Up Regulated Complement and Fc Receptors in Juvenile Idiopathic Arthritis and Correlation with Disease Phenotype

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Abstract

Purpose The progress in identifying immunological mechanisms in juvenile idiopathic arthritis (JIA) has partly been hampered by the fact that the disease is heterogeneous. Here we have investigated complement and Fc receptors, as part of the inflammatory process, in two subgroups of JIA.

Methods Blood from 26 patients with oligoarticular or polyarticular course type JIA and 21 healthy age and sex-matched controls were investigated by FACS and immunoassays.

Results Increased numbers of monocytes and augmented plasma levels of C-reactive protein, C3a and IgG were found in both JIA subgroups. However, only polyarticular patients exhibited increased expression of Fc gamma receptor (FcγR) II and III and complement receptor (CR) 1 on monocytes along with enhanced CR1 expression on B cells. A correlation was observed between degree of receptor expression and C3a levels in the patients.

Conclusions Complement and Fc receptors are up regulated in children with multiple joint involvements, thus highlighting these pathways in the pathogenesis of polyarticular JIA.

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Introduction

Juvenile idiopathic arthritis (JIA) is one of the most common rheumatic diseases during childhood. It is a heterogeneous, autoimmune disorder characterized by chronic arthritis that begins before 16 years of age. The cause of JIA is multifactorial and the disease is believed to be influenced by genetic as well as environmental factors. Various clinical signs, hereditary and serological aspects contribute to different subgroups of the disease. A grand and coarse separation of patients is according to the number of involved joints; the oligoarticular disease is defined as less than five joints with arthritis and polyarticular as five or more. The division of JIA into subgroups is an attempt to get more homogenous groups with similar features and outcomes [1].

The joint inflammation in JIA resembles adult rheumatoid arthritis (RA), with hyperplasia of the synovial lining layer and infiltration of macrophages and lymphocytes. However, known autoantibodies associated with rheumatic disease are sparse in JIA. In population-based studies, anti-nuclear antibodies (ANA) are present in 16–44% of patients [2–4], depending on the method and cut-off value. Rheumatoid factors (RF) are much less frequent in population-based studies of JIA, with an occurrence of 2–16% [2, 4, 5]. So far no population-based study is presented with the occurrence of anti-cyclic citrullinated peptide (anti-CCP) antibodies in patients with JIA, but the prevalence is low and anti-CCP antibodies are particularly found in the RF-

positive, polyarticular JIA subset [6, 7]. Nonetheless, a role of B cells in JIA pathology is indicated by the higher numbers of IgG-secreting B cells in comparison to healthy individuals, implying a B cell hyper-reactivity [8]. Indeed, in JIA patients the presence of autoantibodies correlates with increased concentrations of immune complexes (ICs), which may trigger inflammation via complement and Fc receptors [9, 10]. The Fc receptors play an important bridging role between innate and adaptive immunity, where they direct and regulate antibody responses. IgG antibodies exert their effector function via Fc receptors for IgG (FcγRs), which are found in three classes, FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16), of which FcγRII and FcγRIII exist in a, b or c isoforms. The activating FcγRI, FcγII and FcγIII are involved in phagocytosis, antigen presentation, antibody-dependent cellular cytotoxicity and cytokine secretion. The inhibitory FcγRIIb (CD32b) regulates IC-mediated inflammation induced by activating FcγR. In addition, due to its expression on B cells, FcγRIIb regulates B cell activation, antibody production and self tolerance. The FcγRs have been shown to be essential in autoimmune inflammation [11, 12], where enhanced expression of FcγRI, FcγRII and FcγRIII on monocytes and macrophages is demonstrated in RA patients [13–15], while FcγRIIb on peripheral blood B cells is reduced [16] . Complement activation is another route whereby ICs can trigger inflammation and in fact increased titers of activated complement fragments have been detected in JIA plasma and synovial fluid [17–19]. The central C3 component is split into activated complement fragments, which become attached to ICs and enable activation of complement receptors (CR) on leukocytes. CR type 1 (CD35) is activated by C3b, iC3b and C4b fragments, and has a multifunctional role as a complement regulatory protein, a receptor for clearance and in phagocytosis of ICs and regulation of B cell responses and tolerance [20]. CR type 2 (CD21) binds C3d, iC3d, and C3dg-opsonized antigens, lowering the threshold for B cell activation [21, 22].

To better understand the consequences of B cell hyper reactivity in JIA we asked whether FcγR and CR pathways are involved in two subgroups of the disease; oligoarticular and polyarticular JIA. We examined peripheral blood monocytes and B cells as these are central in humoral immune responses. The expression of FcγRI, FcγRII, FcγRIIb, FcγRIII, CR1 and CR2 were examined, and molecules associated with activation of the receptors; C3a, C-reactive protein (CRP), IgM and IgG were analyzed in the plasma. Notably, only JIA patients with polyarthritis expressed increased activating FcγR and CR1 levels on monocytes, and enhanced CR1 expression on B cells compared to age-matched healthy controls. The increased receptor expression coincided with increased immunoglobulin, CRP and complement levels in the polyarticular patients.

Methods

Ethics Statement

The study was approved by the regional ethics committee in Uppsala (also known as the Institutional Review Board at our Institution and hospital) (D number 2007/191). Written information about the study, approved by the ethical committee, was given to the parents and patients. Informed consent was collected from the parents (and from the children as far as possible) and documented individually in the patient's medical journal before entering the study.

