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Terminal monosaccharide screening of synovial immunoglobulins G and A for the early detection of rheumatoid arthritis

Ewa Maria Kratz · Krzysztof Borysewicz ·
Iwona Kątnik-Prastowska

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Abstract The expressions of some terminal glycotopes of synovial immunoglobulins G, A, and M were analysed in relation to rheumatoid arthritis (RA) progression defined according to early and advanced radiological changes in patients' hands. The relative amounts of terminal monosaccharides were determined by lectin-immunoblotting of immunoglobulin preparations using appropriate lectins able to recognize α 2,6-linked (*Sambucus nigra* agglutinin) and α 2,3-linked (*Maackia amurensis* agglutinin) sialic acid, galactose (*Ricinus communis* agglutinin I), *N*-acetylglucosamine (*Griffonia simplicifolia* agglutinin II) as well as α 1,6-linked (*Aleuria aurantia* lectin), α 1,3-linked (*Lotus tetragonolobus* agglutinin), and α 1,2-linked (*Ulex europaeus* agglutinin) fucose. The results indicate differences between early and advanced RA stages in the terminal sugar exposition of synovial IgG and IgA, but not IgM. The galactose-deficient glycotope with exposed *N*-acetylglucosamine of the synovial 33.1-kDa IgG fragment appeared exclusively in the early stage of RA. In contrast, this glycotope of intact synovial IgG and IgA was present in both groups, although with higher proportions in advanced RA.

The proportions of the sialyl and fucosyl determinants of intact synovial A and G immunoglobulins were clearly lower in the early RA group than in the advanced. The analysis of terminal oligosaccharide exposition in IgG, IgG fragments, and IgA present in the synovial fluid of RA patients might be applicable as a stage-specific marker in the diagnosis and therapy of RA patients.

Keywords Immunoglobulins · Rheumatoid arthritis · Sialylation · Galactosylation · Fucosylation · Synovial fluid

Abbreviations

RA	Rheumatoid arthritis
SNA	Lectin from <i>Sambucus nigra</i>
MAA	Lectin from <i>Maackia amurensis</i>
RCA I	Lectin from <i>Ricinus communis</i>
GSL II	Lectin from <i>Griffonia simplicifolia</i>
AAL	Lectin from <i>Aleuria aurantia</i>
LTA	Lectin from <i>Lotus tetragonolobus</i>
UEA	Lectin from <i>Ulex europaeus</i>

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory and systemic disease which leads to joint inflammation and results in bone and cartilage erosion, tendon destruction, and joint dysfunctions and disabilities [1]. The aetiology of disease is not well defined, but a bacterial or viral background as well as an autoimmunological theory in which the immune system is activated after reaction with an unknown antigen is considered. One of the most characteristic symptoms of RA is the production autoantibodies to citrullinated proteins and to the Fc region of IgG, defined as rheumatoid factor (RF) (reviewed in [2]).

Some preliminary experiments described in this work were presented during the 2nd European Conference on Chemistry and Life Sciences, 2007, Wrocław, Poland.

E. M. Kratz (✉) · I. Kątnik-Prastowska
Department of Chemistry and Immunochemistry,
Wrocław Medical University, Bujwida 44a,
50-345 Wrocław, Poland
e-mail: kratz@immchem.am.wroc.pl

K. Borysewicz
Department of Rheumatology and Internal Diseases
of the University Clinical Hospital, Wrocław Medical University,
Borowska 213, 50-556 Wrocław, Poland

Extensive studies have shown that the mechanisms of RA could be associated with changes in IgG glycosylation. The alterations in IgG profile are confined to changes in the level of terminal galactose and number of antennas as well as in the relative proportions of sialic acid, fucose, and bisecting GlcNAc (reviewed in [3–5]). In RA, there is an increase in the number of oligosaccharide structures that lack the terminal galactose residue [3, 5]. Glyco-modifications of IgG molecules have been shown to affect various effector functions (i.e. immune complex formation, activation of complement, and binding to monocytes) and to have a critical role in the regulation of the immune response [5].

