Elevated IgG autoantibody production in oligoarticular juvenile idiopathic arthritis may predict a refractory course

M.L. Stoll[1,2], Q.-Z. Li[3], J. Zhou[4], M. Punaro[1,2], N.J. Olsen[5]

[1]Department of Pediatrics, UT Southwestern Medical Center, Dallas, TX;
[2]Department of Rheumatology, Texas Scottish Rite Hospital for Children, Dallas, TX;
[3]Department of Immunology, and [4]Microarray Core Facility, UT Southwestern Medical Center, Dallas, TX;
[5]Department of Rheumatology, Penn State Hershey Medical Center, Hershey, PA, USA.

Abstract

Objective

Although oligoarticular juvenile idiopathic arthritis (oJIA) is considered to carry the best prognosis among the JIA subtypes, many children evolve to a chronic course. A few studies have identified clinical risk factors for disease extension, and recent studies have evaluated synovial fluid markers. However, the only biological marker from the serum studied to date is the anti-nuclear antibody (ANA), regarding which there is mixed data regarding prognosis. No studies have evaluated whether additional autoantibodies may affect the articular prognosis of oJIA.

Methods

Microarrays containing candidate autoantigens were printed on slides, which were used to profile 36 children with oJIA and 18 controls. Unsupervised cluster analysis was used to identify distinct subgroups of JIA patients. Response to therapy after a mean interval of 4.9 months was evaluated.

Results

Cluster analysis revealed two subgroups of oJIA patients, with identical clustering observed when children with onset over age six were excluded. Cluster 1 had higher levels of multiple autoantibodies compared to both cluster 2 as well as controls, including antibodies against several extracellular matrix (ECM) and nuclear antigens. Although the two patient clusters were similar with respect to clinical features and treatment decisions, children in cluster 1 were less likely to have attained remission by the follow-up visit.

Conclusion

Antibodies against ECM and possibly other antigens may identify a sub-group of children with oJIA who will require more aggressive therapy to attain control of the arthritis.

Key words
oligoarticular, juvenile idiopathic arthritis, antibodies.

Matthew L. Stoll, MD, PhD
Quan-Zhen Li, MD, PhD
Jinchun Zhou, PhD
Marilynn Punaro, MD
Nancy J. Olsen, MD

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Since this paper was accepted, Dr Stoll has left UT Southwestern Medical Center, Dallas. His new contact details are below.

Please address correspondence and reprint requests to:
Dr Matthew Stoll,
Children's Hospital of Alabama,
University of Alabama at Birmingham,
Children's Park Place, Ste 210,
1601 4th Avenue South,
Birmingham, AL 35233, USA
E-mail: mstoll@uab.edu

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Introduction

Oligo-articular juvenile idiopathic arthritis (oJIA) has long been considered to carry the best prognosis among the subtypes of JIA (1). Indeed, a large number of children with this diagnosis respond to conservative therapy consisting of non-steroidal anti-inflammatory drugs or local corticosteroid injections (2). However, historically, at least 50% of this population has extended to poly-articular disease (3, 4), and even years into the disease course, 40–50% continue to have periods of active disease (5), and one-third develop erosive changes (3).

There is no reliable prognostic marker within oJIA. Clinical predictors of disease extension include upper extremity involvement, elevated inflammatory markers, and more than one involved joint at baseline (3, 4). One study showed that early age of onset predicted longer active disease duration (6). There are a few studies that have identified biomarkers that may predict extension. Hunter *et al*. (2010) reported that decreased synovial fluid (SF) CD4: CD8 ratios and increased SF expression of multiple inflammatory markers were associated with an increased likelihood of extension (7). Similarly, Gibson *et al*. (2009) performed proteomic analysis of SF proteins to identify patterns associated with an extended oligoarticular *versus* a persistent oligoarticular course (8). With the exception of the ANA, no studies have evaluated more accessible serum biomarkers, including antibodies.

