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ORIGINAL ARTICLE

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Soluble CD21 in sera and synovial fluid of arthritic patients

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Abstract Soluble CD21 (sCD21) is the ectodomain of the CD21 glycoprotein released by shedding from the cellular membrane. The ectodomain of CD21 is capable of binding complement fragments, Epstein-Barr virus (EBV) and CD23. Functionally sCD21 can activate monocytes and abrogate B-cell/follicular dendritic cell interaction, thereby inhibiting antibody production by antigen primed B cells. Levels of sCD21 vary in several clinical conditions. Here we analyzed sCD21 in synovial fluids and sera in arthritic patients. sCD21 concentrations were consistently lower in synovial fluids compared to paired sera samples from the same patients. In contrast to healthy donors, sCD21 levels are significantly reduced in rheumatoid arthritis patient's sera. Potential causes and consequences of the data are discussed.

Keywords Complement receptor 2 · Shedding · CD21 · Arthritis · Synovial fluid

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Introduction

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease. The innate immune system may play an important role in the complex pathophysiology [1, 2] because complement activation products are elevated in RA [3, 4].

Proteolytic cleavage of membrane bound proteins, known as shedding, releases the ectodomain part of the molecule from the cell surface. Usually these ectodomains retain the capability to bind to all known ligands [5, 6].

CD21 is the receptor for C3d complement fragments bound to microbial surfaces or immune complexes, and the Epstein-Barr virus (EBV). CD21 amplifies the signal through the B cell receptor and is required for B cell activation to low affinity antigen and survival of B cells during the germinal center reaction [7]. CD21 is shed above the cellular membrane and is found in the circulation. It has been found at higher concentrations in patients with certain B lymphomas, EBV infections [8, 9] and was shown to activate monocytes through its ligand CD23 [10]. Furthermore, soluble CD21 (sCD21) could be a modulator of immunity because it can block follicular dendritic cell (FDC) dependent B cell activation [11, 12].

Surface expression of CD21 is reduced in synovial lymphocytes as compared to peripheral blood lymphocytes and in activated T cells [13, 14]. We have recently shown that the concentration of sCD21 is low in systemic lupus erythomatodes (SLE) [15], Sjögren's syndrome [15] and RA serum [16] compared to healthy persons' sera but not in juvenile arthritis [15]. Analysis of paired synovial fluids and sera from juvenile arthritis patients showed low sCD21 concentration in synovial fluids compared to sera [15]. Therefore we were interested to analyze sCD21 concentrations in other forms of arthritis synovial fluids as well. Using an ELISA we measured sCD21 in synovial fluids and sera of the same patients, and

furthermore we extended our previous analysis of RA patients and healthy individuals.

Patients and methods

Human samples, cells, antibodies and reagents

Nine consecutive patients with different forms of arthritis, the majority of them with RA, requiring removal of joint fluid were recruited from the out-patient clinic of the Division of Rheumatology at the Kantonsspital St. Gallen after obtaining informed consent. All patients suffered from a well-defined arthritic disorder diagnosed, as in the case of RA, using the ACR-classification criteria [17]. Undifferentiated arthritis was defined as non-remittent oligoarthritis in the absence of rheumafactor, anti-CCP antibodies and without evidence for spondarthropathy, psoriasis or reactive arthritis. Human sera from RA patients ($n = 83$) were collected from out-clinic patients at the Freiburg University Medical Center and from healthy donors ($n = 63$). In all instances patients gave informed consent. Mononuclear cells were counted and neutrophil granulocytes differentiated from lymphocytes and monocytes using standard methods.

Monoclonal anti-CD21 antibodies BU32 (IgG1) and THB5 (IgG2a) were purified by affinity chromatography with protein-G sepharose (Amersham, Freiburg, Germany). BU32 was biotinylated using Biotintag micro kit (Sigma-Aldrich, Switzerland). For standardization, aliquots of a human sera were used. Values are given in arbitrary units. A sandwich ELISA was performed to quantitate sCD21 levels in human serum/plasma. The monoclonal antibodies THB5 and biotinylated BU32 were used as capture antibody and revealing antibody respectively. Briefly, THB5 was coated onto an ELISA plate (TPP, Trassadingen, Switzerland) at a concentration of 2 μg/ml in PBS for 12–15 h at 4°C. Plates were blocked with 3% bovine serum albumin in PBS for 2 h at room temperature. The serum/plasma samples at appropriate dilutions were added in triplicates to the plates along with the standard. The plates were incubated at 4°C for 12–15 h, washed twice and then incubated for 2 h with BU32-biotin. After four washes, streptavidin coupled to horseradish peroxidase was added for 1 h and then H_2O_2/*o*-phenylenediamine as substrate/coloring agent for 30 min. The enzyme reaction was quantified by taking OD at 450 nm in an ELISA reader (Tecan, Switzerland) and sCD21 concentrations were calculated extrapolating from the standard graph.

