Identification of antihistone antibodies in subsets of juvenile chronic arthritis

MONIKA ØSTENSEN,¹ KNUT FREDRIKSEN,² ERIK KÅSS,¹ AND OLE-PETTER REKVIG²

From the ¹Oslo Sanitetsforening Rheumatism Hospital, Oslo; and the ²Department of Immunology, University of Tromsø, Norway

SUMMARY Antihistone antibodies (AHAs) as measured by an enzyme linked immunosorbent assay (ELISA) were detected in the sera of 58 (48%) of 121 unselected patients with juvenile chronic arthritis (JCA). AHAs were found in 28 (93%) of 30 patients with JCA with uveitis but in only 30 (33%) of 91 patients with JCA without uveitis. AHA positivity was unrelated to the type of joint involvement, disease activity, and drug regimen. When the AHA positive group was divided into 28 patients with JCA with uveitis and 30 patients with JCA without uveitis a distinct response pattern of AHA was detected in each group. Anti-H3 dominated in the JCA/uveitis group, whereas a more heterogeneous AHA pattern was shown in the group without uveitis. The results indicate that subtyping for AHA reactivity may define patients who are highly susceptible for the development of anterior uveitis.

Key words: anterior uveitis.

Juvenile chronic arthritis (JCA) contains a subset of patients with circulating antinuclear antibodies (ANAs).¹ The group consists mainly of girls with pauciarticular disease, disease onset before the age of two, and is associated with a greatly increased risk of developing anterior uveitis.² In fact, about 80% of children with JCA and uveitis are ANA positive.³ Thus ANAs can be employed as a marker for predicting the risk for anterior uveitis in patients with JCA. Despite the close relation between ANAs and uveitis associated with JCA the antigenic specificity of this ANA is not known so far.

In the present study of 121 patients with JCA we studied the presence of antihistone antibodies (AHAs), with special emphasis on their pattern in patients with anterior uveitis.

Patients and methods

One hundred and twenty one patients (90 girls, 31 boys), consecutively admitted to the inpatient or outpatient clinic of the Oslo Sanitetsforening Rheumatism Hospital, were studied. All patients

had an established diagnosis of JCA according to American Rheumatism Association criteria,⁴ with disease onset before the age of 16 and a mean disease duration of 6.5 years. The mean age was 11.0 years (range 1–21) in boys and 12.8 years (2–23) in girls. Eleven patients had monarticular JCA, 53 had pauciarticular JCA, and 57 had polyarticular JCA at the time of investigation. Table 1 gives further clinical details.

All patient records were reviewed by one of us (MØ) and evaluated for the type of disease onset, extra-articular manifestations, results of tests for autoantibodies, and actual drug treatment. Evaluation of disease activity was based on a global assessment, including the active joint count, duration of morning stiffness, functional impairment, and laboratory parameters such as erythrocyte sedimentation rate (Westergren), haemoglobin, and platelet counts. Disease activity was graded as none (0), moderate (1), or severe (2). No separate grading of the activity of eye involvement was made in patients with anterior uveitis.

A serum sample was taken from each patient and analysed for the presence of autoantibodies immediately. An additional serum sample was kept frozen at –20°C until used.

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Correspondence to Dr Monika Østensen, Oslo Sanitetsforening Rheumatism Hospital, Akersbakken 27, Oslo 1, Norway.

Table 1 Clinical data on 121 patients with juvenile chronic arthritis at entry to the study

Sex	Number	RF* positive	ANA* positive	History of uveitis	Type of onset†				Course†		
					1	2	3	4	1	2	3
F	90	4	45	21	19	33	32	6	5	40	45
M	31	2	16	9	7	15	5	4	6	13	12
Total	121	6	61	30	26	48	37	10	11	53	57

*RF=rheumatoid factor; ANA=antinuclear antibody.
†1=monarticular; 2=pauciarticular; 3=polyarticular; 4=systemic, febrile.

ANTINUCLEAR ANTIBODIES

The presence of ANAs in serum was tested by indirect immunofluorescence using HEP₂ cells as substrate and fluorescein isothiocyanate conjugated rabbit antihuman Fc antibodies.

ANTI-HISTONE ANTIBODIES AND ANTI-dsDNA ANTIBODIES

Serum samples with a titre >1/16 in the fluorescence ANA test were examined for antibody activities against individual histone classes (H1, H2A, H2B, H3, and H4) and against double stranded DNA by an indirect solid phase enzyme linked immunosorbent assay (ELISA) (Neosystem, Laboratoires SA, Strasbourg, France) as described by Rekvig *et al.*⁵

Results

CLINICAL PARAMETERS

Of the 121 patients, 28 (23%) received no drug treatment, 36 (30%) were treated with non-steroidal anti-inflammatory drugs alone, and 57 (47%) were

treated with disease modifying drugs including cytostatic drugs together with non-steroidal anti-inflammatory drugs or corticosteroids. None of the patients received D-penicillamine or sulphasalazine, which have been shown to induce antihistone antibodies.⁶ Twenty six (21%) of the patients had inactive disease, 62 (51%) had moderate, and 33 (27%) severe disease activity. Severe disease activity was almost exclusively found in the polyarticular group.

Eighty eight (73%) patients had no organ involvement, one had had pleuropericarditis, three had amyloidosis, one had mononeuritis, and 30 (25%) had previous or ongoing anterior uveitis with severe impairment of vision in six cases.

AUTOANTIBODIES

ANAs had been or were still (10 patients) present in 61 (50%) of all patients, whereas only 10 (8%) were positive for rheumatoid factor. None of the ANA positive patients had anti-native DNA antibodies.

