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ANTIBODIES DIRECTED TO INDIVIDUAL HISTONES IN JUVENILE CHRONIC ARTHRITIS. ASSOCIATION WITH UVEITIS

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The trials for identification of the specific target antigen for antinuclear antibodies (ANA) in juvenile chronic arthritis (JCA) revealed that a significant number of patients produce antibodies directed to individual histones and histone peptides. Fifty JCA patients, 58 healthy children and 58 children with autoimmune and rheumatologic disorders were studied for a presence of IgG- and IgM-antibodies against histone 1, histone 2 and histone 3 measured by ELISA. The levels of IgG- and IgM-antibodies directed to histone 1, 2 and 3 were elevated in JCA as compared to the healthy controls. IgG-antibodies to histone 2 and IgM-antibodies to histone 3 also were elevated in comparison with the disease controls. IgG- and IgM-antibodies against histone 1 were found to be positive in 30 % and 26 % of JCA patients, respectively, in significant association with ANA (p = 0,038 and p = 0,03, respectively) and uveitis (p = 0,02 and p = 0,016, respectively). The same prevalence of IgG- and IgM-antibodies to histone 2 was established but only the IgG-isotype showed significant association with uveitis (p = 0,018). Anti-histone 3 IgG- and IgM-antibodies were found in 34 % and 27 % of JCA patients, respectively. IgM-antibodies to histone 3 were proved to be significantly associated with uveitis (p = 0,009). It was concluded that antibodies to histone 1, histone 2, and histone 3 represented a common serological feature of JCA. Their presence was related to the manifestation of chronic anterior uveitis, associated with JCA.

Key-words: Histones, antinuclear antibodies, chronic juvenile arthritis, uveitis, ELISA

Antinuclear antibodies (ANA) are universal finding in juvenile chronic arthritis (JCA). They are most typical of the pauciarticular form and those patients whose disease is associated with chronic anterior uveitis. During the last decade many investigators put their attention on the specific target antigen

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of ANA in JCA (3,4,7). Using various immunologic methods a number of studies have been conducted in this field but the nature of the antigen is still unclear.

The objective of this study is to investigate the prevalence of antibodies to individual histones - histone1 (H1), histone 2 (H2), and histone 3 (H3) and their clinical associations.

MATERIAL AND METHODS

Fifty patients with pauciarticular (30) and polyarticular (20) form of JCA were enrolled in this study. Thirty of them had active disease and 20 - inactive disease at the time of the investigations. Seventeen JCA patients (1/3 of the group) were ANA-positive and 10 had chronic anterior uveitis. The age of the patients ranged from 1,5 to 16 years. Fifty-eight healthy children and 58 disease controls were simultaneously studied. The disease control group included 38 children with rheumatologic and autoimmune diseases such as reactive arthritis, systemic lupus erythematosus, vasculitidies, autoimmune uveitis, chronic glomerulonephritis as well as 20 patients with inflammatory non-autoimmune diseases.

Clinical evaluation of the results was made in regard to the following criteria: JCA form, disease activity, duration, patient's age at onset and at the time of investigation, ANA-positivity and association with uveitis. ELISA-method for detection of IgG and IgM antibodies to histone 1, histone 2, and histone 3 was performed as described Cohen et al (2). Highly purified calf histones (H1, H2a+H2b, and H3) from Sigma Chemical Co. were used as antigen. $X \pm 2$ SD of the normal values were chosen for the optimal cut off optical density levels. Statistical methods included variation analysis (by mean values), χ^2, Fisher's exact test, correlation analysis (Pearson's or Spearman's coefficient "*r*").

RESULTS AND DISCUSSSION

IgG antibodies against H1 were found in 30 % of JCA patients in significantly higher prevalence as compared to the healthy children (9 %), p = 0,011, but with no significant difference with the disease controls (15 %) (Fig. 1). These antibodies correlated with ANA (r = 0,35, p = 0.02) and uveitis (r = 0,31, p = 0,038). The prevalence of IgG antibodies to H1 did not depend on the disease form, activity, duration, and patient's age at onset.

