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Relative sialylation and fucosylation of synovial and plasma fibronectins in relation to the progression and activity of rheumatoid arthritis

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Abstract The expressions of terminal sugars in synovial and plasma fibronectins were studied in relation to rheumatoid arthritis (RA) progression defined according to the early, established and late radiological changes in the patients' hands. The relative amounts of sialic acid and fucose were analyzed by lectin-ELISA using appropriate sialic acid-linked α2-3 (*Maackia amurensis*) and α2-6 (*Sambucus nigra*) lectins as well as fucose-linked α1-6 (*Aleuria aurantia*), α1-2 (*Ulex europaeus*), and α1-3 (*Tetragonolobus purpureus*). In the early RA group, the synovial fibronectin reactivities were the lowest with the all lectins used. In the established and late groups, relative sialylation and fucosylation significantly increased. However, sialylation negligibly decreased, whereas fucosylation remained at nearly the same level in the late group. Moreover, the expression of α1-6-linked fucose was found to be related to disease activity. In contrast, plasma fibronectin reactivity with lectins showed different dynamic alterations. In the early RA group, the reactivity of fibronectin with the lectins used was similar to that of healthy individuals, whereas it increased significantly in the established RA group compared with the early and normal plasma groups. In the late RA group it decreased to a level similar to that of the normal group. The lower expressions of terminal sugars in synovial fibronectin were mainly associated with the early degenerative processes of RA. In conclusion, such alterations may be applicable as a stage-specific marker for diagnosis and therapy of RA patients. The higher expression of terminal sugars in fibronectin could be associated with repair and adaptation processes in longstanding disease.

Keywords Fibronectin · Rheumatoid arthritis · Sialylation · Fucosylation · Synovial fluid

Abbreviations

RA	rheumatoid arthritis
FN	fibronectin
MAA	lectin from *Maackia amurensis*
SNA	lectin from *Sambucus nigra*
AAA	lectin from *Aleuria aurantia*
LTA	lectin from *Tetragonolobus purpureus*
UEA	lectin from *Ulex europaeus*

Introduction

Fibronectin (FN) is a large, modular, multi-domain and multifunctional glycoprotein ubiquitous in the extracellular matrix of tissue as an insoluble macromolecular fibrillar component and present in the form of a soluble compact dimer in plasma and body fluids including synovial fluid [9]. Plasma FN is produced in the liver, whereas cellular FN is produced by fibroblasts and many other cell types. In tissue, FN is secreted as a dimer, which is subsequently captured by cell surface integrin receptors and then irreversibly assembled into the fibrillar matrix via interactions with other matrix components and/or with itself [41]. The interaction of soluble

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fibronectin with a cell surface and the extracellular matrix is mediated by a carbohydrate-to-carbohydrate interaction [43].

Each monomer of FN consists predominantly of three types of repeating segments, which are organized in a series of functional domains with specific binding affinities for biologically active molecules, including collagen, heparin, fibrin and cell surface receptors. The binding of FN with ligands allows FN to function as a connecting molecule by mediating cell–matrix and matrix–matrix interaction. FN participates in such critical cellular processes as adhesion, migration, cell spreading, matrix remodeling, tissue repair, wound healing, and in the communication between extracellular matrix proteins and components of the immune system [41]. FN is also involved in biochemical pathways of the regulatory processes of cartilage metabolism [4, 16].