Subjects

Twenty-six patients with JIA (Table I), and 21 healthy controls were recruited from the Uppsala University Hospital, Sweden. Patients were included during an active phase of the disease, before initiation or change of treatment. The onset type of the disease, as well as the disease course type, was defined according to the ILAR Edmonton criteria [1]. Onset type of disease is a description of the first 6 months of disease in contrast to course type. Patients were grouped according to the number of active joints during disease course and at sampling. Sixteen of the JIA patients had 1–4 actively inflamed joints during disease course and at sampling and were defined as oligoarthritis. Ten patients had ≥5 affected joints during disease course and at sampling and were defined as having polyarthritis. The number of active joints was confirmed by an experienced pediatric rheumatologist (LB). Four of the oligoarticular patients were on NSAID or methotrexate at the sample collection and eight of the polyarticular patients were on NSAID, prednisolone or etanercept (one). The disease duration at sampling was 0.3 (0.1–14.0) (median and range) years in patients with oligoarticular disease and 0.7 (0.2–9.0) years in those with a polyarticular disease. The female/male ratio was 9/7 in patients with oligoarticular and 9/1 in polyarticular disease. Median age and range (years) at sampling was 8.0 (1.7–16.2) and 9.0 (1.3–14.6) respectively. Previous routine autoantibody analyses showed positive ANA in 15 of the 26 JIA patients (58%), evenly distributed in the two groups, while only three patients with oligoarthritis and none of the patients with polyarticular disease had positive RF.

The age-matched control group consisted of 21 healthy children, 9 boys and 12 girls. The median age and range (years) at sampling was 6.3 (1–16.3). All patients were undergoing minor elective surgery and peripheral blood samples were collected before the surgery was performed. Exclusion criteria were rheumatic disease, inflammatory disease or infectious disease and having a first degree relative with rheumatic or psoriatic disease.

Table I Characteristics of JIA patients

Sex	Age	Onset type according to ILAR criteria	Course type according to ILAR criteria	Disease duration (months)	Active joints at sampling	Therapy	ANA	RF
OLIGOARTHRITIS (1-4 active joints during disease course and at sampling)								
Female	7	Oligoarticular	Oligoarticular persistent	3.5	1	–	Pos	Neg
Male	2	Oligoarticular	Oligoart. pers.	2	1	–	Pos	Neg
Male	16	Oligoarticular	Oligoart. pers.	5	1	–	Pos	Neg
Female	4	Oligoarticular	Oligoart. pers.	2.5	1	–	Neg	Neg
Female	3	Oligoarticular	Oligoart. pers.	1.5	1	NSAID	Pos	Neg
Female	1.5	Oligoarticular	Oligoart. pers.	1.5	1		Neg	Neg
Male	2	Oligoarticular	Oligoart. pers.	8.5	1		Neg	Neg
Female	7	Oligoarticular	Oligoart. pers.	3.5	1	–	Pos	Neg
Female	15	Polyarticular RF[-]	Oligoarticular[a]	148	1	Mtx	Pos	Neg
Female	10	Oligoarticular	Oligoart. pers.	5.5	1	–	Pos	Pos
Male	9	Oligoarticular	Oligoart. pers.	2.0	2	Mtx	Pos	Neg
Female	16	Oligoarticular	Oligoart. pers.	168	2	–	Pos	Pos
Male	13	Other arthritis[b]	Other arthritis[b]	3.5	2	–	Neg	Neg
Male	4.5	Oligoarticular	Oligoart. pers.	2.5	2	–	Neg	Pos
Male	10	Oligoarticular	Oligoart. pers.	9.5	3	NSAID	Neg	Neg
Female	15	Oligoarticular	Oligoart. pers.	5	3	–	Neg	Neg
POLYARTHRITIS (5 or more active joints during disease course and at sampling)								
Female	13	Polyarticular RF[-]	Polyarticular RF[-]	28	5	Mtx	Neg	Neg
Female	1	Polyarticular RF[-]	Polyarticular RF[-]	7	6	NSAID	Neg	Neg
Female	13	Polyarticular RF[-]	Polyarticular RF[-]	7.5	6	NSAID	Pos	Neg
Female	7	Oligoarticular	Extended oligoart.	33	7	Mtx	Pos	Neg
Female	14	Oligoarticular	Extended oligoart.	108	7	Prednisolone	Pos	Neg
Female	12	Polyarticular RF[-]	Polyarticular RF[-]	8.5	10	Etanercept, Mtx, NSAID	Pos	Neg
Female	9	Polyarticular RF[-]	Polyarticular RF[-]	2.5	16	–	Pos	Neg
Female	9	Oligoarticular	Other arthritis[c]	82	18	Mesalazin	Neg	Neg
Male	7	Polyarticular RF[-]	Polyarticular RF[-]	2.5	19	NSAID	Neg	Neg
Female	15	Polyarticular RF[-]	Polyarticular RF[-]	2	23	–	Pos	Neg

[a] Polyarticular disease during the first 9 months after onset. Since then oligoarticular disease. [b] Could be classified as oligo- as well as enthesitis-related arthritis during the first 6 months as well as during course of disease, excluded from both because of first degree heredity for psoriasis. [c] Can be classified as polyarticular RF[-] as well as juvenile psoriatic arthritis

Purification of Blood Mononuclear Cells

Fresh peripheral blood samples were collected from the patients and controls at inclusion and analyses were started within 4 h. The EDTA-treated blood was centrifuged for 20 min at 1,800 rpm and the plasma was collected and stored at −80°C until analysis. Peripheral blood mononuclear cells (PBMCs) were purified from the remaining cell pellet by density centrifugation using Ficoll-Paque (GE Healthcare, Uppsala, Sweden). The PBMCs were washed twice with cold PBS-EDTA and centrifuged for 8 min at 1,200 rpm at 4°. The cell pellet was subsequently re-suspended in cold PBS and the viability and cell concentration was determined by Trypan blue staining using a Bürker chamber and a light microscope (Leitz Laborlux 12).