IgM antibodies against H1 were found in 26 % of JCA patients. This prevalence was significantly higher than that in the healthy children (p = 0,0004) and disease controls (p = 0,009) (Fig. 2). IgM antibodies against H1 significantly correlated with ANA (r = 0,36, p = 0,016) and uveitis (r = 0,36, p = 0,023). No other significant clinical associations were proved.

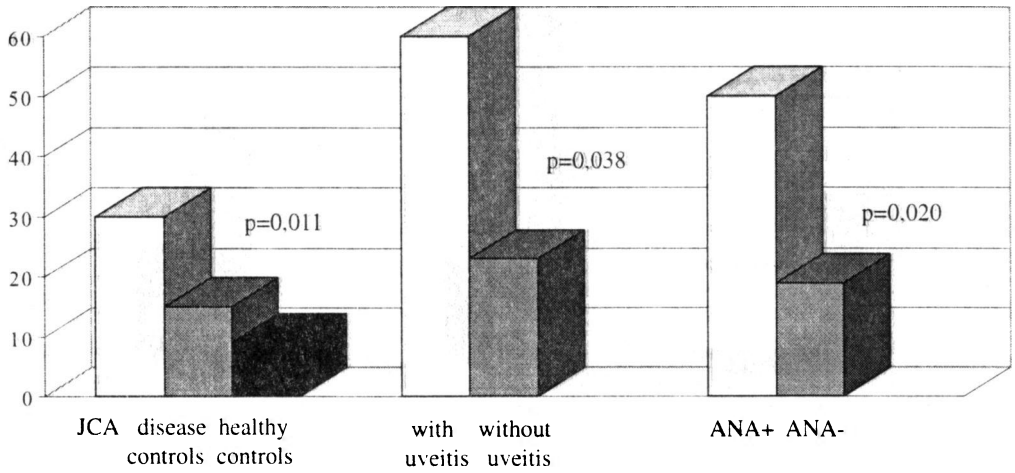

Fig. 1. *Prevalence and clinical associations of IgG antibodies against histone 1*

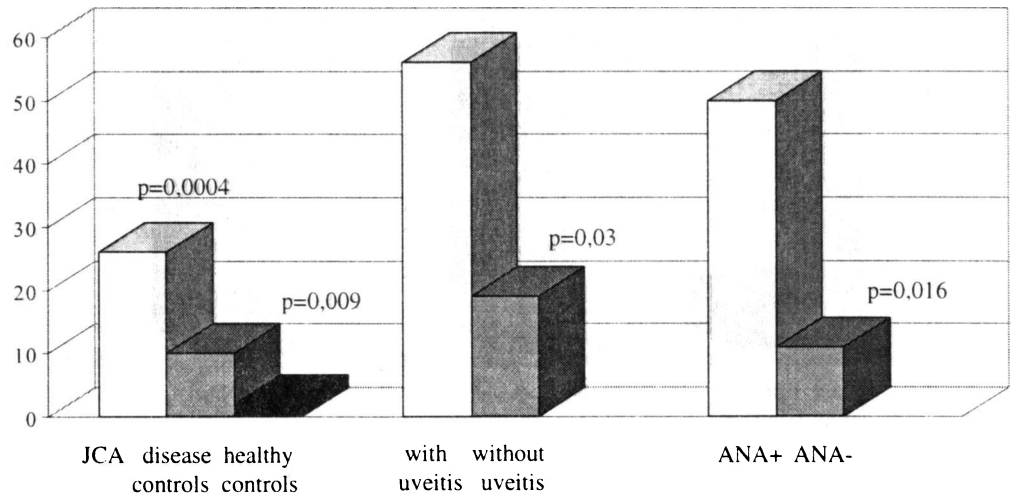

Fig. 2. *Prevalence and clinical associations of IgM antibodies against histone 1 in JCA*

IgG antibodies against H2 were established in 26 % of JCA patients. There were no control patients with positive IgG anti-H2 antibodies (Fig. 3). The only clinical association established in JCA patients was that one with uveitis.