The significant heterogeneity of FN arises from alternative splicing of the primary transcript in the ED–A, ED–B, and V, or variable (also named IIICS), regions. A cartilage-specific isoform lacking of internal peptide sequences in its IIICS segment has also been recently described [8, 46]. FN polymorphism is also due to post-translational glycosylation. FN consists of 5–9% of carbohydrates attached by N- and O-glycosidic bonds, however the extent and type of its glycosylation varies depending on the tissue source and cell type [18]. Characteristic glycosylation profiles have been identified for the plasma and cell matrix [7] and embryonal forms of FN [24]. Most of the N-glycans of human plasma FN are conserved and have seven potential N-glycosylation sites, which are located on the II and III modules in cellular-binding domain, and three in collagen-binding domain [26, 37]. The major N-glycan of cellular and plasma FNs is a bi-antennary complex type structure, although significant minor micro-heterogeneity has been detected between these isoforms. The heavily sialylated bi-antennary N-glycans of plasma FN have α2-6-linked sialic acid at the terminal galactose residue, whereas cellular FN is less sialylated and contains sialic acid attached by α2-3 linkage [12, 23]. Only two N-glycans of plasma FN undergo fucosylation, whereas cellular FN contains fucose residues at six positions of N-glycosylation [37]. However, cellular FN contains α1-6-linked fucose to the innermost *N*-acetylglucosamine of the chitobiosyl core which is nearly absent in plasma FN [12]. O-glycosidic-bound glycans were detected in both the N- and C-terminal halves of cellular and plasma FNs [7], and a new one at Thr279 in the connecting segment between the fibrin–heparin binding domain and the collagen-binding domain [37]. Moreover, a hexapeptide of a unique variable, extra-IIICS region contains α2-3-sialylated O-glycan, which is claimed to be characteristic of oncofetal tissues [25, 37]. Synovial FN derived from patients with RA differs from plasma FN in charge, molecular weight, carbohydrate content (6.9%), and the presence of expressed splice variants [7, 27, 46]. It contains an oncofetal glycosylated segment [24], at least one more N-glycan unit per monomer, and another O-glycosylation site, as well as the glycans are less sialylated [5].

Rheumatoid arthritis (RA) is a complex autoimmune disease characterized by persistent inflammation of the synovial joint, which leads to progressive cartilage destruction by matrix degradation products [46]. In inflamed RA tissue FN is readily degraded into fragments by proteinases. Several studies have shown that its proteolytic fragments may greatly contribute to the pathological state of RA [4, 16, 17]. Our last study have showed [28], that the profound degradation of FN and the low immunoreactivity of some of its domains with monoclonal antibodies were associated with early, but not with late, destructive changes observed in radiographs of RA patients' hands.

In the present study the expressions of sialyl- and fucosyl-glycotopes in FN of synovial fluid and blood plasma were studied in relation to RA progression specified with respect to the radiographs of the patient's hands as well as to RA activity determined by C-reactive protein level. The relative amount of sialic acid linked to FN glycans by α2-3 and α2-6 isomeric linkages and of fucose attached to glycans by α1-6, α1-3 and α1-2 isomeric linkages were determined by enzyme-immunosorbent assay (Lectin-FN-ELISA) using lectins with well-known sugar specificity from *Maackia amurensis* and *Sambucus nigra* and from *Aleuria aurantia*, *Tetragonolobus purpureus* and *Ulex europaeus*, respectively. Our intention was not to determine the "true" structure of the carbohydrate units on human fibronectins, but alterations in the relative amounts of accessible glycotopes for reaction with lectins. Such an observation mimics a similar type of interaction, which could occur between sialyl- and fucosyl-glycoconjugates and their specific receptors in vivo.

Material and methods

Patients and samples

Fifty-eight patients (21–78 years old, mean age 52±14 years), who were attending the Rheumatology Clinic of the Wrocław Medical University and who fulfilled the 1987 American Rheumatism Association criteria for RA [1] were included in the present study after they had given their informed consent. The study was approved by the local ethics committee (approval no 780/2004). All patients were evaluated from the results of clinical examination, plain X-rays of the hands, and routine laboratory blood plasma parameters including CRP determination as a measure of disease activity [13]. The duration of disease was from 2 months to 20 years. Patients who had traumatic, septic, or microcrystalline arthritis, previous joint surgery, or isotopic synovectomy within the 12 months before the study were excluded.

Synovial fluid and blood were drawn from the RA patients into plastic syringes and placed directly into tubes containing 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma Chemical, St Louis, MO, USA). The samples were centrifuged and the synovial fluid supernatants and blood plasma were stored in small aliquots at −78°C until used.

The samples were divided into three groups with respect to the radiographic outcome by scoring the X-rays of the patients' hands [31] and to the immunoblotting pattern (Fig. 1) of the RA synovial fluids described earlier [28]:

1. The early group (15 synovial fluids and 15 plasmas): the patients had early radiological changes, described as soft-tissue swelling symmetrically around the involved joints, subtle juxta-articular osteoporosis, and small erosions of the "bare" areas of bone. All patients had suffered from 2 months up to 2 years, 60% of them had C-reactive protein (CRP) concentration lower than 5 mg/l, and 47% were RF positive. As is shown in Fig. 1 and described earlier [28] 80% of the synovial samples (Fig. 1, lane 2) contained FN totally degraded to FN fragments having molecular weights from 65 to 150 kDa and the remaining 20% had half of the molecules intact and the rest were degraded to fragments with molecular weights ranging from 100 to 200 kDa.