Antibodies

The following monoclonal antibodies to human antigens were used for flow cytometry: PE or FITC conjugated anti-CD14 (clone 61D3 and TÜK4 respectively) (eBioscience, San Diego, USA and DAKO, Glostrup, Denmark), anti-CD19 (clone HD37) (DAKO), anti-CD64 (clone 10.1) (BioLegend, San Diego, USA), anti-CD32 (clone AT10) (AbD Serotec, Oxford, United Kingdom), anti-CD16 (clone DJ130c) (DAKO), anti-CD35 (clone E11) (BD Biosciences, Pharmingen) and anti-CD21 (clone LT21) (BioLegend). In addition, biotinylated anti-CD32b (clone GB3), a novel antibody that separates the detection of FcγRIIb from FcγRIIa [15] (kindly provided by Dr Uwe Jacobs, SuppreMol, Martinsried, Germany) was used followed by streptavidin-

PE (BD Biosciences, Pharmingen). All antibodies were of IgG1 isotype except anti-CD14 FITC, which was an IgG2a antibody. Thus, corresponding isotype controls were mouse IgG1 (DAKO) conjugated to FITC or PE and FITC-conjugated IgG2a (DAKO), as well as biotinylated mouse IgG1 (BD Pharmingen).

Flow Cytometry

Two hundred thousand PBMCs were suspended in 100 μl 0.5% BSA (Roche Diagnostics GmbH, Mannheim, Germany) in PBS (FACS buffer) and were stained for 30 min on ice using different combination of the antibodies described above. The cells were thereafter washed in FACS buffer, centrifuged for 8 min at 1,200 rpm at 4°, and washed again twice. A secondary staining for additional 30 min on ice was performed with streptavidin conjugated to PE on samples stained with biotinylated antibodies. After further washing, the cells were analyzed by FACScan and Cell quest software (both from BD biosciences). Gates for monocytes and lymphocytes in the PBMCs were set according to cellular size and granulation in a SSC and FSC diagram (Fig. 1a). The monocyte gate was further used when analyzing CD14+ monocytes and the lymphocyte gate used when analyzing CD19+ B cells.

Serology

Previous measures of ANA and RF in the JIA patients were obtained from the medical records. In each patient at least two analyses of ANA with at least 3 months in between had been analyzed. ANA was demonstrated by indirect immunofluorescence on HEp-2 cells (Bio-Rad, Stockholm, Sweden) in a screening dilution of 1:200, with a secondary

antibody to IgG (Dako A/S, Glostrup, Denmark). IgM RF was analyzed using nephelometry (Beckman Image, Beckman Coulter, Fullerton,California, USA) and was recorded as a qualitative (positive/negative) value.

CRP, total IgM, total IgG and C3a levels in plasma from the patients and healthy controls were analyzed at the Department of Clinical Chemistry and Clinical Immunology, Uppsala University Hospital using routine methods. Briefly, the CRP, IgM and IgG analyses were done with turbidimetric immunoassays using the CRP *Vario* (Abbott Scandinavia AB, Solna, Sweden), the IGM2 and the IGG2 kit (Roche Diagnostics) respectively. C3a levels were analyzed with a C3a sandwich ELISA accordingly: plasma was diluted in working buffer and incubated in microtiter plates coated with the monoclonal antibody 4SD17.3. Captured C3a was detected with biotinylated polyclonal rabbit anti-C3a, followed by horseradish peroxidase-conjugated streptavidin (GE Healthcare, Uppsala, Sweden). Zymosan-activated serum, calibrated against purified C3a, served as standard.

The plasma was also analyzed for IgG anti-CCP antibodies using a semi-quantitative anti-CCP ELISA (Immunoscan CCPlus, Euro-Diagnostica, Malmö, Sweden). According to the manufacturer protocol, samples >25 U/ml were defined as positive.

Statistics

Differences in CRP, C3a, IgG, IgM and receptor expression between patients and healthy controls were analysed using Student's *t*-test. GraphPad Prism 5 (GraphPad Software Inc.) was used to analyse Pearson correlation between C3a plasma levels and receptor expression. $p < 0.05$ was considered statistically significant.

Fig. 1 Augmented levels of inflammatory markers and immunoglobulins in JIA subsets. Mean plasma levels ± SEM of CRP (**a**), C3a (**b**), IgM (**c**) and IgG (**d**) in oligoarticular ($n=16$), polyarticular ($n=10$) JIA and in healthy controls ($n=21$). *$p<0.05$, ** $p<0.01$
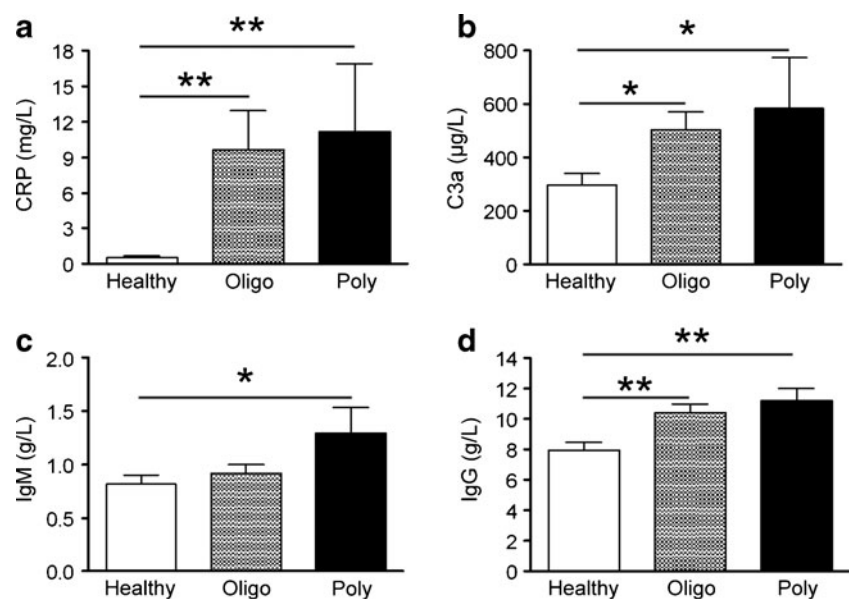
Results