Statistics

Statistical calculations and graphical illustrations were performed using Instat/Prism software. Mann–Whitney tests to obtain nonparametric two-tail *P* value were performed.

Results

Soluble CD21 in synovial fluid and serum

We analyzed sCD21 concentrations in synovial fluids and sera of the same patients. sCD21 concentration in synovial fluids ranged from 0 to 73 units sCD21/ml (mean 49.24) (Fig. 1). Serum levels of sCD21 in this cohort ranged from 119 to 169 units sCD21/ml (mean 142.58), and were in all cases higher compared to the synovial fluid of the same patients (Fig. 1). Using the non-parametric Mann–Whitney test, the difference between the synovial fluid and serum sCD21 concentrations was calculated as being highly significant ($P = 0.0001$). Thus, the concentration of sCD21 differs significantly between synovial fluid and blood.

Soluble CD21 concentrations in sera of patients with rheumatoid arthritis

We have previously shown comparatively low levels of sCD21 in the sera of RA patients [16]. In the present study this analysis was extended using 63 sera samples from a different set of RA patients and compared these results to 80 sera from a different set of healthy donors. Values of RA sera ranged from 59 to 518 unit sCD21/ml (mean 159.60) and in HD sera from 93 to 570 unit sCD21/ml (mean 196.70). The sCD21 concentrations were found to be lower in RA than in normal controls revealing a statistically highly-significant reduction ($P = 0.0004$)(Fig. 2).

Methotrexate (MTX) stimulates adenosine release from connective tissue cells [18]. This could stimulate purinergic P2 receptors, which are known to induce shedding of CD62L and CD23 [19]. The same mechanism might induce CD21 shedding as well. Therefore we

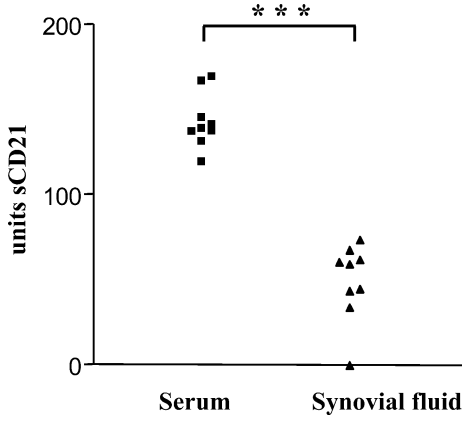

Fig. 1 Soluble CD21 concentration in synovial fluid and sera. Sera and synovial fluid were tested for sCD21 concentration by ELISA. 11 sera (*filled triangle*) and 11 synovial fluids (*filled square*) from patients detailed in Table 1 were analyzed for sCD21 content. Sera and synovial fluid were collected at the same time. Differences between synovial fluid and sera were highly significant ($P = 0.0008$)

Table 1 Patients with different kinds of arthritis

Sample name	DOB	Kind of arthritis	sCD21	Gender	Duration of disease	DMARD/pred	Cell-number in SF
TSG 01 - SF	1953	UA	33.81	F	1 year	MTX/pred	1200/7
TSG 01 serum	1953	UA	168.69	F			
TSG 03 - SF	1953	RA	0	F	30 year	Leflu/pred	7800/57
TSG 03 serum	1953	RA	136.72	F			
TSG 05 SF	1927	RA	67.22	F	16 year	MTX/inflixi/pred	83000/80
TSG 05 serum	1927	RA	137.08	F			
TSG 10 SF	1924	RA	60.18	M	7 year	MTX/inflixi/pred	25400/55
TSG 10 serum	1924	RA	166.08	M			
TSG 11 SF	1979	RA	61.61	F	2 year	None	26300/68
TSG 11 serum	1979	RA	131.08	F			
TSG 13 SF	1955	SpondA	59.16	M	4 weeks	None	39650/53
TSG 13 serum	1955	SpondA	119.25	M			
TSG 14 SF	1945	RA	44.65	M	3 year	None	35400/nd
TSG 14 serum	1945	RA	138.64	M			
TSG 15 SF	1926	RA	73.02	F	5 year	Pred	ND
TSG 15 serum	1926	RA	144.94	F			
TSG 16 SF	1926	RA	43.49	F	5 year	Pred	ND
TSG 16 serum	1926	RA	140.7	F			