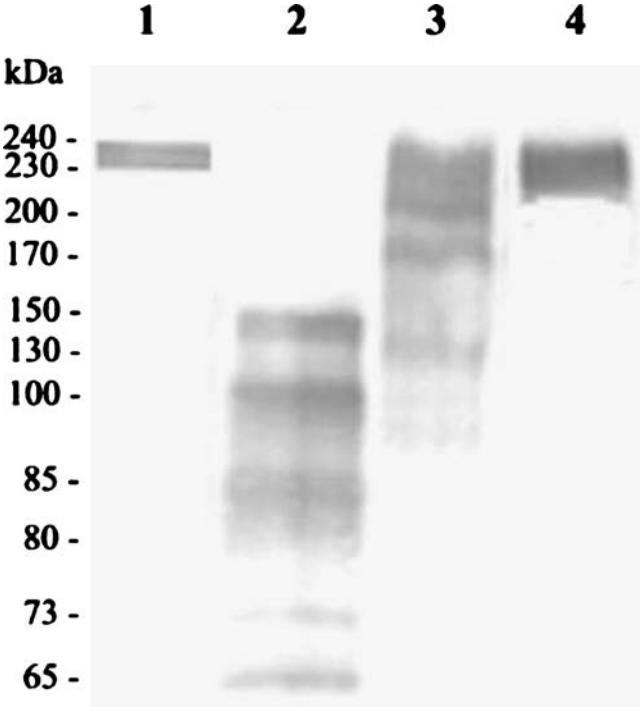

Fig. 1 Characteristic pattern of synovial and plasma FN immunoblotting [28]. SDS-PAGE in 7.5% gel was done under reducing conditions. The immunoblots were developed with monoclonal antibody directed to cellular domain of FN (TaKaRa Biomedicals, Tokyo, Japan). The lanes represent the patterns of normal plasma (*lane 1*), the synovial FN of RA patients with early (*lane 2*), established (*lane 3*) and late (*lane 4*) changes classified according to radiographs of the patients' hands [31]

2. The established group (15 synovial fluids, 25 plasmas): the radiological changes were as in early RA, but the erosions were established. The patients had suffered from 4 months to 20 years with 70% suffering more than 2 years. CRP concentration was lower than 5 mg/l in 66% of the samples and 34% of the samples were RF positive. All analyzed synovial samples contained a portion (68%) of intact FN (bands 240 and 230 kDa) and a portion of FN degraded up to 5 fragments showing molecular weights from 100 to 200 kDa (Fig. 1, lane 3).
3. The late group (11 synovial fluids, 18 plasmas): the patients had progressive radiological changes such as joint-space loss, decrease in soft-tissue swelling, diffuse osteoporosis, large subchondral erosions, subluxation and fibrous ankylosis of the digits. All patients had suffered more than 2 years. CRP concentration was lower than 5 mg/l in 34% of the samples and 66% were RF positive. All 11 synovial samples (Fig. 1, lane 4) contained the intact FN molecule and 3 of them revealed additionally one FN fragment having a molecular weight of 200 kDa (not shown).

A normal group was formed by collecting blood plasma from 15 healthy individuals, Wrocław Medical University research workers, 20–57 years old, mean age: 45±9 years, median age: 45 years. All plasma samples from healthy individuals (Fig. 1, lane 1) and RA patients showed a wide FN band as in normal plasma (not shown).

Quantification of FN

The FN concentration was determined based on the immunoreactivity with mouse monoclonal antibody (TaKaRa Biomedicals, Tokyo, Japan) specific to the cellular FN domain (FN 30-8; M010) by sandwich type solid phase-enzyme linked immunoassays (FN-ELISA) according to the procedure described earlier [28].