Increased Complement and Immunoglobulin Levels in JIA

Prior the FcγR and CR expression studies we analyzed the plasma of the patients for immunoglobulin, complement and CRP, proteins that are associated with receptor activation. CRP is a clinically important classical acute phase protein that interacts with FcγR and C3a is an early marker of complement activation [23, 24]. As expected, we found that children with JIA compared to healthy controls had significantly higher levels of CRP, confirming the ongoing inflammation in these children (Fig. 1a). This was further supported by the higher C3a levels seen in the patients compared to healthy controls (Fig. 1b). However, the level of inflammation measured by CRP or C3a could not be associated with the number of involved joints, as no difference was seen between children with poly- and oligoarthritis. We were also interested in measuring total IgM and IgG, as both can activate complement and IgG can further activate FcγR. In previous studies it has been reported that JIA is associated with higher levels of immune complexes [9]. Notably, in our study JIA patients with polyarthritis had higher IgM levels compared to healthy controls ($p \leq$ 0.05), while patients with oligoarthritis had similar IgM levels as controls (Fig. 1c). However, both patient groups had significantly higher plasma levels of IgG ($p \leq 0.01$) (Fig. 1d). The enhanced IgG production led us to investigate if the JIA patients exhibited IgG anti-CCP antibodies, which are strongly associated with RA in adults. However, very few of the JIA patients were positive for anti-CCP antibodies. One out of 16 patients with oligoarthritis and 1 out of 10 with polyarthritis had IgG anti-CCP antibodies. Also, 1 out of 21 healthy controls was positive for anti-CCP antibodies. However, all positive samples (2 patients and 1 control) showed very low concentration of anti-CCP antibodies (33–40 units/ml), where values >25 units/ml are regarded as positive according to the anti-CCP ELISA manufacturer. Thus, anti-CCP antibodies were not common in our study population.

Increased Monocyte Numbers in JIA and Enhanced Fcγr and CR1 Expression in Polyarticular Patients

The enhanced immunoglobulin production observed in JIA may trigger inflammatory reactions via complement and FcγR. Thus, we explored whether FcγR and CR are affected in leukocytes from JIA. PBMC were isolated from JIA patients and healthy control children and further analyzed using flow cytometry. We found that the frequency of CD14+ monocytes was significantly higher in JIA patients compared to healthy controls, an effect seen regardless of number of inflamed joints involved ($p < 0.01$–0.001) (Fig. 2a). The percentage of monocytes expressing activating FcγR did not differ between JIA patients and healthy controls. Almost all monocytes expressed FcγRI and FcγRII, while FcγRIII was expressed on approximately 25% of the monocytes (Fig. 2b). The inhibitory FcγRIIb was expressed on a small population of monocytes, which did not differ between JIA and healthy subjects. However, we observed that the FcγRIIb expressing monocyte population was increased in patients with polyarthritis compared to patients with oligoarthritis ($p < 0.01$) (Fig. 2b). Further, the frequency of monocytes expressing CR1 was significantly enhanced in JIA patients with polyarthritis, but not with oligoarthritis, compared with healthy controls ($p < 0.001$) (Fig. 2b). Moreover, when analyzing the receptor expression (mean fluorescence intensity; MFI) we observed a distinct pattern (Fig. 3). The group of patients with polyarthritis displayed significantly higher expression of FcγRII and FcγRIII on their monocytes compared to healthy controls ($p < 0.05$ and $p < 0.01$ respectively) (Fig. 3b, c). There was also a trend toward higher expression of FcγRI, although not significant ($p = 0.08$) (Fig. 3a). Concerning monocytes from oligoarthritis patients the expression level of FcγRI, II and III did not differ compared with monocytes from healthy controls. No difference was seen in the MFI of FcγRIIb among the groups (Fig. 3d). However, the MFI value of CR1 on monocytes was significantly increased in JIA patients with polyarthritis compared with healthy controls ($p < 0.01$) (Fig. 3e). This effect was not seen in JIA patients with oligoarthritis, which displayed a CR1 MFI value ranging between polyarthritis patients and healthy controls.