Autontibodies may play a role in oJIA. Although not pathognomonic, a positive ANA is seen in about two-thirds of patients and has long been recognised to be a risk factor for chronic uveitis; there is mixed data on whether it affects the articular prognosis (3, 6, 9). Additional data suggests that antibodies to histones or chromatin may also be associated with an increased risk of uveitis (10, 11). The observation that antibodies offer prognostic information with respect to the uveitis and possibly the overall prognosis associated with oJIA raises the question as to whether as yet unidentified antibodies likewise affect the articular prognosis. To evaluate for this possibility, we screened a population of children with oJIA for the presence of antibodies against an array of autoantigens.

Patients and methods

Patients

Thirty-six children meeting the International League of Associations for Rheumatology (ILAR) criteria for oJIA were evaluated at Texas Scottish Rite Hospital for Children (TSRHC) (12). 18 children evaluated at TSRHC with a chief complaint of joint pain but found to have a non-inflammatory etiology (*e.g*. benign hypermobility or amplified pain syndrome) were enrolled as controls. Information on patient demographics, disease duration, routine laboratory studies, joint count, and medication use were recorded. IRB approval from the UT Southwestern Medical Center was obtained in advance of the study, and informed consent was obtained from the legal guardians of all enrolled patients; assent was obtained from children age 10 and older as per local regulations.

Antibody profiling

5–10 ml of blood was obtained from each subject and stored in aliquots at -80°C. Antigens were purchased from a variety of sources, and the optimal priming concentrations had previously been determined. A complete listing of the antigens used, their sources, and the priming concentrations is available from the authors upon request. The array consists of 105 proteins: 101 autoantigens and 4 controls. A MicroGrid II microarrayer was used to spot the proteins onto Nitrocellulose-coated 16-pad FAST™ slides (Whatman Schleicher & Schleicher BioScience, Keene, NH), with 16 arrays printed per slide. Antigens were printed in duplicate and randomly distributed on the slides. Details of the hybridisation procedure were as described previously (13). Briefly, after pre-treatment with DNAse, serum samples were added to the arrays for 60 minutes and washed, after which Cy3 labeled anti-human IgG (Jackson Immuno Research, West Grove PA; 1:500 dilution) was applied. After washing, a Genepix 4000B scanner with laser

wavelengths 532nm (cy3) and 635nm (cy5) was used to generate Tif images which were analysed with Genepix Pro 6.0 software. Net fluorescence intensities (nfi), defined as the spot minus background fluorescence intensity of the auto-antigen divided by the spot minus background intensity of the control antigens, were calculated; data obtained from duplicate spots were averaged. The signal: noise ratio for each nfi was calculated; ratios less than three were considered negative. For the analysis, we filtered the data to include only antigens targeted by at least 25% of the overall population.

Outcome assessment

The outcome assessment was obtained at the first follow-up visit that was at least three months following study enrolment. Information on interim medication use, including intraarticular corticosteroid injections at the study visit, was obtained, as well as a complete joint count. Arthritis in a joint was defined by the presence of swelling or limited range of movement accompanied by pain or tenderness and observed by a physician (12). Inactive arthritis was defined as an active joint count of zero, as well as the absence of dactylitis.

Data analysis

In order to detect associations that may not otherwise be evident, cluster analysis was performed on the data. Specifically, open-source Cluster software (Cluster 3.0, available for download at http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/) was used to perform unsupervised hierarchical cluster analysis. A dendrogram of the data, clustered by both antigen and subject, was viewed using the open-source Java Treeview software (14).

The Chi squared or Fisher exact test, as appropriate, were used to evaluate proportional data. Two-way continuous data was evaluated with the Student's *t*-test. Comparisons of three groups were performed with one-way ANOVA; back-testing was performed with the Bonferroni correction. SPSS Version 16 was used for these analyses. Because this was an exploratory analysis, we set α to 0.05.

Table I. Profile of study patients.