Lectin-FN- ELISA for differentiating sialyl- and fucosyl- glycoforms

Sialyl- and fucosyl-glycotope expressions on FN were determined by lectin-FN-ELISA using biotinylated lectins (Vector Laboratories, Burlingame, USA) with well-defined binding preference according to a procedure described earlier [15]. FN was caught from synovial and plasma samples directly on ELISA plate by partly deglycosylated anti-FN antibodies [21]. Sialic acid linked to N- and O-glycans by α2-3 [22] and/or, α2-6 [35] isomeric glycosidic linkages were recognized by *Maackia amurensis* agglutinin (MAA) and SNA (*Sambucus nigra* agglutinin)- respectively. Fucosylation was analyzed by three lectins: AAA (*Aleuria*

aurantia agglutinin), UEA (*Ulex europaeus* agglutinin, lectin I), and LTA (*Tetragonolobus purpureus* agglutinin), but their binding abilities are substantially different. AAA recognizes α1-6-linked fucose to the core *N*-acetylglucosamine of N-glycans, but it also interacts less strongly with α1-2- and α1-3-linked fucoses to outer oligosaccharides of N- and O-glycans [44]. LTA (*Tetragonolobus purpureus* agglutinin) and UEA (*Ulex europaeus* agglutinin) are known to have main binding preference to fucose linked by α1-3 [45] and α1-2 [2] glycosidic bonds in N- and O-glycans respectively. However, the terminal sialic acid limits fucose binding by UEA [2] and sialylation as well as α1-2 fucosylation prevent fucose-linked α1-3 binding by LTA [42, 45].

The experimental details of lectin-FN- ELISA are as follows: rabbit anti-human FN antibodies partly deglycosylated by oxidation with sodium periodate [21] were diluted in 10 mM Tris, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 8.5, and coupled to a polystyrene microtiter ELISA plate and used to specifically bind FN of the sample. For the test, 100 μl of synovial fluid or blood plasma samples were taken which were prediluted in 10 mM Tris, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% Tween 20, and 0.5% glycerine, pH 7.4, to a FN concentration of 5 mg/l. The presence of the respective glycotope on FN was detected by reaction with specific biotinylated lectin. The FN-lectin complex was quantitated with phosphatase-labeled ExtrAvidin (Sigma, St. Louis, MO, USA) and then detected by the reaction with di-sodium 4-nitrophenyl phosphate (Merck, Darmstadt, Germany). The absorbance was measured on a Stat Fax 2100 Microplate Reader (Awareness Technology, USA) at 405 nm with the reference filter at 630 nm. All samples were analyzed in duplicate.

Control measurements were performed to demonstrate the specificity of a lectin as well as the absence of detectable endogenous reactive materials. The positive controls consisted of: an asialohaptoglobin preparation derived from ovarian cancer fluid [19] for AAA, LTA, UEA, transferrin for SNA (Boehringer Mannheim from Glycan Differentation Kit, and mouse glycophorin for MAA (a gift from Dr. Hubert Krotkiewski, Institute of Immunology and Therapiae Experimantalis, PAN, Wrocław, Poland). The negative control was a human albumin preparation (Sigma, St.Louis, MO, USA) included in the test instead of an synovial fluid sample. The background intensity was low (~0.05 AU) when the TBS was included in the tests instead of reagents: (1) lectin, (2) ExtrAvidin-AP, and (3) synovial or plasma samples, respectively. The background absorbances at $A_{405\ nm}$ (with buffer instead of synovial fluid or blood plasma, but with all other reagents) ranged from 0.06–0.09 for SNA, from 0.09–0.15 for MAA, from 0.08–0.1 for AAA, form 0.04–0.05 for LTA, and from 0.04–0.06 for UEA, depending on the microtiter plate and day of experiment.

Statistics

Data are presented as means ± standard deviations (SD). Comparisons between groups were performed by means of the Mann–Whitney *U* test. p values <0.05 were regarded as significant. Correlations were calculated by the Spearman test.

Results

The FN glycoform expressions based on analyses of the relative reactivities of synovial and plasma FN with sialyl- and fucosyl-specific lectins in relation to RA progression are shown in Tables 1 and 2 and in relation to disease activity in Table 3.

Sialylation

In the synovial fluid (Table 1) of the early RA group, most of the samples showed very low FN reactivity with MAA and SNA, lectins specific to sialic acid linked to glycans at the terminal position by α2-3 (0.05±0.05 AU) and α2-6 glycosidic linkages (0.15±0.2 AU), respectively. In the established RA group, the relative FN reactivities with both sialic acid-specific lectins increased significantly to the values of 0.28±0.3 AU (p<0.02) and 0.54±0.4 AU (p< 0.01), respectively. In the late RA group, the FN reactivities decreased slightly to the values of 0.12±0.2 AU with MAA and 0.24±0.3 AU with SNA.