There is no single laboratory test available that helps to distinguish rheumatic diseases or the degree of RA progression, although certain serological tests seem to be associated with specific disease-related mechanisms. For the characterization of RA it is very important to distinguish between early and advanced RA stages and to determine progression and remission periods. Since the glycosylation of glycoproteins is sensitive to alterations in cellular function, changes in glycosylation can be used as a diagnostic value in a number of diseases, including rheumatoid arthritis [4–6]. A decreased level of glycan galactosylation in serum and synovial fluid IgG is known to be associated with disease progression and is used as a prognostic indicator for RA. Patients with arthritis and high levels of hypogalactosylated IgG have poor prognosis [4–7]. Finally, Pasek et al. [8] and Croce et al. [9] provided experimental evidence that the treatment of the RA patients with methotrexate or a combination of methotrexate and infliximab also improves the degree galactosylation of serum IgG and, in their opinion, the analysis of IgG galactosylation in RA patients may have clinical relevance.

The aim of this study was to investigate whether the main classes of immunoglobulins undergo degradation in the synovial fluid of RA patients in relation to rheumatoid arthritis progression defined according to early and advanced radiological changes in the patients' hands. Moreover, semiquantitative alterations in the expressions of the terminal glycotopes of synovial IgG, IgA, and IgM were studied in relation to RA progression. The relative amounts of terminal monosaccharides were analysed by lectin-immunoblotting of salted-out immunoglobulins using appropriate lectins able to recognize α 2,6-linked (SNA: *Sambucus nigra* agglutinin) and α 2,3-linked (MAA: *Maackia amurensis* agglutinin) sialic acid, galactose (RCA I: *Ricinus communis* agglutinin I), *N*-acetylglucosamine (GSL II: *Griffonia simplicifolia* agglutinin II) as well as α 1,6-linked (AAL: *Aleuria aurantia* lectin), α 1,3-linked (LTA: *Lotus tetragonolobus* agglutinin) and α 1,2-linked (UEA: *Ulex europaeus* agglutinin) fucose. However, the

data did not show the “true” glycan structures, but rather reflected the exposition of the conformationally accessible terminal monosaccharides of synovial immunoglobulins.

Materials and methods

Patients and samples

In the present study, the synovial samples were from 38 patients (22–72 years old, mean age 49 ± 12 years) who were attending the Rheumatology Clinic of Wrocław Medical University. The patients fulfilled the American Rheumatism Association criteria for RA [10] and they gave their informed consent. The patients were evaluated on the basis of a clinical examination, plain X-rays of the hands, and standard laboratory blood plasma parameters, including CRP determination as an indicator of disease activity. The duration of RA was from 2 months to 15 years. Patients who had isotopic synovectomy, previous joint surgery, or traumatic, septic, or microcrystalline arthritis within the 12 months before the study were excluded. The normal blood plasma samples (26 healthy volunteers; 37–79 years old; mean age 54 ± 15 years) were positive controls for analysis of the synovial immunoglobulin reactivities with lectins. The study was approved by the local ethics committee (approval no. 501/2006).

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

The patients' synovial samples were divided into two RA groups with respect to the radiographic outcome by scoring the X-rays of their hands [11]. The first was the early RA group (15 samples). 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 the patients had suffered from 2 months to 2 years, 47% were RF positive, 60% had a C-reactive protein (CRP) concentration lower than 5 mg/l, and, as was shown previously [12], fibronectin was degraded to fragments reactive with the monoclonal antibody against the cellular domain of fibronectin. The second group, with advanced RA, consisted of 23 patients with disease duration of from 2 months to 15 years and 83% of patients suffered more than 2 years. Fifty percent of the samples were RF positive and in 66% of the samples the CRP concentration was higher than 5 mg/l. They had established or late radiological RA changes. In most cases the patients had changes such as joint-space loss, decrease in

soft-tissue swelling, large subchondral erosions, subluxation, diffuse osteoporosis, and fibrous ankylosis of the digits. Forty-eight percent of the samples contained high-molecular fibronectin and the rest partially degraded fibronectin [12].