Fig. 1 Serum concentrations of antihistone antibodies in 28 patients with juvenile chronic arthritis (JCA) with uveitis (group A) and 30 patients with JCA without uveitis (group B). Serum antibodies to five histone classes—H1, H2A, H2B, H3, and H4—were measured by an enzyme linked immunosorbent assay (ELISA). ●=group A; ○=group B.

ANTIHISTONE ANTIBODIES

AHAs were detected in altogether 58 patients, 45 of whom had also been positive for ANAs at some point of their disease. The AHA positive group was divided into 28 patients with uveitis (group A) and 30 patients without uveitis (group B). The two groups were comparable with regard to disease activity and drug regimens. An equal distribution of ANA titres recorded during the disease was found, with a median ANA titre of 64 (range 16–1024) in 27 patients in group A and 18 in group B.

Figure 1 and Table 2 show the pattern of AHAs in the two groups of patients with JCA. When groups A and B were compared it was found that 9 v 22 patients had AHAs which showed reactivity to only one histone, 16 v 6 had AHAs directed against two histones, and 3 v 2 had AHAs directed against three or more histone classes. The highest reactivity was found to be directed against H3 in group A (Fig. 1). In group B the reactivity to anti-H1 and anti-H3 was equally frequent and of the same magnitude (Fig. 1, Table 2).

When the 30 patients with JCA and uveitis were compared with the 91 patients with JCA without uveitis (Table 3) the uveitis group differed from the non-uveitis group by an excess of ANA and AHA positivity, a predominantly pauciarticular type of

onset, a lower age at disease onset, and longer disease duration. The differences in ANA and AHA positivity were significant ($p < 0.0001$) by applying the χ^2 test with Yates's correction.

Discussion

We used an ELISA to detect AHAs in 58 (48%) of an unselected group of 121 patients with JCA. Forty five of the AHA positive patients had also been positive for ANA at some stage of their disease. More than 90% of the AHA/ANA positivity was detected in the JCA subgroup with uveitis, contrasting with only about 35% positivity in the patients with JCA without uveitis.

Few reports on the prevalence of AHAs in JCA exist.⁷ Preliminary observations with immunoblotting have suggested the presence of anti-H1 in 18 (56%) of 32 sera of pauciarticular JCA.⁸ Weill and Menkes found a higher percentage of AHA and ANA positivity in their study of 47 patients with JCA with mono/pauciarticular disease.⁹ They did not comment on the presence or absence of uveitis, however, nor did they report the antihistone response pattern. In another study 6 (15%) of 41 patients with JCA displayed antihistone reactivity, mainly against H2B.¹⁰ In our study the prevailing antibody in the entire AHA positive group was anti-H3.

A high proportion of ANA positive patients with JCA, yet not all, develop anterior uveitis.⁷ We therefore analysed an AHA positive subgroup of 28 patients with JCA with a history of anterior uveitis (group A) and compared them with 30 AHA positive patients with JCA without uveitis (group B). Despite minor differences in the presenting clinical picture, including actual disease activity, type of joint affection, and drug treatment, a distinct autoimmune response to histones was found in the two groups. Analysis of the pattern of AHAs showed a predominance of anti-H3, which alone or

Table 2 The antihistone response in 28 patients with juvenile chronic arthritis (JCA) with uveitis (group A) and 30 patients with JCA without uveitis (group B)

Antihistone antibody	No (%) positive	
	Group A	Group B
Anti-H1	7 (25)	14 (47)
Anti-H2A	2 (7)	4 (13)
Anti-H2B	3 (11)	6 (20)
Anti-H3	26 (93)	14 (47)
Anti-H4	10 (36)	4 (13)

Table 3 Clinical data on 30 patients with juvenile chronic arthritis (JCA) and uveitis and 91 patients with JCA without uveitis

Group	Female/male	Positivity (%) of:			Mean disease duration (range)	Mean age at onset (range)	Type of onset†				Course†		
		RF*	ANA*	AHA*			1	2	3	4	1	2	3
With uveitis	21/9	0	97	98	8.7 (2–14)	3 (1–9)	5	22	2	1	0	17	13
Without uveitis	69/22	8	35	33	6.7 (2–14)	7 (1–15)	18	27	36	10	10	34	47

*RF=rheumatoid factor; ANA=antinuclear antibody; AHA=antihistone antibody.

†1=monarticular; 2=pauciarticular; 3=polyarticular; 4=systemic, febrile.

combined with other AHAs was detected in 26 (93%) of the 28 patients with JCA with uveitis. By contrast, the 30 patients with JCA positive for AHAs but without uveitis showed a more heterogeneous pattern of AHA. Most of group A showed reactivity to more than one histone class, whereas patients in group B reacted mostly to one histone only. In the present study the presence and titre of AHAs were unrelated to the activity of joint inflammation, ANA positivity or titre, and drug treatment. Extended studies are warranted to investigate the temporal relation between the AHA response and the onset of uveitis and to discover whether the AHA pattern shown by us is specific for uveitis without JCA.

Disease related patterns of antihistone response have been shown for rheumatoid arthritis, idiopathic systemic lupus erythematosus, and drug induced systemic lupus erythematosus⁶ but have not been demonstrated for JCA. Despite the surprisingly high proportion of anti-H3 reactivity in the uveitis group a causative role of AHAs, and especially those against H3 in uveitis, is unlikely for the following reasons: 13% of the patients with JCA/uveitis did not display anti-H3 reactivity and 35% of all anti-H3 positive patients with JCA did not have uveitis.

To conclude, the present data show a high prevalence of circulating AHAs in JCA, mainly directed against H3 and H1. Our results suggest a distinction between the ANA specificities in ANA positive JCA associated with uveitis versus JCA without uveitis. As the mere presence of ANAs is not predictive for the development of anterior

uveitis in JCA, subtyping the patients for AHA reactivity may define an ANA positive subgroup which is highly susceptible for uveitis.

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