In the blood plasma (Table 2) of the early RA group, the relative reactivities of FN with MAA (0.49±0.7 AU) and SNA (0.58±0.8 AU) were at the same level as those derived from healthy individuals (0.42±0.4 AU and 0.63±0.6 AU, respectively). The FN reactivity with MAA and SNA was found to be significantly higher in the established RA group (0.76±0.5 AU, p<0.01 and 1.02±0.7 AU, p<0.007, respectively) than those in the early group and similar to those in the late RA group (0.52±0.5 AU and 0.81± 0.7 AU, respectively).

Fucosylation

In the synovial fluid (Table 1) of the early RA group, the relative FN reactivities with AAA, UEA and LTA, lectins specific to core α1-6-linked fucose (0.43±0.4 AU) and the outer fucoses linked by α1-2 (0.03±0.04 AU) and α1-3 (0.14±0.1 AU) bonds were significantly lower than those in the established (AAA, 0.72±0.3 AU, p<0.03; UEA, 0.12± 0.1 AU, p<0.007; LTA, 0.24±0.1 AU) and late groups (AAA, 0.86±0.4 AU, p<0.009; UEA, 0.12±0.1 AU, p< 0.007; LTA, 0.24±0.2 AU), respectively.

In the blood plasma (Table 2) of the early RA group, the relative reactivities of FN with AAA (0.62±0.3 AU), UEA

Table 1 Relative synovial FN sialylation and fucosylation in relation to RA progression

Lectin and its binding preference	Relative reactivity of synovial FN with lectin in groups (AU)		
	Early RA (n=15)	Established RA (n=15)	Late RA (n=11)
MAA sialic acid-linked α2-3	0.05±0.05	0.28±0.3[a] p<0.02	0.12±0.2
SNA sialic acid-linked α2-6	0.15±0.2	0.54±0.4[a] p<0.01	0.24±0.3
AAA fucose-linked α1-6<α1-2<α1-3	0.43±0.3	0.72±0.3[a] p<0.03	0.86±0.4[a] p<0.009
UEA fucose-linked α1-2<α1-3<α1-4	0.03±0.04	0.12±0.1[a] p< 0.007	0.12±0.1[a] p<0.007
LTA fucose-linked α1-3	0.14±0.1	0.24±0.1	0.24±0.2

The early, established and late groups were determined according to radiographs of the patients' hands [31]. The relative reactivity of FN was measured by lectin-FN-ELISA [15] using sugar-specific lectins. The results are expressed in absorbance units as the absorbance difference at 405 nm (ΔAU, mean value ± SD) of the sample and the appropriate background absorbance.
[a] Significantly different from the early RA group.

(0.11±0.1 AU), and LTA (0.15±0.1 AU) were similar to those in normal (0.58±0.2 AU, 0.13±0.1 AU, and 0.21±0.1 AU, respectively) and late (0.75±0.3 AU; 0.14±0.1 AU, and 0.21±0.2 AU, respectively) RA groups. However they were lower than those in the established RA group (AAA, 0.93±0.4 AU, p<0.01 vs early and p<0.00002 vs normal; UEA, 0.24±0.2 AU, p<0.002 vs early and p<0.004 vs normal; LTA, 0.42±0.3 AU, p<0.0004 vs early and p<0.0005 vs normal). In the late RA group the FN reactivities with LTA (0.21±0.2 AU, p<0.01) and with AAA and UEA (0.75±0.3 AU and 0.14±0.1 AU, respectively) were lower than those in the established group.

FN glycoform expression in relation to disease activity

The relative synovial and plasma FN reactivities (Table 3) with sialic acid- (MAA and SNA) and fucose-specific (UEA and LTA) lectins did not differ when the results were compared in groups defined according to CRP concentration (lower than 5 mg/l and higher than 5 mg/l). Only synovial FN reactivity with AAA, but not plasma FN, was significantly lower (0.46±0.3 AU) in the group with CRP concentration below 5 mg/l than in the group with CRP concentration above 5 mg/l (0.77±0.4, p<0.02), and the relative amount of α1-6 linked-fucose in synovial FN showed a positive correlation (r=0.4) with the CRP level.

