Autoantibodies to chromatin: Prevalence and clinical significance in juvenile rheumatoid arthritis

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ABSTRACT

Objective. *To determine the prevalence of anti-chromatin antibodies (Abs) in juvenile rheumatoid arthritis (JRA) and to assess any association between the presence of anti-chromatin Abs and clinical subsets of the disease.*

Methods. *IgG anti-chromatin Abs and anti-extractable nuclear antigens (ENA) Abs were detected by an enzyme-linked immunosorbent assay (ELISA), and antinuclear Abs (ANA) by indirect immunofluorescence in sera of 89 children with JRA. Ten children with systemic, 32 with polyarticular and 47 with pauciarticular disease onset (uveitis occurred in 17/47 children) were studied. As a control group, 12 sera of patients suffering from idiopathic uveitis and 31 age- and-sex-matched healthy children (HC) were examined.*

Results. *Abs to chromatin were detected in 14/47 (29.8%) of children suffering from pauciarticular onset JRA and in this group the higher prevalence of anti-chromatin Abs has been found in children with chronic uveitis (p = 0.002). Anti-chromatin positivity was observed in 2/10 (20%) of systemic and in 3/32 (9.3%) of polyarticular onset JRA. Furthermore, none of the patients with idiopathic uveitis and HC had Abs to chromatin. anti-chromatin Abs titers remained relatively stable over a 6-month control period.*

Conclusion. *Our results confirm previous data about the presence of circulating anti-chromatin Abs in juvenile arthritis. Interestingly, anti-chromatin Abs were significantly higher in the group of patients with pauciarticular onset with past or present history of uveitis, than in patients without ocular involvement. A long-term follow-up study could be useful to demonstrate the potential utility of these autoantibodies in diagnosing, classifying and treating children affected.*

Introduction

Juvenile rheumatoid arthritis (JRA) is a chronic autoimmune disease, which is characterized by destructive inflammation of the joints. The diagnosis is primarily based on clinical manifestations and JRAis categorized in different subsets according to the disease onset and course. Unlike in adult rheumatoid arthritis (RA), rheumatoid factor is positive only in a small percentage of children with polyarticular disease onset, while antinuclear antibodies (ANA) are relatively frequent, particularly in patients with pauciarticular onset and anterior uveitis (1).

In addition, several other studies have shown the presence of many other autoantibodies in JRA sera, including anticardiolipin Abs (2), antiperinuclear factor (4), antikeratin Abs (4) and anticyclic citrullinated peptide Abs (5), however none of them appears to have diagnostic and/or clinical value. Moreover, some studies have shown that reactivity to nuclear, cytoplasmic or nonhistone antigens are rarely found (1).

The only laboratory assay routinely used is the presence ANA, even if their antigenic specificities are not well defined; these Abs have been established as a marker present in more than 50% of JRA children (10-20% with polyarticular and 30-50% with oligoarticular disease onset) and in around 80% of JRA patients with anterior uveitis (6). JRAsera, when assayed for ANAby indirect immunofluorescence, usually display staining to the chromosomal regions of metaphase/anaphase cells, suggesting that chromatin or nucleosomal constituents may be the antigenic targets. Based on these findings, previous studies have reported contrasting data, maybe due to technical differences in the procedures, regarding the presence of Abs to chromatin, individual histones (H1, H2A, H2B, H3 and H4) or histone peptide fragments in JRA patients (7-11).

To further explore the role of anti-chromatin Abs in JRA children, we investigated the prevalence of IgG anti-chromatin Abs in a cohort of patients with JRA; and we sought to estimate the clinical significance of these Abs.

Patients and methods

Study population

89 consecutive Italian JRApatients (61 girls and 28 boys) who attended the Pediatric Unit of Rheumatology at the Istituto Ortopedico Gaetano Pini (Milan, Italy) were included in the study. These patients fulfilled the criteria proposed by

the American College of Rheumatology (12) and they were grouped according to the disease onset: 47 had pauciarticular, 32 had polyarticular and 10 had systemic disease. Their mean age was 15.5 years (range 2.6-18) and the mean disease duration was 4.5 years (range 1.5-10.5). Eighteen JRApatients were further examined three times over a period of six months. For the purpose of this study, the disease was considered active (active joints and abnormal laboratory test) and inactive (no active joints). None of children with polyarticular or systemic disease onset had ocular involvement, while 17 of 47 patients with pauciarticular disease had associated chronic anterior uveitis. At the time of sampling, all these patients had inactive uveitis. The presence of uveitis was diagnosed by slit lamp examination performed routinely and active uveitis was defined as the presence of cells and flare in the anterior chamber. 31 age- and sex-matched HC sera were obtained, after informed parental consent, from children who were hospitalized for surgical orthopedic procedures at the Istituto Ortopedico Gaetano Pini (Milan, Italy). 12 adult patients with idiopathic uveitis were also included in the study; they had been referred to the Rheumatology Department for the purpose of ruling out associated disease.