Equal volumes of synovial and blood plasma samples were pooled in each group and the immunoglobulin fractions were precipitated with ammonium sulfate to 40% saturation. The pellets were centrifuged for 30 min at 4,000g and the precipitates were washed twice using cold 1.75 M ammonium sulfate. Then, the precipitates were dissolved in 100 µl of water and the immunoglobulin concentration was measured by UV spectrophotometry and calculated using the molar absorption coefficient of 0.7.

SDS-electrophoresis and Western blot

The solution containing 2 µg of immunoglobulin preparation was denatured at 100°C for 5 min in the presence of 7.5% β-mercapto-ethanol and subjected to SDS-PAGE in a 12.5% gel, according to Laemmli [13]. After electrophoresis, the separated samples were transferred onto nitrocellulose (Serva Electrophoresis GmbH, Heidelberg, Germany) according to Towbin et al. [14] for 1.5 h (120 mA) and then blocked with 3% milk in TBS, pH 7.5. The blotted immunoglobulins were analysed in the reaction with specific antibodies and biotinylated lectins.

The reactivity of immunoglobulins with specific antibodies

To identify the immunoglobulin band positions in the lectin-blotting pattern, Western immunoblotting was performed as previously described [15]. Briefly, after the SDS-electrophoresis, protein transfer, and blocking steps, the respective blots were incubated with rabbit anti-human immunoglobulin M (IgM) (DAKO, Denmark; 2.5 µg/ml), rabbit anti-human immunoglobulin A (IgA) (DAKO, Denmark; 1.64 µg/ml), or the goat anti-human F(ab')₂ fragment of immunoglobulin G (IgG) (Jackson ImmunoResearch Laboratories, USA; 1 µg/ml) antibody. Then the blots were incubated with the respective anti-animal immunoglobulins conjugated with horseradish peroxidase (Sigma, St. Louis, MO, USA) diluted 1:5,000 with 3% milk in TBS, pH 7.5. The colour reaction was developed with diaminobenzidine. The molecular masses of the antibody-reactive bands were estimated using a standard mixture of seven proteins with defined molecular weights (Protein Test Mixture 6 for SDS PAGE; SERVA Electrophoresis, Germany): phosphorylase B (97.4 kDa), albumin bovine (BSA; 67 kDa), albumin egg (45 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (soybean; 21 kDa), cytochrome C (12.5 kDa), and trypsin inhibitor (bovine lung; 6.5 kDa).

The lectin-blotting of immunoglobulins

The reactivities of IgM, IgA, and IgG with lectins were analysed by Western blot using biotinylated lectins (Vector Laboratories Inc., Burlingame, USA). *Sambucus nigra* agglutinin [16] and *Maackia amurensis* agglutinin [17] are commonly used for α2,6-linked and α2,3-linked sialic acid determination, respectively. *Ricinus communis* agglutinin I [18] and *Griffonia simplicifolia* lectin II [19] are known to react with terminal galactose and *N*-acetylglucosamine residues, respectively. None of the fucose-specific lectins has an absolute specificity; however, *Aleuria aurantia* lectin [20] mainly reacts with the innermost α1,6-linked fucose to a core *N*-acetylglucosamine of *N*-glycans and with lower affinity with α1,2- and α1,3-linked fucoses of the outer arms, whereas *Lotus tetragonolobus* agglutinin [21] and *Ulex europaeus* agglutinin [22] are known to recognize α1,3-linked and α1,2-linked fucoses to the galactose or *N*-acetylglucosamine of the antennas, respectively. However, the terminal α2,3-sialic acid limits the binding of LTA to α1,3-linked fucose of Lewis^x structure [21] and the appearance of a α1,2-fucosylated structure reduces the attachment of α2,3-sialic acid to glycans [23].