Increased CR1 Expression on B Cells in Polyarticular JIA

We next investigated if FcγR and CR expressing B cells were affected in JIA. The B cells were labeled with anti-CD19 antibodies and FACS analyses demonstrated no differences in B cell numbers between the two JIA patient groups and healthy controls (Fig. 4a). Neither were there any differences in the percentage of B cells expressing CR1, CR2 or FcγRIIb (Fig. 4b-d). However, the receptor expression was affected as we found a significantly higher expression of CR1 on B cells in polyarthritis patients compared to oligoarthritis patients and healthy controls ($p < 0.05$ and $p < 0.01$ respectively) (Fig. 4b). Further, there was a trend toward higher expression of CR2 in patients with polyarthritis compared to healthy controls ($p < 0.07$) (Fig. 4c). Notably, the enhanced CR expression on B cells was exclusive in the polyarthritis patient group, as oligoarthritis patients had almost identical expression of CR1 and CR2 as the healthy controls. The MFI value of FcγRIIb on B cells did not differ
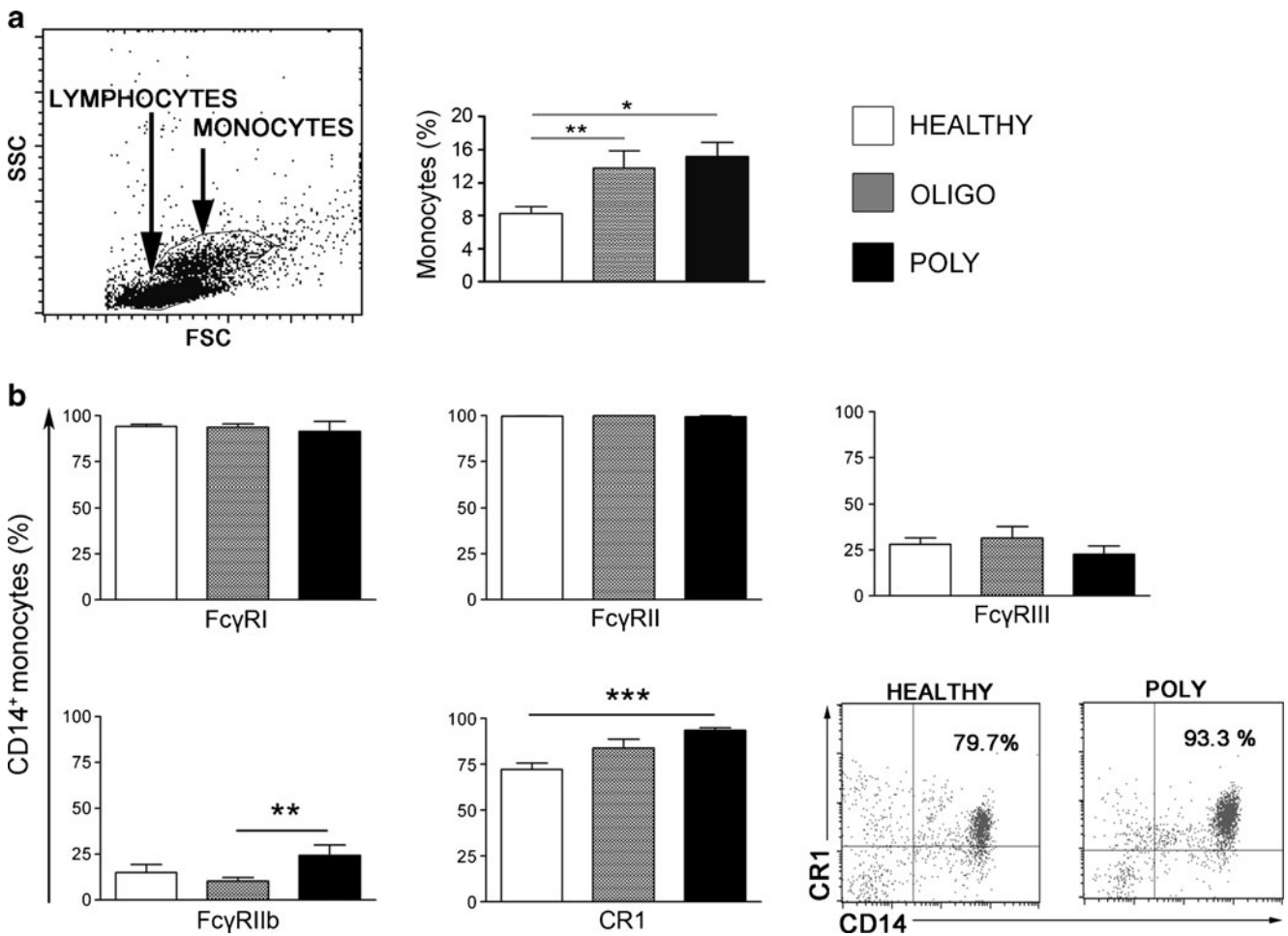
Fig. 2 Monocyte numbers are increased in JIA subsets. Monocyte numbers and FcγR and CR expressing monocytes were analyzed in oligoarticular ($n=16$) and polyarticular ($n=10$) JIA and in healthy controls ($n=21$) by FACS. **a**. Monocytes were defined by size and granulation (*left* diagram) and by the expression of CD14; the mean percentage CD14 positive monocytes ± SEM out of total peripheral blood mononuclear cells was calculated (*right* diagram). **b**. The mean percentage ± SEM of FcγRI, FcγRII, FcγRIII, FcγRIIb and CR1 positive monocytes out of total monocytes. Diagram and representative dot plots showing increased percentage of CR1 positive monocytes in polyarticular JIA compared to healthy control. *$p<0.05$, ** $p<0.01$ and ***$p<0.001$

between any of the JIA subgroups and healthy controls (Fig. 4d).

Correlation between C3a Levels and Receptor Expression in JIA

We further analyzed whether the FcγR and CR expression in the JIA patients was related to the amount of inflammatory molecules and immunoglobulin in the patients. The results demonstrated a correlation between C3a levels and CR1 and FcγRI expression on monocytes ($p=0.032$, $r^2=0.211$ and $p=0.003$, $r^2=0.356$ respectively) (Fig. 5a-b). The C3a levels also correlated with the CR1 expression ($p=0.009$, $r^2=0.283$) and in particular with the CR2 expression ($p=0.0001$, $r^2=0.539$) on B cells (Fig. 5c-d). The immunoglobulin levels nor CRP correlated with the expression of the different FcγR and CR in the JIA patients (data not shown).

Discussion

To our knowledge this study is the first evaluating the involvement of Fc- and complement receptors in JIA, in particular in comparison with a unique age and sex-matched healthy control group. Although JIA is a heterogeneous disease we present a study population with two homogeneous groups of patients regarding the number of active joints during course of the disease. The patients were in a disease flare during sample collection and the subtype of JIA at sample collection was representative for the disease course in each patient. In all subjects the analyses of the blood was started within a few hours and significant results were obtained despite the limited study population.

