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Relationship between urinary sialylated saccharides, serum amyloid A protein, and C-reactive protein in rheumatoid arthritis and systemic lupus erythematosus

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SUMMARY The urinary excretion of sialic-acid-containing oligosaccharides, total sialic acid, serum amyloid A protein (SAA), and C-reactive protein (CRP) has been studied in 48 patients with rheumatoid arthritis (RA) and in 17 patients with systemic lupus erythematosus (SLE). Linear regression analysis revealed a close positive correlation between serum SAA and CRP levels in both RA ($r=0\cdot71$, $p<0\cdot001$) and SLE ($r=0\cdot86$, $p<0\cdot001$). The urinary excretion of sialyl lactose showed a positive correlation with the serum levels of SAA and CRP in RA ($r=0\cdot45$ and $r=0\cdot45$, respectively, $p<0\cdot01$) but not in SLE ($r=0\cdot05$ and $r=0\cdot10$ respectively). Changes in serum total sialic acid levels paralleled those in CRP and SAA in RA as well as in SLE. Patients with very active RA had higher urinary sialyl oligosaccharide excretion ($p<0\cdot001$), higher CRP levels ($p<0\cdot01$), and higher SAA levels ($p<0\cdot05$) than those with moderately active disease.

Endogenous sialylated low-molecular-weight saccharides are normal constituents of human urine.[1–3] Excretion of these oligosaccharides is considerably increased in tissue injury and inflammation.[3–5] We recently demonstrated that urinary sialyl-oligosaccharide content correlates closely with disease activity in rheumatoid arthritis (RA)[6 7] and in systemic lupus erythematosus (SLE).[8] In fact the results of our studies suggest that sialyl lactose and sialyl-N-acetyllactosamine may be low-molecular-weight acute phase reactants. The relationship between these oligosaccharides and serum acute phase proteins is not known.

C-reactive protein (CRP) is an acute phase reactant synthesised in the liver.[9] Its serum concentration rises in response to tissue injury and inflammation. CRP is structurally related to serum amyloid P component[10 11] and may play a role in modulating the inflammatory response.[12 13] In active RA increases in the serum level of CRP closely reflect disease activity.[14] In SLE the concentration of CRP is only modestly raised even in patients with very active disease.[15 16]

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Serum amyloid A protein (SAA), thought to be the precursor of the secondary amyloid fibril protein,[17 18] was recently shown to behave like an acute phase reactant.[19 20] High serum concentrations were detected in infections and inflammatory and neoplastic diseases.[19] Moreover, SAA levels seem to be a good indicator of disease activity in RA.[19 21]

We report here the relationship between the urinary content of sialic-acid-containing oligosaccharides and the serum levels of CRP and SAA in 48 patients with RA and in 17 patients with SLE.

Patients and methods

We studied 37 women and 11 men aged 28 to 82 years (mean 51 yr) with classical or definite RA, and 17 women aged 19 to 59 yr (mean 36 yr) with SLE. All patients fulfilled the American Rheumatism Association criteria for RA[22] or SLE.[23] All had normal serum creatinine levels. From clinical assessment (severity of symptoms, duration of early morning stiffness, joint status) and laboratory results (ESR and platelet count) the rheumatoid disease was considered to be very active in 25 patients, moderate in 13, and mild or almost inactive in 10. Ten of the 17 patients with SLE had a mild or inactive disease

on clinical (severity of symptoms) and laboratory (complement and anti-DNA antibody levels) assessment. Six had moderately active disease and one had very active disease. At the time of serum and urine sampling most patients were receiving some form of medication, the major drugs being steroids, cyclophosphamide, gold or nonsteroid anti-inflammatory drugs, or a combination of these.