IgM antibodies to H2 were detected in 30 % of JCA patients. This was a significantly higher prevalence than that in the healthy children ($p = 0,023$) and disease controls ($p = 0,012$) (Fig. 4). These antibodies were more typical of the polyarticular form ($p =$

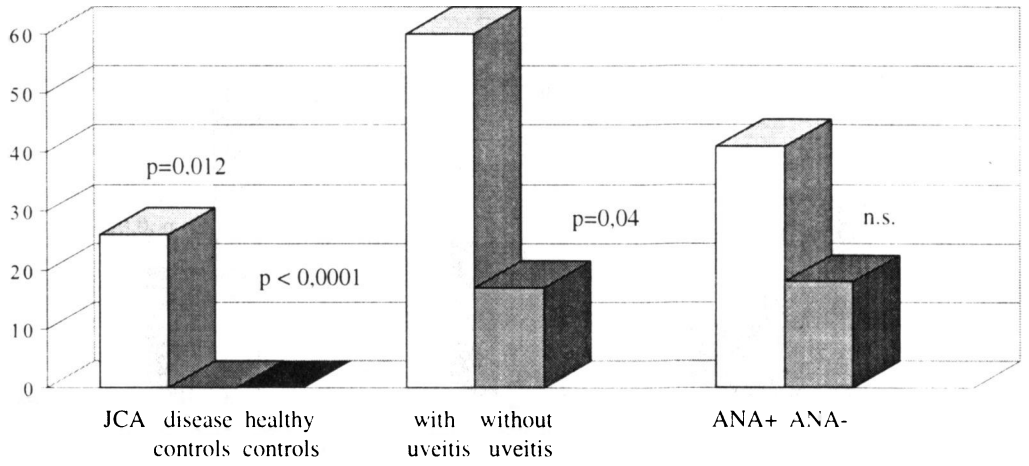

Fig. 3. *Prevalence and clinical associations of IgG antibodies against histone 2 in JCA*

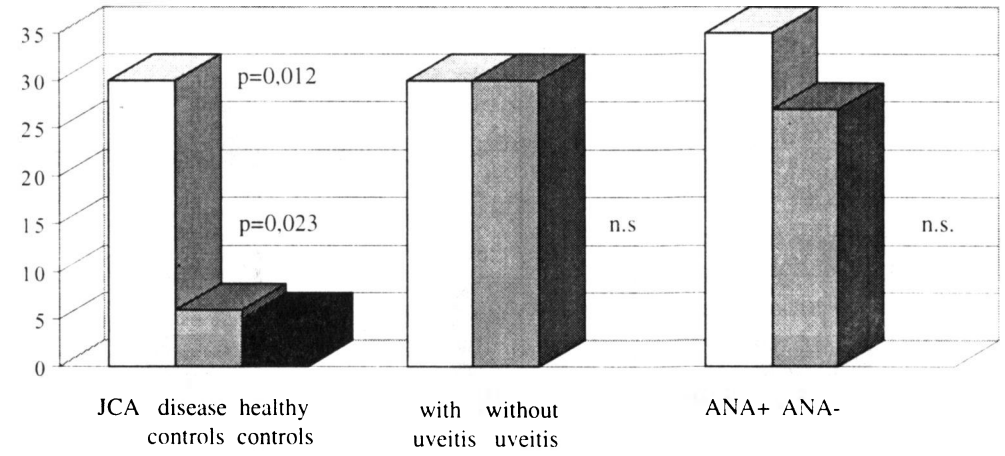

Fig. 4. *Prevalence and clinical associations of IgM antibodies against histone 2 in JCA*

0,03), active disease (p = 0,042) and the age interval between 6 and 12 years (p = 0,032). IgM antibodies against H2 did not correlate with ANA and uveitis.

IgG antibodies directed to H3 were established in 34 % of JCA patients in a significant difference as compared to the healthy group (p = 0,019) and disease controls (p = 0,024) (Fig. 5). They did not significantly correlate with any clinical parameters of the disease.