Discussion

A previous study from our laboratory [28] claimed that the profound degradation of synovial FN and lower expression of some of its domains were only associated with early degenerative changes visible in the radiographs of the rheumatoid arthritis patients' hands. Here, analyzing the same patients' samples, we found that the expression of terminal sugar residues on glycans of the synovial FN was also related to the RA stages, *i.e.* the degree of the relative sialylation and fucosylation of synovial fibronectin was low in early stage of rheumatoid arthritis.

Many authors have reported that carbohydrate antigens, particularly those terminated by sialic acid and fucose are essential for cell–cell and cell–substrate interactions and for receptor-mediated intracellular signaling and that they can exhibit diverse function, promote a growth and differentiation of the tissues, and can also regulate the secretion and sorting of hepatic glycoproteins into circulating ducts [5,

Table 2 Relative plasma FN sialylation and fucosylation in relation to RA progression

Lectin and its binding preference	Relative reactivity of plasma FN with lectins in groups (AU)			
	Early RA (n=15)	Established RA (n=25)	Late RA (n=18)	Normal (n=46)
MAA sialic acid-linked α2-3	0.49±0.7[a] p<0.01	0.76±0.5[b] p<0.001	0.52±0.5	0.42±0.4
SNA sialic acid-linked α2-6	0.58±0.8[a] p<0.007	1.02±0.7[b] p<0.006	0.81±0.7	0.63±0.6
AAA fucose-linked α1-6<α1-2<α1-3	0.62±0.3[a] p<0.01	0.93±0.4[b] p<0.00002	0.75±0.3	0.58±0.2
UEA fucose-linked α1-2<α1-3<α1-4	0.11±0.1[a] p<0.002	0.24±0.2[b] p<0.004	0.14±0.1	0.13±0.1
LTA fucose-linked α1-3	0.15±0.2[a] p<0.0004	0.42±0.3[b] p<0.0005	0.21±0.2[a] p<0.01	0.21±0.1

For experimental details, see the footnotes to the Table 1.
[a] Significantly different from the established RA group.
[b] Significantly different from the normal group.

Table 3 Relative synovial FN and plasma sialylation and fucosylation in relation to RA activity

Lectin and its binding preference	Relative reactivity of FN with lectins (AU)			
	Synovial fluid		Blood plasma	
	CRP <5 mg/l (n=18)	CRP >5 mg/l (n=24)	CRP <5 mg/l (n=37)	CRP >5 mg/l (n=21)
MAA sialic acid-linked α2-3	0.15±0.2	0.14±0.2	0.68±0.6	0.5±0.5
SNA sialic acid-linked α2-6	0.3±0.3	0.31±0.4	0.91±0.8	0.72±0.6
AAA fucose-linked α1-6<α1-2<α1-3	0.46±0.3 p<0.02	0.77±0.4	0.80±0.4	0.78±0.4
UEA fucose-linked α1-2<α1-3<α1-4	0.06±0.07	0.11±0.1	0.2±0.2	0.13±0.1
LTA fucose-linked α1-3	0.16±0.1	0.22±0.1	0.33±0.3	0.21±0.2

CRP <5 mg/l was assumed to represent low activity and CRP >5 mg/l was assumed to represent active disease.

14, 34]. Modified glycosylation of many glycoproteins is reported to be connected with diseases [10]. In rheumatoid arthritis high degrees of glycan variation were found in AGP [32, 36], IgG [3], haptoglobin [20] and FN [6, 7, 30]. The majority of the reported changes in glycosylation are related to the degree of glycan branching and the type of substitution of the N-linked glycans with sialic acid and/or fucose [39]. Fucosylation of synovial glycoconjugates is considered a clinical marker for differentiating joint disease [11]. Our results suggest that glycans in FN may play quite different roles at the beginning of RA and in the late phase.

It is well-known that the molecular events happening in the early and late phases of RA are quite different. In the early stages of RA, highly activated inflammatory cells, particularly macrophages are able to produce and release proinflammatory cytokines, nitric oxide, arachidonic acid metabolites, as well as matrix hydrolases [38]. All these factors penetrate cartilage leading to a cascade of events promoting inflammation and matrix destruction [16, 29]. The extracellular matrix components of cartilage become easily degraded [46]. Synovial FN, but not plasma FN, was found to be digested to a mixture of FN fragments in the early, but not late RA group. The fibronectin degradation was accompanied not only by low collagen, fibrin and C-terminal FN domain expressions [28], but also, as the present data indicate, by low expressions of α2-3- and α2-6-sialyl- and α1-6– α1-3-, and α1-2-fucosyl-glycotopes in synovial FN (Table 1).

