, how monocyte ST3Gal-1, Neu3, and α -2,3-SIA interact altogether in RA patients is an intriguing and important topic to pursue in the future, though α -2,3-SIA is definitely produced by ST3Gal-1³³ and removed by Neu3.³⁴ Nonetheless, what kinds of molecular mechanism to explain such difference between RA and SLE patients (SLE monocyte ST3Gal-1/Neu3 ratios correlated with SLE disease activity index at $\rho = 0.307$, but $p = 0.154$, and Liou and Huang¹⁷ with SLE B-cell ST3Gal-1/Neu3 ratios correlating significantly with SLE disease activity index) waits for a future sophisticated study³⁵ to decipher.

Monocyte Neu1 levels correlated with DAS28 in RA patients having DAS28 >5.1, but not in all RA patients (Results). In contrast, the correlation between ESR and CRP with DAS28 was

seen in all RA patients (Results), but not in RA patients having DAS28 >5.1 (Results). These results indicate that monocyte Neu1 correlated with DAS28 in RA patients with high disease activity, similar to monocyte Neu3 levels (Tables 2 and 3, Results, and Fig. 1) and B cell ST3 and Neu 3 levels.¹⁷

Good correlations between monocyte ST3Gal-1, Neu3, ST6Gal-1, and Neu1 levels (Results) and corresponding enzyme levels of RA B and T cells suggests a close interrelationship among the three immune cell types. These observations for monocyte and B cell ST3Gal-1 and Neu3 levels were not found in lupus patients.¹⁷ The mechanism underlying the difference between RA monocytes and B cells and the corresponding monocytes and B cells in patients with systemic lupus is worthy of further studies. Interestingly, cytokine IL-1 β revealed obvious differential effects on expression and correlation of ST3Gal-1 and α -2,3-SIA levels between RA and SLE patients (Fig. 3). The high responsiveness of some RA monocytes (the middle bar RA patient in Fig. 3C had DAS28 scores higher than 4.5, contrary to others with DAS28 scores <4.0), in contrast to SLE monocytes, to such a CRP-inducing cytokine may be partly explained by the cytokines being consistently enriched in the serum of RA patients.²¹ Future studies on these findings are waiting for further exploration.

In conclusion, monocyte ST3Gal-1 and Neu3 levels correlated with DAS28, analyzed by simple correlation analysis and linear regression analysis at different time points. Moreover, monocytes, B cells, and T cells correlated with each other for ST3Gal-1, Neu3, ST6Gal-1, Neu1, α -2,3-SIA, and α -2,6-SIA levels, indicating a close interrelationship. Therefore, monocyte ST3Gal-1 and Neu3 levels may be used as adjunctive biomarkers to monitor RA disease activity, pending further validation.

ACKNOWLEDGMENTS

This work was supported by Chang Gung Medical Foundation, Taiwan (CMRPG3C1641 and CMRPG390162). The funding source has no role in study design, in data collection and analysis, preparation of the manuscript, or decision to publish.

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