IgM antibodies to H3 were found out in 27 % of JCA patients in a significantly higher prevalence as compared to the healthy controls but without any reliable difference with the disease control group (Fig. 6). They cor-

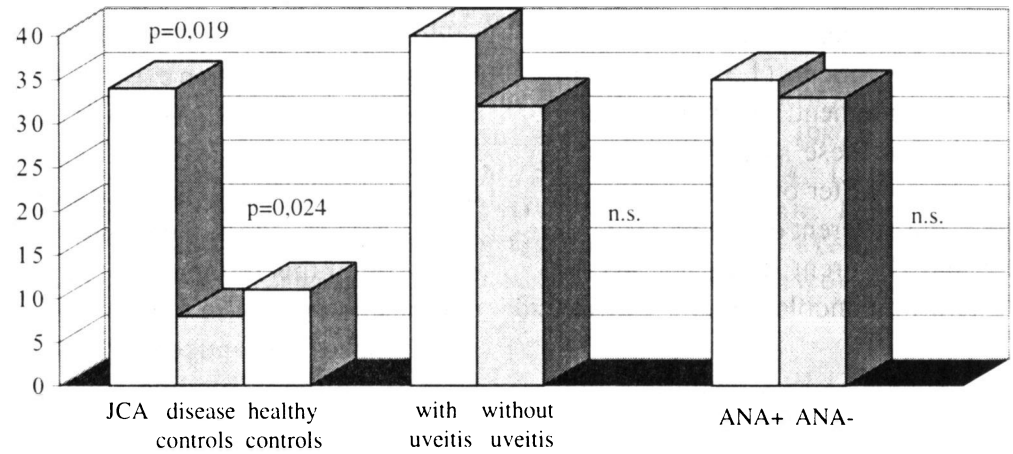

Fig. 5. *Prevalence and clinical associations of IgG antibodies against histone 3 in JCA*

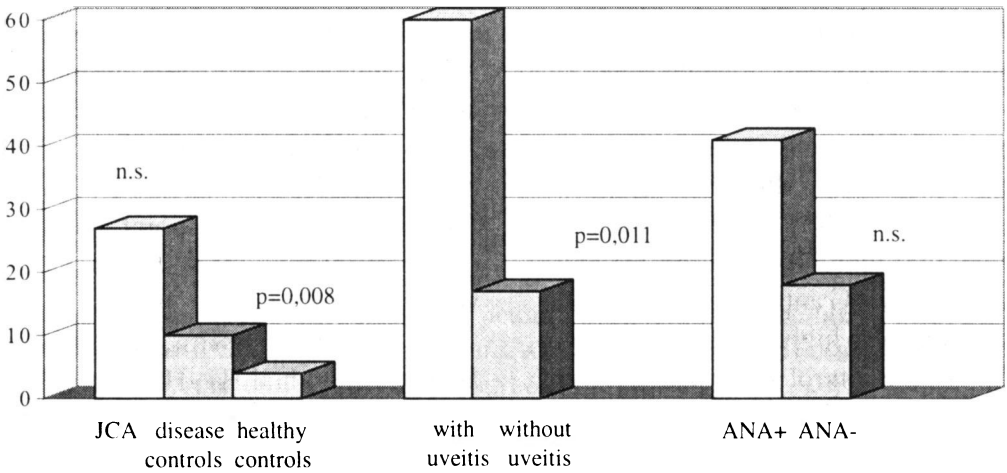

Fig. 6. *Prevalence and clinical associations of IgM antibodies against histone 3 in JCA*

related with the development of uveitis ($r = 0,38$, $p = 0,009$). No other clinical associations were established.

Antinuclear antibodies are common findings in JCA and most typical of the patients with pauciarticular form and uveitis. At presence, little is known about the specificity of these antibodies in JCA. Early studies of ANA found in JCA failed to show the specific autoantibodies which were associated with other connective tissue diseases, e. g., anti-Sm, -SS-A, -SS-B, -RNP, -dsDNA, -ssDNA, etc. (4,7). The possibility that ANA associated with JCA were directed against histones was suggested by Rosenberg et al (cited by 7) but the evidence for this was equivo-

cal. More recently, antibodies against histones were demonstrated in almost 50 % of JCA patients (1,5,6). Although in broad agreement, there are disparities between these studies due to the generally low titer of antihistone antibodies, the different detecting reagents used and different detecting assays (Western immunobloting and ELISA) (1,7).