The lectin-blotting details are as follows: after the SDS-electrophoresis, transfer onto nitrocellulose, and blocking and washing steps, the respective blots were incubated at room temperature for 1 h with biotin-labelled lectins diluted with TBS containing 0.1% Tween 20, pH 7.5 (TBS-T), to the following final concentrations: SNA 1 µg/ml, MAA 5 µg/ml, RCA I 0.5 µg/ml, GSL II 1 µg/ml, AAL 5 µg/ml, LTA 10 µg/ml, and UEA 10 µg/ml. The formed lectin-immunoglobulin complex was detected by the reaction with phosphatase-labelled ExtrAvidin (Sigma, St. Louis, MO, USA) diluted 1:10,000 in TBS-T. The colour reaction was developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma, St. Louis, MO, USA) diluted in TBS containing 0.05 M MgCl₂·6H₂O, pH 9.5, and stopped by deionised water. The blots were dried, scanned, quantified, and analysed. The lectin-blotting was done with 3–6 repetitions for each synovial RA preparation. The immunoglobulin bands of the lectin reactivity were quantified by densitometric analysis using GelScan Software version 1.45 (Kucharczyk Electrophoretic Techniques, Poland). The relative content of a lectin-reactive immunoglobulin band was expressed as the percentage of the total number of pixels in a lane. Non-immunoglobulin proteins (not reactive with anti-IgM, anti-IgA, and anti-IgG antibodies) were not analysed. The specificity of the lectins was checked in the reaction with positive controls, i.e. transferrin for SNA (the Glycan Differentiation Kit, Boehringer Mannheim, Germany), mouse glycophorin for MAA (a gift from Prof. Hubert Krotkiewski, Institute of Immunology and Experimental Therapy, Polish Academy

of Sciences, Wrocław, Poland), and an asialo-haptoglobin preparation derived from ovarian cancer fluid [24] for AAL, LTA, and UEA. A human albumin preparation (Sigma, St. Louis, MO, USA) was used as a negative control.

Results

The lectin-immunoblotting pattern of synovial fluid (Fig. 1) using SNA, MAA, RCA I, GSL II, AAL, LTA, and UEA shows the bands terminated, respectively, by α 2,6- and α 2,3-linked sialic acids, galactose, *N*-acetylglucosamine, and α 1,6-, α 1,3-, and α 1,2-linked fucoses expressed in the heavy chains of IgM (μ = 88 kDa), IgA (α = 65.3 kDa), and IgG (γ = 54.3 kDa) as well as in the 58-kDa IgA, 33.1-kDa IgG and 29.8-kDa IgG forms [15] and in the light chain of immunoglobulins (27.4 kDa) of patients with early and advanced stages of RA.

Table 1 comprises the results of the percent participation of the lectin-reactive bands of the synovial immunoglobulins (Fig. 1) in the early and advanced groups of RA.

The reactivity of synovial IgM with lectins

The relative content of the SNA-reactive bands of the μ chain (88 kDa) of IgM was similar in the early and advanced RA groups (13.3 and 13.2%, respectively), whereas the content of MAA-reactive bands was marginal in the early (2%) and advanced RA (0%) groups.

The RCA I- and AAL-reactive bands of IgM heavy chain were detected in both RA groups, and their proportions were slightly lower in the early RA group (8.6 and 10.6%, respectively) than in the advanced RA group (10.6 and 15.5%, respectively). In contrast, no reactivity of the μ -chain band with GSL II, LTA, or UEA was observed in either RA group (Table 1).

The reactivity of synovial IgA with lectins

In the early RA group the proportions of the 65.3-kDa IgA heavy chain band reactive with SNA (27.5%), MAA (8.3%), RCA I (22.3%), AAL (11.6%), and LTA (14.3%) were slightly lower than in the advanced RA group (36.5, 13.1, 32.1, 19.7, and 18.5%, respectively) (Table 1). However, the proportions of the GSL II- and UEA-reactive 65.3-kDa IgA heavy chain band were significantly lower in early RA (9.3 and 9.7%, respectively) than in the advanced RA (24.8 and 22.4%, respectively).

The relative content of the SNA-reactive 58-kDa IgA band was slightly lower in early RA than in the advanced RA (10.4 and 15.4%, respectively). In contrast, the relative content of 58-kDa IgA reactive with MAA was higher in

early RA than in advanced RA (10.6 and 8.4%, respectively). Reactivity with RCA I, LTA, and UEA was observed in early RA only (11.8, 5.3, and 8.3%, respectively), but not in advanced RA. No reactivity of 58-kDa IgA was observed with GSL II or AAL in either RA group (Table 1).