The result demonstrates a common disturbance in monocyte numbers in the JIA patients, regardless of joint counts. Thus, an increased proportion of CD14+ monocytes were seen in patients with oligoarthritis and polyarthritis in

Fig. 3 Increased CR1 and
FcγR expression on monocytes
in polyarticular JIA. FcγR and
CR expression on monocytes
was analyzed in oligoarticular
(*n*=16) and polyarticular
(*n*=10) JIA and in healthy
controls (*n*=21) by FACS.
a. Diagram showing mean
fluorescence intensity (MFI) of
FcγRI ± SEM and
representative histogram
displaying FcγRI expression. b.
Diagram showing MFI of
FcγRII ± SEM and
representative histogram
displaying FcγRII expression.
c. Diagram showing MFI of
FcγRIII ± SEM and
representative histogram
displaying FcγRIII expression.
d. Diagram showing MFI of
FcγRIIb ± SEM and
representative histogram
displaying FcγRIIb expression.
e. Diagram showing MFI of
CR1 ± SEM and representative
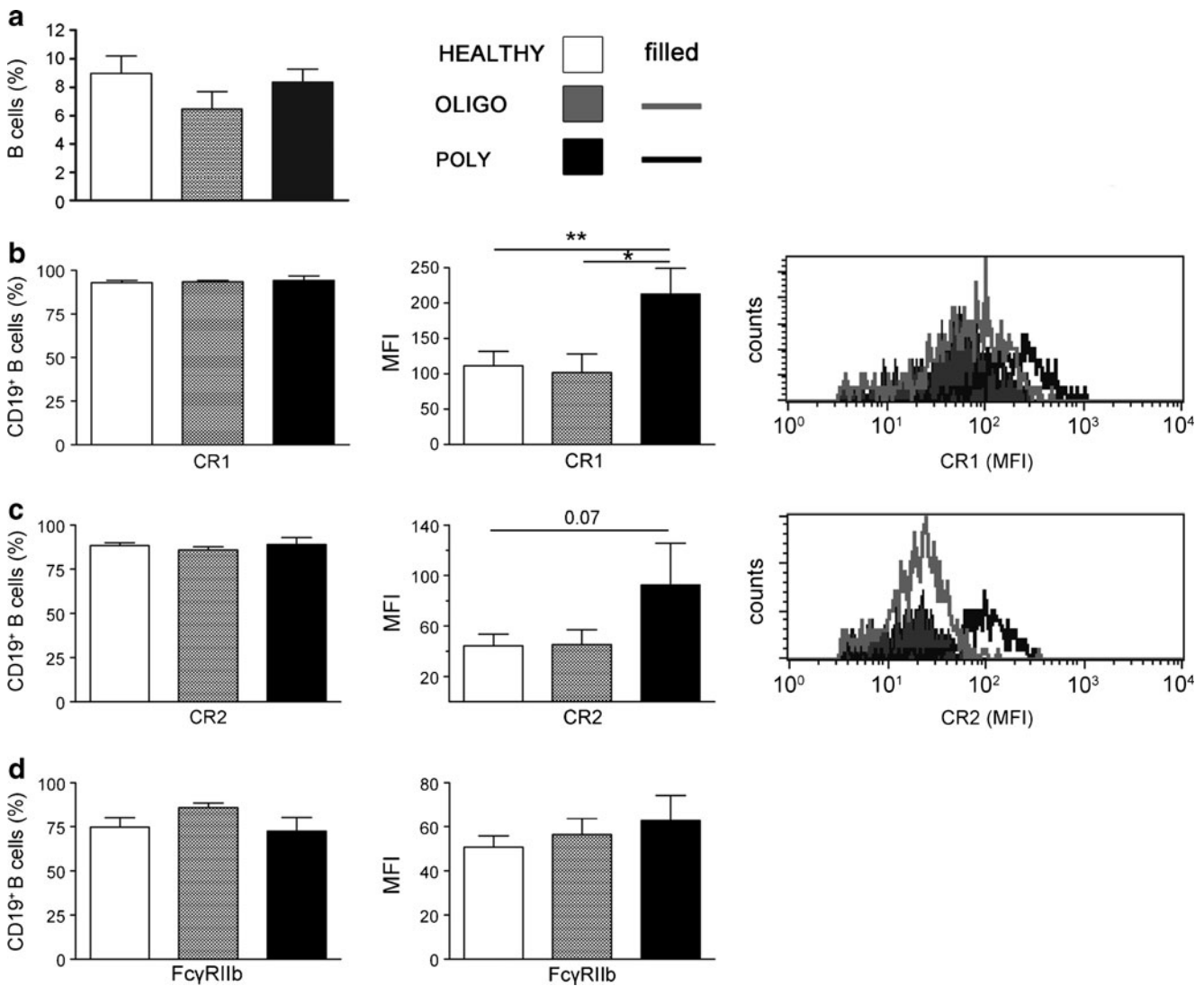histogram displaying CR1
expression. *$p<0.05$,
** $p<0.01$
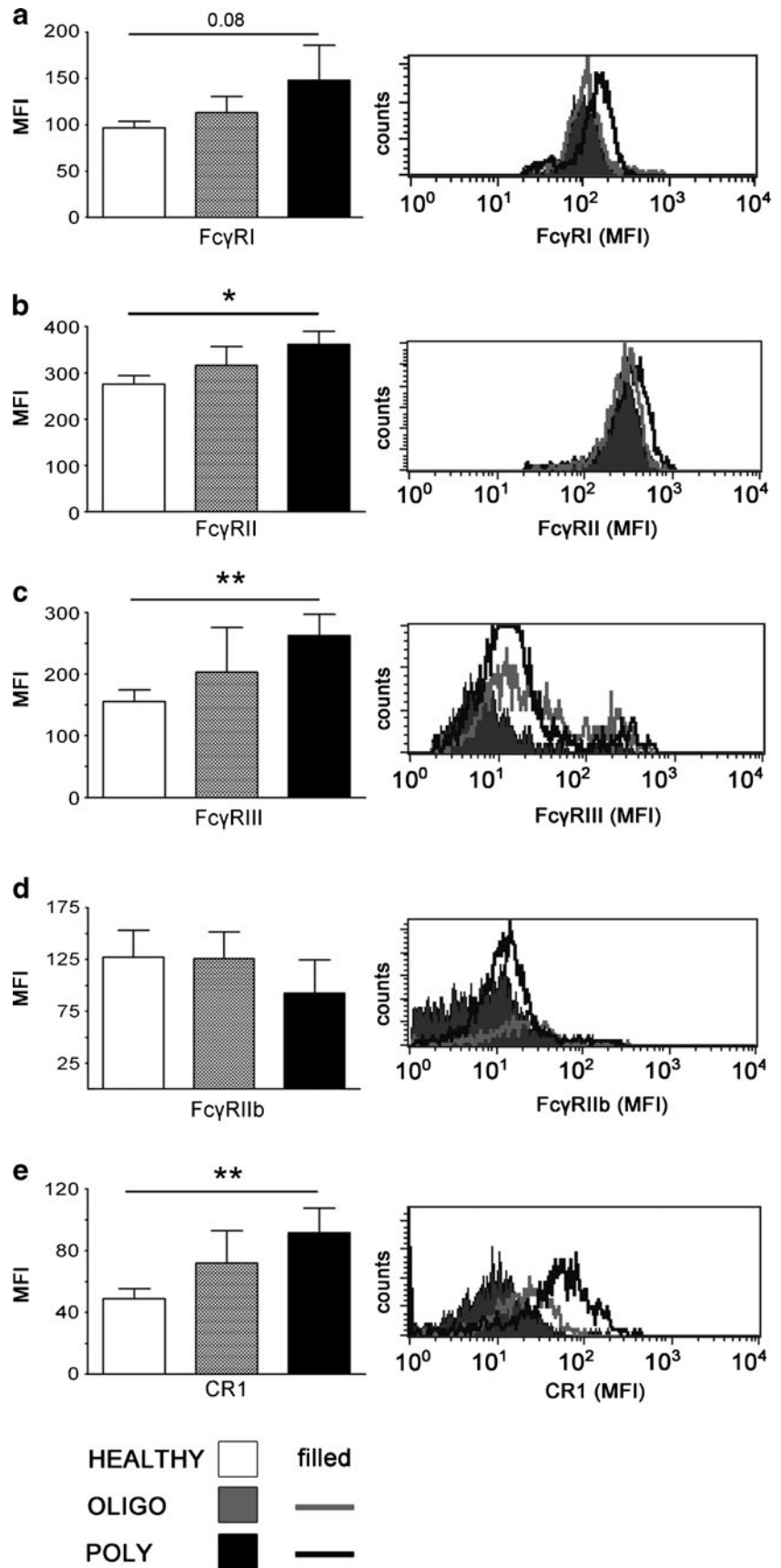
Fig. 4 Increased CR1 expression on B cells in polyarticular JIA. B cells numbers, and FcγR and CR expressing B cells were analyzed in oligoarticular ($n=16$) and polyarticular ($n=10$) JIA and in healthy controls ($n=21$) by FACS. **a**. B cells were defined by the expression of CD19 and the mean percentage ± SEM of B cells out of total peripheral blood mononuclear cells was calculated. **b**. Diagrams showing the mean percentage ± SEM of CR1 positive B cells out of total B cells, and the MFI of CR1 expression ± SEM. Representative histogram displaying CR1 expression. **c.** Diagrams showing the mean percentage ± SEM of CR2 positive B cells out of total B cells, and the MFI of CR2 expression ± SEM. Representative histogram displaying CR2 expression. **d.** Diagrams showing the mean percentage ± SEM of FcγRIIb positive B cells out of total B cells, and the MFI of FcγRIIb expression ± SEM. *$p<0.05$, **$p<0.01$