Item	Patients	Controls
N	36	18
Female sex (%)	69.4	55.6
Hispanic (%)	5.6	27.8
African-American (%)	2.8	16.7
Age of onset of symptoms (years)	5.3 ± 2.8*	7.7 ± 3.8
Age at study entry (years)	7.6 ± 3.7	9.7 ± 4.1
Newly diagnosed (%)	36.1	88.9
Laboratory studies		
ANA	23 / 36 (63.9%)	4 / 14 (28.6%)
RF†	4 / 34 (11.8%)	0 / 15
HLA-B27	1 / 31 (3.2%)	0 / 12
Treatment (%)		
Methotrexate	13.9	0
TNF-α inhibitors	5.6	0
Prednisone	0	0
Prior IAC	33	0

*Continuous data is shown as means ± SD. IAC: intra-articular corticosteroids; TNF: tumour necrosis factor. †All four children had only a single positive RF study, so were not excluded from the diagnosis of oJIA as per the revised ILAR criteria (12).

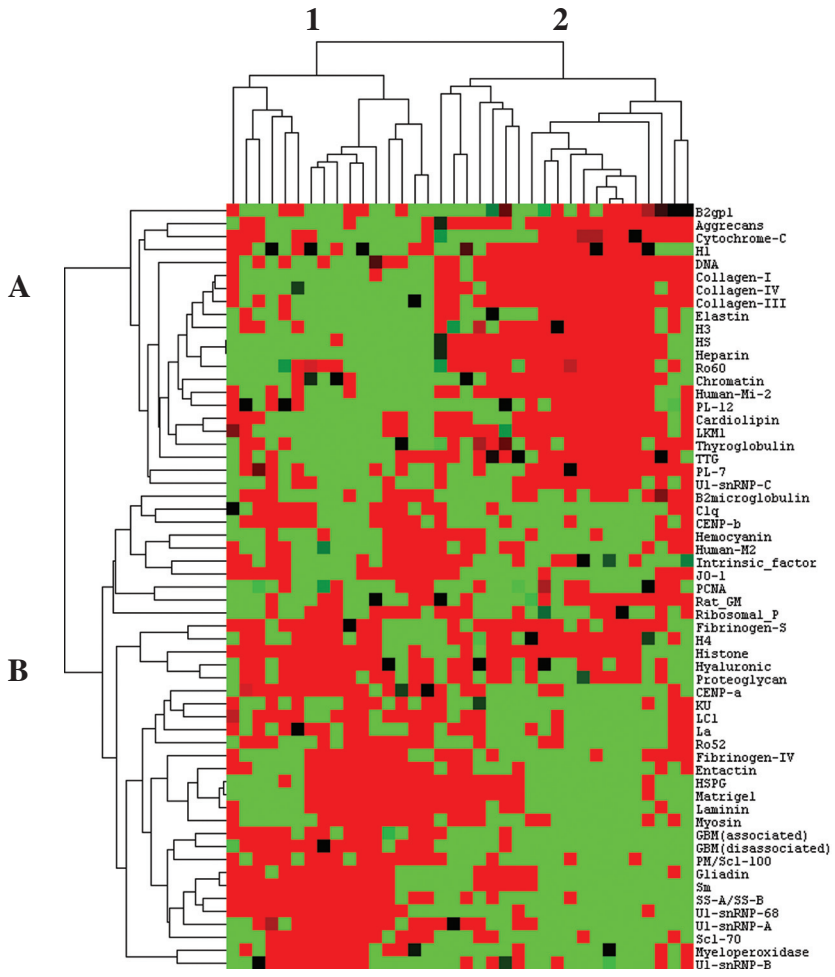

Fig. 1. Unsupervised clustering heat map of the 59 IgG autoantibody reactivities in serum samples of oJIA patients. Each row consists of an antigen, and each column represents a unique patient. The net fluorescent intensity data were used to generate the heat map. For each antigen, the intensities are displayed on a relative scale: reactivities above the mean are coloured red, reactivities below the mean are coloured green, and reactivities near the mean are coloured black. The left margin dendogram depicts two antibody clusters (**A, B**), while the patient clustering (**1, 2**) is depicted above.

Table II. Phenotype of oJIA clusters at baseline study visit.