Using ELISA-technique and highly purified calf thymus histones we tried to determine the prevalence and clinical significance of IgG and IgM-class antibodies against H1, H2, and H3 in JCA. Prevalence of the tested antibodies varied between 26 and 34 %. Although the antibody levels in JCA were generally low (results are not shown), significant difference as compared to the healthy children was observed. IgM antibodies against H1 and H2 as well as IgG antibodies against H2 and H3 had also higher prevalence than in the disease control group. Despite our findings that antihistone antibodies were not associated with clinical criteria of the disease such as its form, activity, duration, age at onset and patient's age, an important relationship between the presence of some antibody isotypes to individual histones and the clue criteria (ANA-positivity and uveitis) was established. Although histone proteins are chromatin components and part of the nucleus, a significant association between the presence of antihistone antibodies and ANA are seldom reported (5-7). According to our data, such an association exists, but only concerning IgG and IgM anti-H1 antibodies. The antibodies to H2 and H3 are found in JCA patients regardless of their ANA-positivity. One possible explanation of this finding is the superficial position of H1 in the nucleosome which makes this antigen more accessible to antibody binding (including ANA-binding).

We established a weak correlation between the presence of some antihistone antibodies (IgG and IgM anti-H1 and IgM anti-H3) and the development of uveitis. These correlations are of less strength as compared to the ANA-correlation with uveitis ($r = 0,47$, $p < 0,001$, data not shown), but confirm the controversial point about the role of antihistone antibodies for the development of uveitis in JCA.

In conclusion, antibodies against individual H1, H2, and H3 are often found in JCA patients irrespectively of the disease parameters such as form, activity, duration, age at onset of the disease and are not age-dependent. The clinical significance of some of them (against H1 and H3) consists in their association with uveitis. Antibodies to H1 are associated with ANA which could lead to the strong suspicion that H1 is a possible candidate for a specific nuclear antigen of ANA in JCA.

REFERENCES

1. Burlingame, R., R. Rubin, A. Rosenberg. *Arthrit. Rheumat.*, **36**, 1993, 836-841.- 2. Cohen, M., J. Webb. *Arthrit. Rheumat.*, **32**, 1989, 1319-1324.- 3. Leak, A., P. Woo. *Ann. Rheumat. Dis.*, **50**, 1991, 653-657.- 4. Leak, A. *Ann. Rheumat. Dis*, **47**, 1988, 178-182.- 5. Monestier, M., J. Losman, T. Fasy et al. *Arthrit. Rheumat.*, **33**, 1990, 1836-1841.- 6. Ostensen, M., K. Frederiksen, E. Kaas et al. *Ann. Rheumat. Dis.*, **48**, 1989, 114-117.- 7. Southwood, T. R., P. Malleson. *Ann. Rheumat. Dis.*, **50**, 1991, 595-598.

Антитела срещу индивидуални хистони при ювенилен хроничен артрит. Асоциация с увеит

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Резюме: Опитите за идентификация на специфичния нуклеарен антиген на антинуклеарните антитела (ANA) при ювенилен хроничен артрит (ЮХА) доведоха до откритието, че част от болните с това заболяване позитивират антитела срещу индивидуални хистони и хистонови пептиди. Ние изследвахме 50 болни с ЮХА, 47 здрави деца и 46 деца с автоимунни и ревматологични заболявания за наличие на антитела от клас IgG и IgM срещу хистон 1, хистон 2 и хистон 3 с помощта на ELISA. Антитела срещу хистон 1 от клас IgG и IgM бяха установени при съответно 30 % и 26 % от болните с ЮХА, в асоциация с ANA ($p = 0,038$ и $p = 0,03$) и увеит ($p = 0,02$ и $p = 0,016$). IgG- и IgM-антителата срещу хистон 2 бяха открити съответно при 26 % и 30 % от децата с ЮХА, като наличието на IgG-антителата корелираше с прояви на увеит ($r = 0,38$, $p = 0,048$). Антитела срещу хистон 3 от клас IgG и IgM бяха установени при съответно 34 % и 27 % от изследваните с ЮХА. IgM-антителаат срещу хистон 3 корелираха с увеит ($r = 0,038$, $p = 0,009$). Антителата срещу хистон 1, хистон 2 и хистон 3 са характерно отклонение в хуморалния имунен отговор на болните с ЮХА. Наличието на тези антитела е свързано с прояви на хроничен преден увеит, често придружаващ ЮХА.