comparison with immunologically healthy controls. This implies that the increase in monocytes is shared among JIA subtypes and corresponds well with the fact that monocytes are a potential source of pro-inflammatory cytokines such as TNF, IL-1 and IL-6. The monocytes in polyarticular JIA did not only differ in number, they also displayed higher surface expression of activating FcγR, particularly FcγRII and FcγRIII, as the increase in FcγRI did not fully reach statistical significance. The enhanced FcγR levels were unique to polyarthritis patients, implying that the amount of activating FcγR is associated with the degree of joint inflammation. In accordance, elevated expression of activating FcγR on monocytes/macrophages is also reported in

RA, which is characterized as a polyarticular disease [13–15]. Unlike the activating FcγR, the inhibitory FcγRIIb was only expressed on a minority of the monocytes in the JIA patients and healthy controls. However, the polyarthritis patients had more FcγRIIb expressing monocytes than oligoarthritic patients and greater FcγRIIb expression than healthy children, possibly as an attempt to regulate immune complex-mediated FcγR activation. The enhanced protein levels of FcγR are supported by microarray data from Griffin T.A. et al. showing increased FcγR gene expression in PBMCs from children with polyarticular JIA [25]. This may suggest that the increased FcγR expression in polyarticular JIA is due to elevated transcription in the patients.