sCD21 in ng/ml; numbers of mononuclear cells per µl/percentage of neutrophils are shown in column "cell-number in SF"
SF synovial fluid, *DOB* date of birth, *UA* undifferentiated arthritis, *RA* rheumatoid arthritis, *SpondA* Spondarthropathy with peripheral arthritis, *OA* osteoarthritis, *DMARD* disease-modifying antirheumatic drug, *pred* prednisone, *leflu* leflunomide, *MTX* ethotrexate, *inflixi* infliximab, *ND* not determined

compared sCD21 levels in patients treated with MTX ($n = 37$) or not treated with MTX ($n = 16$). There was no significant difference between the two groups. Thus low dose MTX did not influence CD21 shedding significantly.

Discussion

This study demonstrates that synovial fluids contain less sCD21 than sera from the same patients, and that the levels of sCD21 in the sera of RA patients were significantly lower than those of healthy donors. Even though clearly not powered to answer this question, clinical data of the paired synovial fluid/sera cohort did not suggest a possible influence of disease duration, or the absence or kind of therapy used on the levels of sCD21 in either sera or synovial fluid, nor did the amounts of inflammatory cells in the synovial fluid.

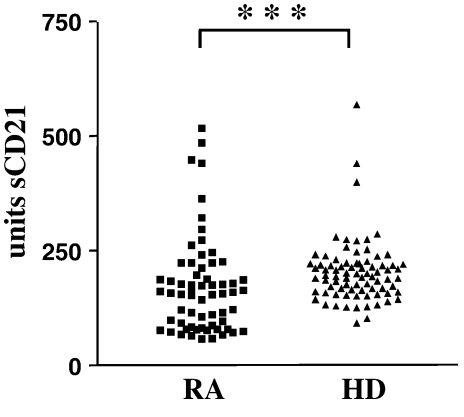

Fig. 2 Serum sCD21 concentration in rheumatoid arthritis. sCD21 concentrations were measured by ELISA from confirmed rheumatoid arthritis patients ($n = 63$, *filled square*) and compared with healthy controls ($n = 80$, *filled triangle*). Differences between RA sera and HD sera were highly significant ($P = 0.0004$)

We have previously shown that the reduction of sCD21 in adult autoimmune diseases is not due to the age of the patients, nor related to the presence of rheumatoid factor [15, 16]. Interestingly, this was not the case in juvenile arthritis where we found normal amounts in sera [15]. Whether this reflects a qualitative or quantitative difference in CD21 shedding is not clear. However, when we compared concentrations of sCD21 according to age we found more sCD21 in sera of donors below age 20 [16]. Low sCD21 in the circulation could be due to increased complement activation products leading to increased clearance of bound sCD21from plasma. In RA patients an even slightly-elevated turnover of C3 (1 g/l in healthy human sera) might be sufficient to "neutralize" sCD21 (about 200–300 µg/l in healthy persons sera). Elevated concentrations of protease inhibitors such as alpha-1-antitrypsin could prevent shedding of CD21 from the lymphocyte surface by inhibiting yet unidentified proteases associated with the cell membrane [20].

The switched memory B cell pool is enlarged in RA patients, and $CD38^+$ plasma-cell-like B cells are present in higher numbers [21]. The lower concentrations of sCD21 are most likely not related to properties of the peripheral blood B cell pool alone, since peripheral blood B cells from RA patients produce amounts of sCD21 comparable to those from healthy individuals [16]. A possible explanation is that the majority of sCD21 is produced by other cell types or by B cells in different locations, for example in the spleen. It is also possible that during autoimmunity the immune system

suppresses further responses. This might lead to lower sCD21 concentrations because activation of B cells leads to CD21 shedding [22]. Our data suggest that low-dose MTX does not significantly influence CD21 shedding.

Lower amounts of sCD21 have been found before in synovial fluids from patients with juvenile arthritis [15]. The protein content of the synovial fluid is in part derived from the pannus tissue and inflammatory cells in the arthritic joint (for example secreted molecules such as cytokines). Previously we reported reduced expression of CD21 in synovial fluid compared to blood-derived mononuclear cells [13]. This could explain the low levels of sCD21 in the synovial fluids observed here.

In conclusion, we found lower levels of sCD21 in synovial fluid compared to sera from the same patients. The reduction of sCD21 serum levels seen in autoimmune arthritis such as RA suggests a role of the innate immune system in autoimmune reactions and possibly in the pathogenesis of these conditions.

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