The reactivity of synovial IgG with lectins

In the early RA group, the relative content of 54.3-kDa IgG heavy chain reactive with SNA was slightly higher (24%) than in advanced RA (19.2%). In contrast, in the early RA preparation the proportions of IgG heavy chain reactive with MAA (34%), RCA I (27.2%), GSL II (50%), AAL (37.6%), LTA (24.2%), and UEA (31.6%) were lower than in the advanced RA group (MAA 45.6%, RCA I 40.7%, GSL II 61.5%, AAL 58%, LTA 32.6%, UEA 36.2%) (Table 1).

Reactivity of the 33.1-kDa IgG band with the used lectins was detectable in the early RA group only (SNA 7.7%, MAA 13.9%, RCA I 16.5%, GSL II 32.7%, AAL 32.8%, LTA 11%, and UEA 12.3%). This band was not recognized by the antibody and lectins in the advanced RA group (Table 1). The proportions of the SNA- and MAA-reactive 29.8-kDa IgG band were similar in the early and advanced RA groups (11.4 and 12.1% for SNA, and 4.5 and 2.6% for MAA, respectively). This band had no reactivity with RCA I or GSL II in either RA groups, but a slight reactivity with AAL in the advanced RA group (2.3%) was observed. In the early RA the proportions of 29.8-kDa IgG recognized by LTA (7.8%) and UEA (6.3%) were low, and even lower for LTA (2.5%), and it lost to bind UEA in advanced RA (Table 1).

The reactivity of synovial light chain with lectins

The immunoglobulin light chain (27.4 kDa) showed reactivity with all tested lectins, but no significant differences were found (Table 1).

Discussion

The results indicate differences between early and advanced RA stages in the terminal sugar exposition of synovial immunoglobulin G and A, but not IgM. The galactose-deficient glycotope with exposed *N*-acetylglucosamine of the synovial 33.1-kDa IgG fragment appeared exclusively in the early stage of RA. Moreover, the 33.1-kDa IgG fragment was also absent in normal blood plasma (Table 1). In contrast, this glycotope of intact synovial IgG and IgA was present in both groups, although in higher relative amounts in advanced RA. Moreover, the relative amounts of the

Table 1 The reactivity of synovial immunoglobulins with lectins in relation to RA progression

Sugar moiety recognized by lectin	Groups of synovial fluid and blood plasma	Quantification of immunoglobulin band-pattern expression revealed by lectin-blotting shown in Fig. 1 Mean value of the relative content (%)						
		IgM (88 kDa)	IgA (65.3 kDa)	IgA (58 kDa)	IgG (54.3 kDa)	IgG (33.1 kDa)	IgG (29.8 kDa)	Light chain (27.4 kDa)
Terminal α 2,6-linked sialic acid	Early RA	13.3	27.5	10.4	24	7.7	11.4	5.7
	Advanced RA	13.2	36.5	15.4	19.2	0 ^a	12.1	3.6
Terminal α 2,3-linked sialic acid	Normal plasma	18.2	27.8	31.7	12	0	7	3.3
	Early RA	2	8.3	10.6	34	13.9	4.5	26.7
	Advanced RA	0	13.1	8.4	45.6	0 ^a	2.6	30.3
Terminal galactose	Normal plasma	5.5	27	0	39.5	0	0	28
	Early RA	8.6	22.3	11.8	27.2	16.5	0	13.6
RCA I	Advanced RA	10.6	32.1	0	40.7	0 ^a	0	16.6
	Normal plasma	12.6	34	0	34	0	0	19.4
Terminal GlcNAc	Early RA	0	9.3	0	50	32.7	0	8
	Advanced RA	0	24.8	0	61.5	0 ^a	0	13.7
GSL II	Normal plasma	0	2	0	96	0	0	2
	Early RA	10.6	11.6	0	37.6	32.8	0	7.4
Fuc α 1,6GlcNAc	Advanced RA	15.5	19.7	0	58	0 ^a	2.3	4.5
	Normal plasma	29.1	26	0	44.7	0	0	0.2
Fuc α 1,3GlcNAc (Le ^x)	Early RA	0	14.3	5.3	24.2	11	7.8	37.4
	Advanced RA	0	18.5	0	32.6	0 ^a	2.5	46.4
LTA	Normal plasma	4.9	25	0	35.8	0	0	34.3
	Early RA	0	9.7	8.3	31.6	12.3	6.3	31.8
Fuc α 1,2Gal, O(H)	Advanced RA	0	22.4	0	36.2	0 ^a	0	41.4
	Normal plasma	19.5	24.6	0	23	0	0	32.9
Fuc α 1,2Gal β 1(Fuc α 1,3)4GlcNAc (Le ^y)	Advanced RA	0	22.4	0	36.2	0 ^a	0	41.4
	Normal plasma	19.5	24.6	0	23	0	0	32.9