	All oJIA patients			Onset under 6 years		
Feature	Cluster 1	Cluster 2	*p*	Cluster 1	Cluster 2	*p*
N	16	20	N/A	12	10	N/A
Newly diagnosed (%)	56.2	20	0.024	50	30	0.415
Age of onset (years)	4.3 ± 2.3*	6.2 ± 2.9	0.040	3.2 ± 1.3	3.8 ± 1.4	0.315
Age at study entry (years)	5.9 ± 3.7	8.9 ± 3.3	0.016	4.6 ± 3.0	6.4 ± 2.8)	0.165
Female sex (%)	81.2	60	0.277	83.3	50	0.172
Involved joints (ever; %)						
Knees	75	80	1.000	75	80	1.000
Ankles	31.2	15	0.422	41.7	0	0.040
Wrists	12.5	10	1.000	16.7	10	1.000
IPJ	37.5	25	0.483	41.7	20	0.381
Uveitis (% evaluated)	16.7	6.2	0.481	20	0	0.385
ANA (% tested)	75	55	0.214	83.3	50	0.172
ESR (mm/hr)	15.1 ± 9.5	11.6 ± 12.2	0.367	15.3 ± 9.9	10.7 ± 9.0	0.267
Treatment (%)						
Methotrexate	12.5	15	1.000	16.7	10	1.000
TNF-α inhibitors	6.2	5.0	1.000	8.3	10	1.000
IAC	25	40	0.343	25	40	0.652

*Continuous data is shown as means ± SD. IPJ: intra-phalyngeal joint (proximal IPs of hands and feet, distal IPs of hands and feet, and IP of first digit of hands and feet).

Results

Patient population

Information on subjects enrolled in this study is summarised in Table I. In general, control subjects were older than the oJIA patients and better reflected the demographics of the state of Texas.

Cluster analysis identifies two oJIA subgroups

Of the 101 autoantigens, 59 had nfi signal: noise ratios greater than or equal to 3 in at least 25% of the patient population. These 59 IgG autoantibody specificities were analysed using unsupervised hierarchical clustering. Children with oJIA fell into two IgG clusters (Fig. 1); their clinical and demographic features are summarised in Table II. Children in the second cluster were older at onset, but were otherwise similar in most of the clinical and demographic features. Because cluster 2 had more children with an older age of onset, some of whom may have represented HLA-B27 negative spondyloarthritis without meeting criteria for enthesitis-related arthritis, we repeated the cluster analysis, limiting it to those 22 children with oJIA with an age of onset less than six years. This age was selected because multiple prior studies have shown it to be an inflection point for the age of onset distribution among JIA patients (15, 16), with younger and older-onset oJIA children clearly distinct from a clinical and genetic basis (17, 18). The cluster assignments of the 22 early-onset children were not changed when the cluster analysis was repeated after excluding the 14 children with an older age of onset (data not shown.) As shown in Table II, analysis of this limited population again demonstrated two demographically and clinically similar populations.

A visual appraisal of Figure 1 identifies two major clusters of antigens, defined as clusters A and B. Cluster B includes several antigens that are found within the extracellular matrix (ECM), including fibrinogen, entactin, heparan sulfate proteoglycan (HSPG), laminin, and Matrigel®, an artificial ECM. As shown in Table III, children in cluster 1 generally had higher antibody titers compared to both their counterparts in cluster 2 as well as to controls, while children in cluster 2 typically had similar titers compared to the controls. The differences between the groups were most pronounced in the antigens comprising cluster B, but are present in both antigen clusters. We pooled the data and calculated mean antibody titers for all of the antigens within each cluster. As shown in Figure 2, children in oJIA cluster 1 had significantly ($p<0.05$, ANOVA) elevated titers in both comparisons, although the titers against the antibodies in cluster A were generally low in both groups. Similar trends were observed in the cohort of children with onset under age six (data not shown). The three patient groups had similar total immunoglobulin levels obtained as part of the array study (nfi 11,850 in cluster 1; 10,179 in cluster 2; and 8915 in controls; $p=0.064$.)