Fig. 5 C3a levels associate with receptor expression in JIA. Correlation of C3a plasma level (μg/ml) with CR1 (**a**) and FcγRI (**b**) expression (MFI) on monocytes, and CR1 (**c**) and CR2 (**d**) expression (MFI) on B cells in oligoarticular and polyarticular JIA (n=19–21)
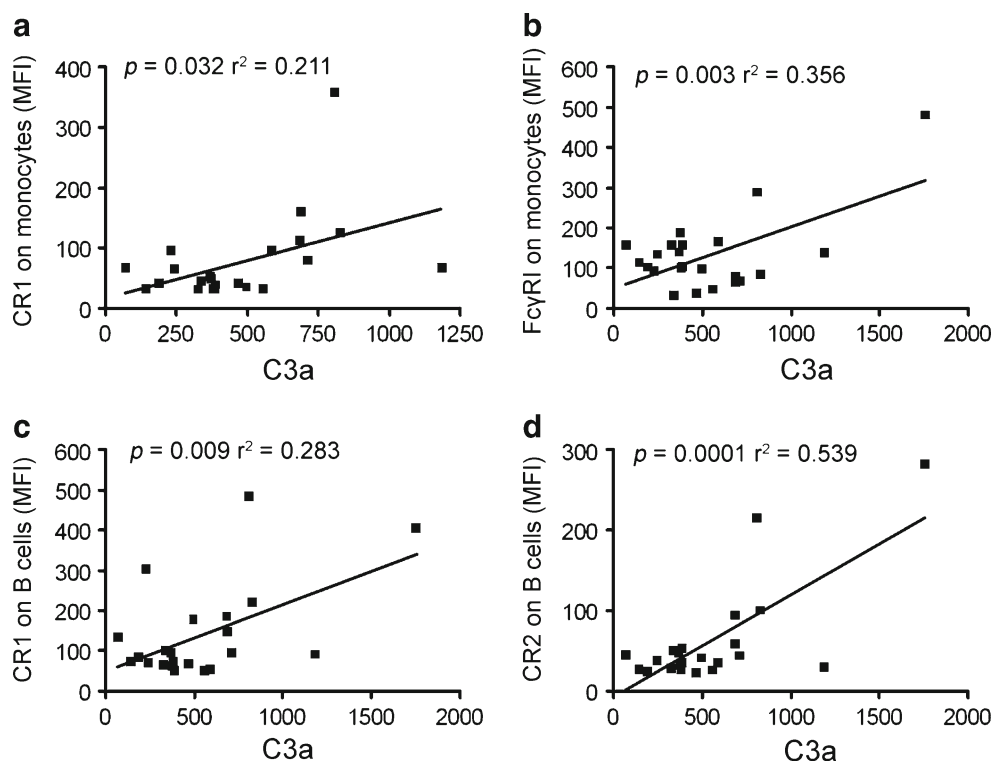
The results propose that studies on FcγR gene polymorphism in JIA might be relevant.

Another monocyte-related finding was the increased CR1 expression in polyarticular JIA. This might be associated with the corresponding expression of activating FcγR, as FcγR and CR1 operate in synergism to promote uptake of particles opsonized by immunoglobulin and complement proteins [26]. Nevertheless, CR1 is a multifunctional receptor, which besides phagocytosis is involved in complement regulation [27]. Thus, the increased CR1 expression may also be an attempt to down regulate the activated complement cascade, indicated by the elevated C3a levels in the JIA patients. Enhanced CR1, as well as CR3, has also been found on monocytes in RA, but not on peripheral blood granulocytes (although activated in RA) [14, 28]. This suggests that monocytic CR1 functions are central in polyarthritis, for example by changing the monocytes ability to respond to opsonized targets. It is noteworthy to mention that besides CR1 also CR3 and CR4 bind iC3b eliciting phagocytosis, however, these receptors were not investigated in this study.

Further, a major site of CR1 expression is on B lymphocytes where it regulates cell activation and facilitates antigen binding and presentation to T cells. In analogy with monocytes, B cells from JIA patients with polyarthritis displayed an enhanced CR1 surface expression. This effect was particularly different from JIA patients with oligoarthritis, which displayed similar CR1 levels on B cells as healthy subjects. Since CR1 generates CR2 ligands by promoting

proteolytic cleavage of C3b to C3dg (a ligand for CR2) it might not be surprising that a tendency of increased CR2 levels on B cells in polyarthritis patients were also observed. The increased protein levels of CR1 and CR2 confirm previous genetic analysis of PBMCs from JIA patients, where the complement cascade, including genes for CR1 and CR2, were up-regulated in JIA subtypes [29]. The level of CR1 expression has been associated with disease pathology in autoimmune disorders. However, CR1 expression on B cells in polyarticular JIA are significantly increased, while reduced on RA B cells [16, 30]. This reveals that the B cell characteristics in polyarticular JIA differ from RA, although both disorders display increased immunoglobulin levels in the circulation. Nevertheless, specific autoantibodies (e.g. RF and anti-CCP) are present in RA, but not frequently seen in JIA (as also shown in our patients). This may suggest that mostly polyclonal and less autoreactive B cells are activated in polyarticular JIA, as up-regulated CR1 and normal FcγRIIb expression will likely support peripheral B cell tolerance. Further studies are needed to define the contribution of the altered B cells in polyarticular JIA to the autoimmune process if we are to fully comprehend the mechanisms involved.

Moreover, we found an interesting positive correlation of C3a levels and CR1, CR2 and FcγRI expression in the JIA patients. C3a is a pro-inflammatory mediator that initiates a wide array of responses through the C3a receptor (C3aR) on immune cells [31]. Thus, it is possible that the CR1 and FcγRI expression on JIA monocytes was stimulated through

signaling from the C3aR. However, B cells lack C3aR, but nonetheless, CR1 and CR2 expression on JIA B cells correlated significantly with the C3a levels in the patients ($p<0.01$–0.001). The reason for this can be two-fold; C3aR-positive cells are activated by C3a and stimulate nearby B cells through soluble mediators, for example TNF and retinoic acid have been reported to up-regulate CR1 on B cells [32]. Secondly, the B cells may express an alternative receptor for C3a as has previously been indicated [33]. The C3a enhancing effect on CR1 and CR2 implies that complement signaling plays a major role in B cell immune responses in JIA. Altogether these findings highlight the complement system as an important pathway in the pathogenesis of polyarticular JIA. This is also evident by the increased amount of complement activating ligands in the patients; IgM, IgG and CRP. Complement components such as C3a and CR1 may serve as useful diagnostic and prognostic markers to monitor the course of polyarticular JIA.

Conclusions

This study shows that oligoarticular and polyarticular JIA share increased monocyte numbers as disease characteristics. However, only patients with polyarthritis display enhanced FcγR and CR1 expression, indicating a role in the pathogenesis of polyarticular JIA.

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