The early and advanced RA groups were determined according to radiographs of the patients' hands [11]. The reactivity with lectins of salted-out immunoglobulin preparations of the early and advanced RA synovial fluid and normal plasma was performed by Western blotting using specific biotinylated lectins (see "Materials and methods"). The lectin reactivity of immunoglobulins was quantified by densitometric analysis of the bands using GelScan Software version 1.45 (Kucharczyk Electrophoretic Techniques, Poland) and the percent participation of an immunoglobulin band in the whole immunoglobulin pattern was calculated. The values of the relative content are the mean of 3–6 determinations

The significant differences are shown in italics

^a The 33.1-kDa band of IgG was recognized by anti-IgG antibody in the synovial early RA group, but not in the synovial advanced RA group and normal plasma

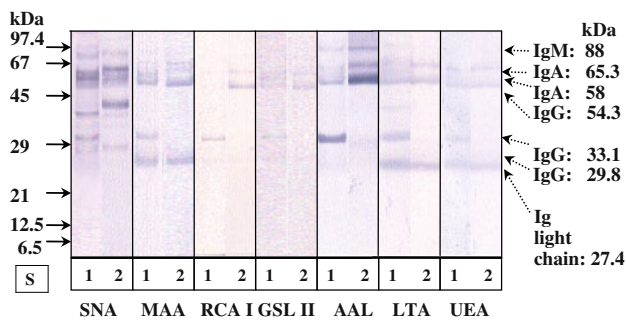


Fig. 1 The reactivities of synovial IgM, IgA, and IgG with specific antibodies and lectins. The synovial immunoglobulin preparations were derived from 1 early and 2 advanced RA stages. The solution containing 2 μ g of immunoglobulin preparation was subjected to SDS polyacrylamide gel electrophoresis. The synovial immunoglobulin bands were identified by Western blot using the respective anti-human antibodies as described in “Materials and methods”. Molecular weight standards (S) (see “Materials and methods”). The immunoglobulin molecular weights are the mean of all determinations. The reactivity of the synovial immunoglobulins with lectins separated by Western blot were analysed using the biotinylated lectins SNA, MAA, RCA I, GSL II, AAL, LTA, and UEA

sialyl and fucosyl determinants of intact A and G immunoglobulins were evidently lower in the synovial early RA and normal plasma groups than in the synovial advanced RA group.

The alterations we observed in terminal monosaccharide expression between early and advanced RA probably resulted from different molecular processes which are known to occur locally in joints during disease progression. In the early stage of RA, the synovium is infiltrated by highly activated immunological cells producing and transiently releasing proinflammatory agents which penetrate the cartilage, leading to a cascade of events promoting inflammation [1, 25, 26]. Various activated proteases and glycosidases may hydrolyze extracellular matrix components and soluble proteins, leading to the presence of fragmented proteins which, if they are glycoproteins, can appear in many glycoforms [27].

Several studies have shown that proteolytic fragments may contribute greatly to the pathological state of RA [12, 28]. For example, the early stage of RA, but not the advanced disease, is accompanied by the presence of fibronectin fragments [12] whose glycans were evidently desialylated and defucosylated [29]. With progression and stabilization of disease, profound metabolic changes have been described, for example, a decreasing cytokine profile, an increase in proteoglycan synthesis, and enhanced anabolic factor release. With persistent inflammation, apart from the still active destructive processes, the enhanced anabolic reparative phase makes cartilage more refractory, leading to further erosive damage [1, 25]. The intensive degradation of proteins consequently comes to a stop and

mainly intact molecules of FN, not degraded proteins, remain in synovial fluid [12].