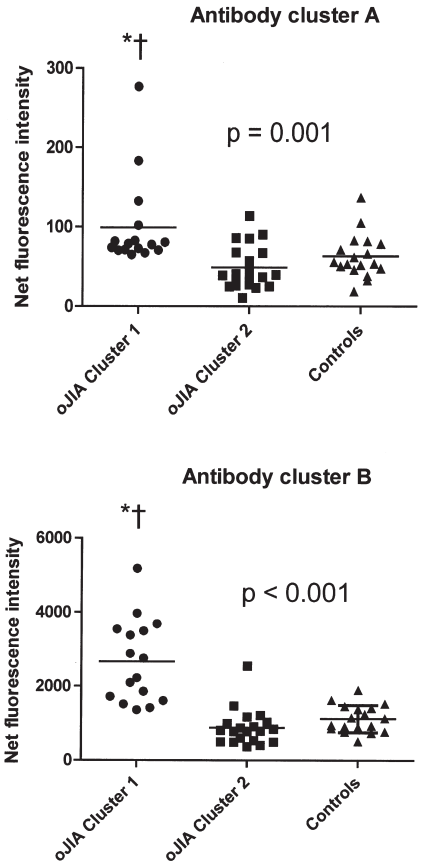

Fig. 2. Average raw net fluorescent intensity data of the autoantibodies. Cluster A consists of the antibodies shown in the upper dendogram of Figure 1, while cluster B represents those shown in the lower dendogram of Figure 1. *Statistically significant difference ($p<0.05$, Bonferroni correction) compared to control patients. †Statistically significant difference ($p<0.05$, Bonferroni correction) compared to Cluster 2.

Children in Cluster 1 were more likely to have active disease at follow-up

Follow-up information obtained after a mean interval of almost 5 months following measurement of autoantibody levels was available on 34 of the children with oJIA, including 21 with onset under age six years (Table IV).

Treatment decisions of the two groups were generally similar; all intra-articular corticosteroids (IAC) injections consisted of triamcinolone hexacetonide. Children in cluster 2 were more likely to be in remission at the time of the follow-up visit among the entire cohort (78.9% *vs*. 40%, p=0.020) as well as among those with onset under age six years (90.0% *vs*. 36.4%, p=0.024.)

Discussion

In this study, we evaluated a cohort of children with oJIA for expression of antibodies against a panel of autoantigens. We demonstrated that this population fell into two separate groups, with one cluster generally demonstrating elevated autoantibody production compared both to the other cluster as well as to control children. To account for possible differences in age, we limited the analysis to oJIA children with onset of arthritis under age six, obtaining similar results. Patient cluster 1 had elevated levels of multiple IgG autoantibodies despite similar total IgG levels and responded relatively poorly to therapy, while cluster 2 patients had low levels of autoantibodies and generally responded well to therapy. In general, children in cluster 2 had similar autoantibody levels compared to the control subjects.

The results of our study may suggest that testing for a limited panel of autoantibodies may help prognosticate oJIA. However, it is not clear which of these antibodies may be most useful. Several of the antigens in the panel were derived from nuclear material, including histones and chromatin, and thus may bear relationship at least with the risk of uveitis, as seen in prior studies (10, 11). Although uveitis may have been more common in Cluster 1, we were underpowered to detect a statistically significant difference. The overall incidence of uveitis in our population was lower than expected, perhaps on account of the short duration of follow-up of some of our patients, as well as use of methotrexate. In contrast to the significant differences in titers against histones and chromatin, the two groups had similar proportions of ANA positivity.

There are also multiple antigens directed against extracellular matrix

Table III. Antibody levels in oJIA patients and controls.