Assays

Venous blood was collected during routine venepuncture performed for periodic assessment of laboratory tests. Permission for drawing extra blood during routine venepuncture was obtained from the parents of all children. Serum samples were stored at -20°C until used and thawed only once, for determinations of anti-chromatin Abs, ANA and ENAreactivity.

The presence of IgG anti-chromatin antibodies was evaluated with commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quanta Lite™ Histone, INOVA Diagnostics, San Diego). The test kit was used following the procedures suggested by the manufacturer.

The presence of ANA was determined by a standard indirect immunofluorescence technique on monolayers of human larynx epidermoid carcinoma cells (Hep-2) at a screening dilution of 1:40 (Delta Biologicals, Miami, Florida).

The presence of IgG Abs to ENA was determined with a commercial ELISA kit (ENA-6 screen, Delta Biologicals, Miami, Florida). The manufacturer's instructions for the kit were followed without modifications.

Statistical analyses

The statistical significance for the various associations was calculated using χ^2 analyses or Fisher's exact test, as appropriate.

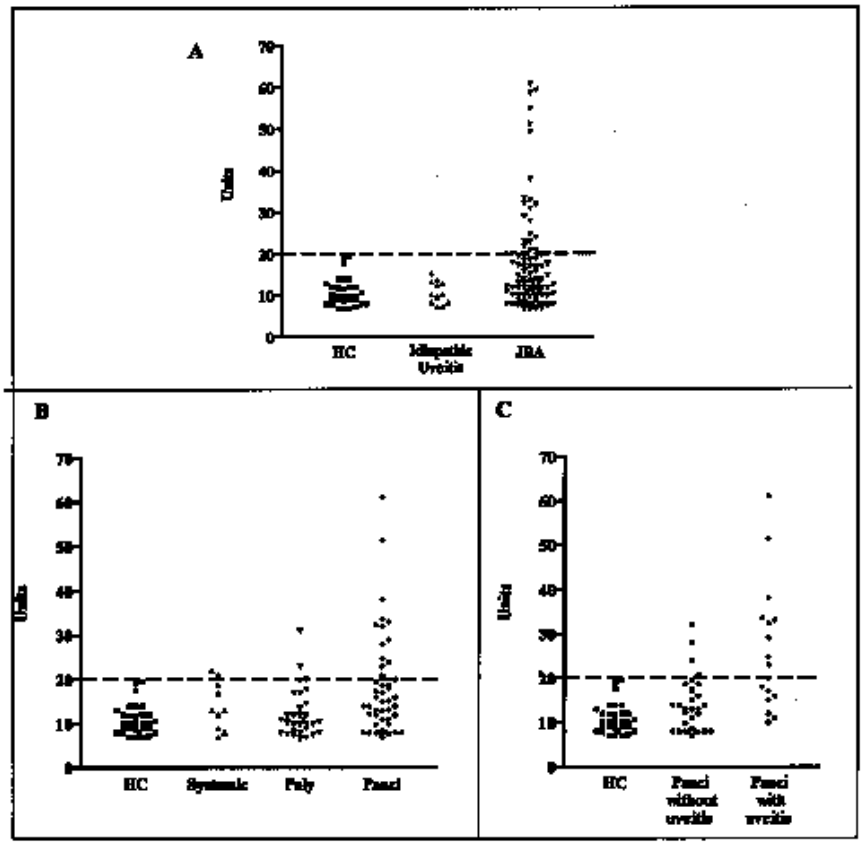

Fig. 1. The cumulative results of anti-chromatin assays in all the patients studied. (**A**) Serum levels of these Abs are shown in the group of patients with JRA, idiopathic uveitis and HC. (**B**) Anti-chromatin Abs values in patients with different JRA disease onsets (polyarticular, pauciarticular and systemic). (**C**) Anti-chromatin Abs values in patients suffering from pauciarticular JRAwith and without ocular involvement. The horizontal dashed line indicates the cut-off value.