The present study indicates that not only matrix proteins, but also synovial IgG undergoes sensitive degradation. As we previously reported [15], two IgG fragments (33.1 and 29.8 kDa) immunoreactive with anti-IgG antibodies appeared in the synovial fluid in significant relative amounts (Fig. 1; Table 1). One of them, with the molecular weight of 33.1 kDa, was exclusively associated with early RA. At the same time synovial IgA and IgM polypeptides were not degraded, most probably because of their 4–6 times higher amounts of sugars [7], which are known to play a protective role against proteolysis. The slight expression of 29.8-kDa IgG band in normal plasma (Table 1) may be a result of undiagnosed age related diseases.

Many authors have reported that the majority of the glycosylation changes in RA are related to the degree of glycan branching and the type of substitution of the N-linked glycans with sialic acid and/or fucose [5]. Our previous and present results suggest that the glycosylation profile of fibronectin [29], as well as of IgG and IgA, was quite different in early and advanced RA. The oligosaccharides of synovial IgG and IgA were evidently degraded in early RA and a mixture of their glycoforms appeared in the synovial fluid. Among them were those which were sialylated and fucosylated as well as those with truncated oligosaccharides, asialylated with exposed galactose, agalactosylated with exposed *N*-acetylglucosamine, and de- or weakly fucosylated glycoforms (Table 1). Two fragments of synovial IgG (33.1 and 29.8 kDa) as well as both molecular forms of IgA (65.3 and 58 kDa) were most probably their glycoforms which differed by the presence of terminal sialic acid, fucoses, and expositions of terminal galactose, or terminal *N*-acetylglucosamine. For example, the 33.1-kDa IgG fragment (but not 29.8-kDa IgG) and 65.3-kDa IgA (but not 58-kDa IgA) elicited the presence of truncated agalacto-oligosaccharides terminating with *N*-acetylglucosamine.

The expression of hypo- and agalacto-glycoforms of IgG in blood plasma of RA patients has been shown to be RA specific [4]. The association between the presence of glycoform lacking galactose but having exposed *N*-acetylglucosamine is well accepted RA marker [4, 5]. Its level increase with disease progression and indicate a poor prognosis [4–7]. Our study indicates that the appearance of the agalacto-glycotope in the 33.1-kDa IgG fragment is exclusively associated with the synovial fluid (but not with blood plasma) and with the early stage of RA only, whereas its relative amount increases in intact IgA and IgG at the advanced stage. However, the mechanism of agalacto-glycoform production can be complex and seems to be different in early and advanced RA. In early RA the extensive actions of extracellular proteases and glycosidases lead to

the appearance of immunoglobulins without terminal sialic acid and galactose. In contrast, in advanced disease, the reduced activity of endocellular galactosyltransferase [30] leads to the synthesis of immunoglobulin G and A molecules with truncated antennas terminated by galactose and/or *N*-acetylglucosamine. The sugar moiety attached to the asparagine-297 residue in the constant domain of IgG is critical for the overall structure and function of the molecule, because removal of this sugar domain leads to the loss of the proinflammatory activity. It suggests that in vivo modulation of antibody glycosylation might be a strategy to interfere with autoimmune processes [31]. Nimmerjahn and Ravetch [32] suggest that high doses of intravenous hyper-sialylated IgG would be a potent antiinflammatory agent for the use in autoimmune diseases. Authors [32] propose that the Fc-sialylated IgGs engage a unique receptor on macrophages that, in turn, leads to the upregulation of an inhibitory Fc γ receptor, thereby protecting against autoantibody-mediated pathology.

In conclusion, the analysis of terminal monosaccharides in molecular forms of the IgG and IgA could be included in the list of rheumatological laboratory tests to distinguish the early stage of RA disease from the advanced. Such information is of importance in making decisions about the best medical treatment.

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