Antibody	oJIA Cluster 1 (n=16)	oJIA Cluster 2 (n=20)	Controls (n=18)	p -value
Antigen Cluster A				
β2 gp1	215 ± 118*†	94 ± 37	114 ± 39	< 0.001
Aggrecan	177 ± 425	21 ± 36	28 ± 60	0.102
Cytochrome C	230 ± 113	230 ± 480	257 ± 501	0.977
Histone 1	196 ± 115*†	65 ± 33	66 ± 32	< 0.001
dsDNA	81 ± 134	27 ± 38	63 ± 87	0.207
Collagen I	29 ± 47	19 ± 27	24 ± 35	0.724
Collagen IV	56 ± 73	19 ± 26	27 ± 30	0.052
Collagen III	49 ± 62	17 ± 32	22 ± 51	0.128
Elastin	69 ± 32	52 ± 44	53 ± 35	0.355
Histone 3	130 ± 62*†	46 ± 35	50 ± 23	< 0.001
Heparin	38 ± 47†	7.3 ± 25	12 ± 27	0.022
Ro60 Kd	106 ± 70†	44 ± 60	77 ± 58	0.016
Chromatin	146 ± 104*†	62 ± 32	67 ± 36	< 0.001
Mi-2	82 ± 122	25 ± 30	41 ± 33	0.058
PL-12	171 ± 83*†	72 ± 57	90 ± 34	< 0.001
Cardiolipin	52 ± 61	39 ± 46	87 ± 73	0.052
LKM1	46 ± 66	41 ± 39*	85 ± 48	0.024
Thyroglobulin	134 ± 83	84 ± 67	105 ± 108	0.235
Transglutaminase	95 ± 100	92 ± 118	89 ± 68	0.984
PL-7	184 ± 79†	108 ± 56	155 ± 119	0.039
U1snRNP-C	210 ± 68*†	112 ± 46	123 ± 36	< 0.001
Antigen Cluster B				
B2-microglobulin	2144 ± 934	1718 ± 837	1532 ± 574	0.081
C1q	656 ± 157	487 ± 403	453 ± 247	0.115
CENP-B	460 ± 165†	235 ± 95*	464 ± 419	0.014
Hemocyanin	413 ± 278	279 ± 188	331 ± 189	0.198
M2	786 ± 975	423 ± 288	579 ± 561	0.252
Intrinsic Factor	503 ± 293*†	246 ± 171	301 ± 140	0.002
Jo-1	2081 ± 5096	446 ± 252	542 ± 340	0.167
PCNA	322 ± 168†	148 ± 87	287 ± 269	0.016
Rat GBM	172 ± 129*†	74 ± 68	95 ± 60	0.005
Ribosomal P	298 ± 141†	169 ± 95	240 ± 124	0.009
Fibrinogen S	235 ± 213*†	36 ± 34	54 ± 49	< 0.001
Histone 4	141 ± 108*†	19 ± 28	38 ± 19	< 0.001
Histone total	291 ± 199*†	88 ± 72	144 ± 66	< 0.001
Hyaluronic acid	295 ± 197*†	85 ± 186	129 ± 148	0.003
Proteoglycan	318 ± 318	123 ± 345	194 ± 379	0.259
CENP-A	636 ± 523*†	266 ± 177	295 ± 92	0.001
KU (p70 / p80)	1933 ± 4859	301 ± 140	496 ± 271	0.154
LC1	681 ± 336†	328 ± 130*	597 ± 429	0.004
La / SS-B	1231 ± 1086	744 ± 1036	597 ± 210	0.099
Ro52 Kd	2681 ± 3639	1199 ± 537	1302 ± 426	0.067
Fibrinogen IV	2691 ± 4010	1000 ± 763	1596 ± 2235	0.154
Entactin	1394 ± 931*†	583 ± 628	649 ± 573	0.003
HSPG	7189 ± 7866†	2740 ± 4417	4002 ± 3119	0.050
Matrigel	8661 ± 9286†	3267 ± 4723	4386 ± 3463	0.032
Laminin	2974 ± 3100	1301 ± 2309	1626 ± 1618	0.103
Myosin	1510 ± 933†	837 ± 642	1342 ± 848	0.038
GBM (associated)	1710 ± 2611†	397 ± 180	497 ± 344	0.017
GBM (disassociated)	903 ± 373*†	343 ± 107	529 ± 335	< 0.001
PM / Scl-100	8180 ± 9394†	1518 ± 742	3803 ± 4018	0.003
Gliadin	4470 ± 4075*†	620 ± 657	838 ± 960	< 0.001
Sm	17099 ± 10429*†	3659 ± 2959	5014 ± 4547	< 0.001
SS-A / SS-B	16847 ± 9708*†	5071 ± 2950	5786 ± 2592	< 0.001
U1snRNP-68 Kd	3435 ± 1651*†	1407 ± 650	2078 ± 843	< 0.001
U1snRNP-A	546 ± 225*†	242 ± 114	270 ± 85	< 0.001
Scl-70	1390 ± 780	821 ± 985	830 ± 422	0.059
Myeloperoxidase	610 ± 224*†	3271 ± 140	360 ± 117	< 0.001
U1snRNP-B	337 ± 130*†	171 ± 65	192 ± 69	< 0.001