Assay of urinary sialyl-oligosaccharides. The quantitative determination of urinary sialyl lactose and sialyl-N-acetyllactosamine was carried out as described elsewhere.[2 4] Briefly, the urine samples (usually 1–2 ml) were diluted and passed through a column system consisting of a Dowex-50 (H^+) mounted on a Dowex-1 (CH_3COO^-) column (size $0{\cdot}9\times3{\cdot}0$ cm). The acidic substances were eluted from the Dowex-1 column with 1 M pyridyl acetate buffer, pH 4·4. The sialyl linkages were then split by mild acid hydrolysis (0·025 M H_2SO_4, 80°C, 1 h). The neutral saccharides purified by anion exchange chromatography were analysed as their trimethyl sailyl derivatives by gas–liquid chromatography (Perkin-Elmer Model 900 gas chromatograph equipped with hydrogen flame ionisation detectors). The column used was 2·2% SE-30 (Applied Science Laboratory). Melibiitol (prepared from melibiose by borohydride reduction[24]) was used as an internal standard. The method permits calculation of the total amount of sialyl lactose and sialyl-N-acetyllactosamine. The values are expressed as monosialyl lactose and monosialyl-N-acetyllactosamine.

Assay of serum and urinary sialic acid. Serum sialic acid was measured without prior purification by the resorcinol method[25] as modified by Miettinen and Takki-Luukkainen.[26] Urinary sialic acid was determined after ion-exchange purification.[27] Serum and 24 h urinary samples were stored at −20°C until processed.

Measurement of CRP and SAA. Determinations were made by radial immunodiffusion in 1% agarose gel (phosphate buffered saline, pH 7·2) containing 2% antiserum against human CRP (Orion Diagnostica, Finland) and 10% antiserum against SAA (Atlantic Antibodies, USA). CRP reference serum (Behringwerke AG, FRG) and purified AA protein, a gift from Dr B. Skogen (Rikshospitalet, Oslo, Norway), were used as standards. The detection limit for CRP was 5 μg/ml and for SAA 1 μg/ml.

Statistical methods. The significance of differences between mean values was analysed by Student's *t* test. With respect to SAA and CRP the results were confirmed by Wilcoxon's rank sum test. Linear regression analysis by the least square method was used to study the relationship between 2 variables. CRP values below 5 μg/ml and SAA values below 1 μg/ml were set to 1 μg/ml in the statistical calculations.

Results

Relationship between serum concentrations of SAA and CRP. There was a highly significant positive correlation between SAA and CRP levels in both RA and SLE (Table 1). Mean concentrations of SAA and and CRP were higher in RA than in SLE (Table 2).

Relationship between urinary excretion of sialyl lactose and serum levels of SAA and CRP. The urinary excretion of sialyl lactose correlated positively with the serum levels of CRP and SAA in RA but not in SLE (Table 1).

Relationship between urinary and serum total sialic acid and serum SAA and CRP. Urinary total sialic acid output correlated with the serum levels of both SAA and CRP in RA (Table 1) but not in SLE (Table 1). Serum total sialic acid showed a linear relationship with both CRP and SAA in RA as well as in SLE (Table 1).

Variation of urinary output of sialyl lactose and serum levels of SAA and CRP with disease activity. Patients with very active RA excreted more urinary

Table 1 *Linear regression analysis of the relationship of serum CRP and SAA to each other and to urinary sialyl lactose, urinary total sialic acid, and serum total sialic acid in 48 patients with RA and in 17 patients with SLE*

	RA		*SLE*	
Variables	*r*	*p*	*r*	*p*
SAA vs CRP	0·71	<0·001	0·86	<0·001
SAA vs U sialyl lactose	0·45	<0·01	0·05	NS
CRP vs U sialyl lactose	0·45	<0·01	0·10	NS
SAA vs U total sialic acid	0·48	<0·001	0·06	NS
CRP vs U total sialic acid	0·33	<0·05	0·12	NS
SAA vs S total sialic acid	0·44	<0·01	0·68	<0·01
CRP vs S total sialic acid	0·65	<0·001	0·70	<0·01

U=urinary. S=serum. NS=not significant.