In our opinion, the destruction of the most exposed, terminal monosaccharides of the glycan units in FN might have a dual biological consequence. Firstly, it could greatly facilitate FN susceptibility to further proteolytic degradation. The FN fragments which appear are known to act as inducers of cartilage destruction, they promote matrix metalloproteinase syntheses and monocyte migration and they stimulate the synthesis of proinflammatory cytokines [17, 46]. On the other hand, Homandberg [16] have shown in experiments *in vitro* that FN fragments could act as potential regulators of cartilage metabolism: high concentrations of FN fragments suppressed cartilage proteoglycan synthesis, while low concentrations enhanced synthesis. Therefore, we can speculate that the desialylated FN glycofragments present in inflamed tissue were removed from the circulation by a specific lectin-receptor for asialoglycoproteins [34] and thus the concentration of FN fragments became lower. Then they could start to act as stimulators of proteoglycan synthesis and could be involved in repair processes. It is known that extensive catabolic processes are transient and, with longstanding disease with inflammation still persistent, anabolic reactions start to develop in addition to the still active destructive processes [29, 38]. In consequence, the degradation of synovial FN comes to a stop and intact molecules of FN were mainly observed in synovial fluid by our group previously [28]. Also, as the results of the present study indicate, the expressions of the sialyl-, and α1-6- and α1-2-fucosyl-glycotopes increased several times more in the established RA group than in the early group (Table 1). According to Rees-Milton et al. [30], TGF-β1 mediated hyper-glycosylation of FN could regulate cartilage metabolism by providing protection of FN from proteolysis, a mechanism that would also favor articular cartilage health. However, at the same time the expression of α1-3-linked fucose, element of Lewis^x, increased only slightly (Table 1). This fact was probably connected with the specificity of LTA, but not connected with the structure of a true glycan. Namely, the heavily sialylated Lewis X structure are unable to react with LTA [45]. Also, the biosynthesis of α1-2-linked fucose, a characteristic monosacharide for bifucosylated Lewis^b and Lewis^y structures, prevents sialyl-Lewis^x formation [42].

In the late RA group, FN is very important in the modulation of cartilage tissue metabolism and is known to participate in tissue repair. FN forms fibrillar aggregates with itself and with a fibrin [33], creating a multimeric network adhering to the synovial surface [41]. FN fragments were not present in the synovial fluid of the late RA group and the FN immunopattern was found to be similar to that of native FN [28]. The expressions of terminal sugars in FN were only slightly modified, and this probably

resulted from a different accessibility of sugar residues for the reaction with the respective lectin. Sialic acid and fucose residues could be involved in the formation of the fibrillar network for matrix rebuilding and their reactivity with lectin was thus slightly lower (Table 1). However, the expression of the innermost fucose (linked by α1-6 bonding) was only found to be clearly related to the activity of RA measured by C-reactive protein concentration, *i.e.* the higher activity of disease in joints, the higher the expression of α1-6-linked fucose (Table 3, correlation coefficient: 0.4). The core type α1-6-fucosylation was reported to be essential for cellular remodeling processes [5] and to regulate epidermal growth factor receptor-mediated intracellular signaling [40].

The dynamics of the FN glycosylation alterations differs significantly between the synovial and plasma forms. In synovial fluid, directly at the site of inflammation, the changes reflect the local metabolic changes in the affected joints, which manifest earlier than in plasma. In contrast, plasma FN originates mainly from the liver and its level and status depends on changes which take place in the whole system. The plasma FN did not undergo degradation and the first observed changes were limited to higher expressions of all the analyzed glycoforms in the established RA group compared with both the early RA and normal control groups. This probably resulted from the mobilization of defensive and adaptive forces in the patients' organism in order to secure homeostasis.

In conclusion, the degree of degradation of the protein and glycan parts of fibronectin resulted from molecular events happening in the joint during the development and progression of RA. The lower expressions of sialic acids and fucoses in synovial fibronectin were mainly associated with the early degenerative processes of RA. The appearance of higher amounts of terminal sugars on synovial and plasma fibronectins could be associated with the repair and adaptation processes of longstanding disease. Analysis of the status of the FN molecule seems to present it as a promising candidate for a biochemical marker to discriminate between the stages of rheumatoid arthritis. Such information is of importance in making decisions about the best medical treatment.

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