Results

Anti-chromatin antibody assay was performed in all the patients and the presence of anti-chromatin Abs were found in sera of 23.6% (21/89) patients with JRA. None of the HC and idiopathic uveitis was positive for anti-chromatin Abs. Titers of these Abs were significantly higher in JRA compared to HC and idiopathic uveitis ($p = 0.003$), as shown in Figure 1A.

Furthermore, we analysed the differences in the prevalence of anti-chromatin Abs between various JRA disease onsets, as shown in Figure 1B. Positivity was found in 3/32 (9.3%) in the group with polyarthritis, 2/10 (20%) in the group with systemic disease and 14/47 (29.8%) in the group with pauciarthritis. Anti-chromatin Abs titers were significantly higher in children with pauciarthritis compared to the other two disease onset groups ($p = 0.05$) and to HC ($p = 0.0009$). In particular, Figure 1C shows that anti-chromatin Abs levels were significantly higher in children suffering from pauciarthritis with ocular involvement compared to the one without uveitis ($p = 0.002$) and to HC ($p=0.0001$).

Interestingly, the ANA prevalence was

Table I. Prevalence of positive anti-chromatin Abs and ANA values in children suffering from pauciarthritis with or without ocular involvement.

	ANA+ Anti-chromatin +	ANA+ Anti-chromatin -	ANA- Anti-chromatin +	ANA- Anti-chromatin -
Pauciarticular JRAwithout ocular involvement	10% (3/30)	33% (10/30)	0% (0/30)	57% (17/30)
Pauciarticular JRAwith ocular involvement	53% (9/17)	18% (3/17)	0% (0/17)	29% (5/17)

found to be 28% in the patients with polyarticular, 10% in the systemic and 48% in the pauciarticular JRA. As shown in Table I, there was a considerable association between anti-chromatin Abs positivity and patients suffering from pauciarthritis with uveitis (p=0.003; RR=5.29; OR=10.12), and the ANA positivity associate with the pauciarthritis disease subtype (p = 0.009). No statistically significant association was found between ANA (p= 0.12; RR= 1.62; OR=3.13) or ENA positivity and pauciarthritis with uveitis. For 18 patients with JRA, time integrated anti-chromatin Abs serum assays were carried out and these levels remained stable throughout baseline and six months.

Discussion

This work describes the reactivity of JRA patient sera with chromatin. Interestingly, we found that these antibody titers were significantly higher in children suffering from JRA and these levels were particularly increased in the pauciarthritis disease subset with ocular involvement. Furthermore, their titers remained relatively stable during a six month period.

In addition, we have confirmed what has already been shown in previous reports concerning ANA and ENA positivity during the course of JRA (1,7). Our results showed a small group of patients ANA+/anti-chromatin Abs-, confirming that ANA are directed against a variety of nuclear proteins, including histones. Moreover, we could not find any relationship between ANA immunofluorescence staining pattern and anti-chromatin Abs positivity. In this study, ANApositivity is a very frequent finding in children with pauciarticular JRA, but not with the pauciarthritis with uveitis disease subtype.

Our study presents data based on an anti-chromatin ELISA, which allows straightforward identification of Abs reacting with chromatin (DNA wrapped around the core histone octamer). In previous studies, different substrate antigens were used for ELISA assays and this could be one of the major causes of variability for contrasting results.

In previous reports regarding Abs to chromatin, individual histones or histone peptide fragments have been studied in JRApatients using different techniques such as indirect immunofluorescence, western blotting assays or ELISA and the results showed notable discrepancies between them, maybe due to different methods (8-11). However, associations with uveitis were not observed in some studies (13,14), JRA children with uveitis (active or inactive) tended to have higher titers of anti-chromatin Abs compared to those with no history of ocular involvement (8,9).

It has been demonstrated in a recent study analysing prognostic factors during the course of pauciarticular JRA that ANA positivity did not predispose these patients to uveitis occurrence, and ANAtiter varied considerably during follow-up (36).

In conclusion our results show that anti-chromatin Abs could contribute to identify the high-risk patients for uveitis. Other analyses will be needed to ascertain the importance of these observations within a clinical setting.

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