Table 2 *Mean (±SE) serum concentrations of CRP and SAA in RA and SLE*

	No. of patients	*CRP μg/ml*	*SAA μg/ml*
RA	48	31·4±4·5*	69·6±13·3†
SLE	17	8·1±3·2	20·2±10·1

* p<0·01. † p<0·05.

Table 3 *Mean (±SE) urinary output of sialyl lactose, and serum levels of CRP and SAA, in 48 patients with RA of varied activity*

RA disease group	*No. of patients*	*Urinary sialyl lactose mg/ 24h*	*Serum CRP µg/ml*	*Serum SAA µg/ml*
A. Very active	25	43·8±3·4*†	48·8±6·1‡†	108·7±21·0§**
B. Moderately active	13	21·2±1·2″	14·6±6·2¶	35·0±15·2¶
C. Mild or inactive	10	17·9±1·6	7·3±3·2	11·0±5·5

* $p<0·001$ as compared to group B.
† $p<0·001$ as compared to group C.
‡ $p<0·01$ as compared to group B.
§ $p<0·05$ as compared to group B.
″ $p<0·05$ as compared to group C.
¶ p NS as compared to group C.
** $p<0·01$ as compared to group C.

Table 4 *Mean (±SE) urinary sialyl lactose, serum CRP, and SAA in patients with SLE*

SLE GROUP	*No. of patients*	*Urinary sialyl lactose mg/24 h*	*Serum CRP µg/ml*	*Serum SAA µg/ml*
Mild/inactive disease	10	22·2±2·0	6·7±2·6	13·1±4·0
Very/moderately active disease	7	59·2±5·7*	9·1±5·9†	27·8±20·1†

* $p<0·001$. † N.S.

sialyl lactose ($p<0·001$), and had higher serum levels of CRP ($p<0·01$) and SAA ($p<0·05$) than patients with moderate disease (Table 3). These differences were even greater when patients with very active disease were compared with those in whom RA was mild or inactive (Table 3).

The mean levels of CRP and SAA did not differ significantly in patients with very or moderately active and mild or inactive SLE. The urinary excretion of sialyl lactose, however, was significantly higher in patients with very or moderately active SLE (Table 4).

Discussion

Results of our earlier studies showed that in both RA[6 7] and SLE[8] the urinary output of 2 sialic-acid-containing trisaccharides, sialyl lactose and sialyl-N-acetyllactosamine, is markedly increased and that this increased excretion is associated with disease activity. These and related observations[3–5 28] suggest that urinary sialyl oligosaccharides may be low-molecular-weight acute phase reactants. The present results reveal important differences in the relationship between these urinary acid saccharides and established acute phase reactants in RA and SLE. In patients with RA changes in sialyl lactose excretion paralleled changes in the serum levels of SAA and CRP. Patients with active, aggressive RA had a significantly higher urinary sialyl lactose content and serum CRP and SAA levels than patients with mild or inactive disease. In SLE sialyl lactose excretion correlated neither with serum CRP nor with SAA. Sialyl lactose excretion did, however, show a positive association with disease activity in SLE. These observations suggest that changes in sialyl lactose output and in the serum levels of CRP and SAA may be controlled by different mechanisms.

A positive correlation between serum levels of CRP and SAA has previously been reported in ethiocholanolone induced inflammation[29] and RA.[21] Scheinberg *et al.*[30] found no clear correlation between CRP and SAA in lepromatous leprosy. Our results, which corroborate those of van Rijswijk *et al.*,[21] indicate a strong positive correlation between CRP and SAA in RA. Our results also suggest a close correlation between serum levels of CRP and SAA in SLE.

Serum and urinary total sialic acid levels behaved differently in RA and SLE. Serum total sialic acid, which mainly represents protein-bound sialic acid, correlated positively with the acute phase proteins in both RA and SLE. This is explained by the fact that in serum protein electrophoresis sialic acid is confined mainly to the α-globulin fractions, which also contain acute phase reactants. In contrast, changes in urinary total sialic acid paralleled changes in urinary sialyl lactose: urinary total sialic acid output correlated with CRP and SAA in RA, but not in SLE.

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