Autoantibody levels for all patients. *Statistically significant difference (p<0.05, Bonferroni correction) compared to control patients. †Statistically significant difference (p<0.05, Bonferroni correction) compared to Cluster 2. Back-tests were only performed when the ANOVA p-value was ≤0.05.

Table IV. Follow-up data on children with oJIA.

	All oJIA patients			Onset under 6 years		
Feature	Cluster 1	Cluster 2	*p*	Cluster 1	Cluster 2	*p*
N	15	19	N/A	11	10	N/A
Time interval (months)	5.0 ± 2.7*	4.8 ± 1.7	0.809	4.3 ± 1.0	5.0 ± 2.1	0.376
Treatment (%)						
None or NSAIDs	13.3	42.1	0.128	9.1	40.0	0.149
Methotrexate	33.3	31.6	1.000	45.5	30.0	0.659
TNF-α inhibitors	6.7	10.5	1.000	9.1	10.0	1.000
PO steroids	0	0	N/A	0	0	N/A
IAC	46.7	26.3	0.218	45.5	30.0	0.659
Inactive arthritis at baseline	6.7	31.6	0.104	9.1	30.0	0.311
Inactive arthritis at follow-up	40.0	78.9	0.020	36.4	90.0	0.024

*Continuous data is shown as means ± SD.

(ECM) structures, including entactin, fibrinogen, heparin sulfate proteoglycan (HSPG), hyaluronic acid, laminin, proteoglycan and Matrigel® (an artificial ECM). These were all present in high titers and were limited to antigen cluster B, underscoring their potential role in the disease process. Likewise, multiple prior studies have shown that ECM antigens such as laminin, entactin, and HSPG are highly expressed in inflamed synovium or otherwise have become antigenic targets in adult or pediatric inflammatory arthritis (19, 20), while fibrinogen and hyaluronic acid are immunogenic in mice, with injections of either one resulting in inflammatory arthritides (21, 22). Thus, we consider it possible that some of these antibodies may mediate oJIA. Findings of a possible role for autoantibodies in oJIA would be consistent with microarray data showing B-cell activation in this population (23), as well as findings of oligoclonal $CD4^+$ T-cell expansion within the synovium of oJIA patients (24).

Our study has several limitations. First, this was not an inception cohort; many of the children had been followed for several years, and a small number were stable on immunosuppressive therapy. Because these studies were only performed once, it is unknown whether antibody titers might have changed over the course of treatment. Future studies will be required to evaluate whether changes in antibody titers longitudinally parallel clinical changes. Additional limitations are the relatively small sample size, the relatively short follow-up period, and the exploratory nature of these analyses. Furthermore, some of the findings may be compromised by multiple comparisons. Thus, our results would need to be validated in future studies.

In summary, we identified two cohorts of children with early-onset oJIA, differing with respect to autoantibody production and course of disease. Confirmation of specific antibodies still needs to be performed by traditional methods, and the array data cannot replace clinical assessment and judgment in the evaluation of each child. However, our work may help identify children with oJIA who may respond poorly to therapy, and may also shed light on the mechanisms involved.

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