

Antibodies against Nuclear Components in Schistosomiasis

Results Compared to Values in Patients with Rheumatoid Arthritis, Systemic Lupus Erythematosus, and Osteoarthritis

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Occurrence of autoantibodies against nuclear material was compared in groups of patients with rheumatoid arthritis (RA $n = 22$), systemic lupus erythematosus (SLE $n = 24$), osteoarthritis (OA $n = 25$), and chronic schistosomiasis mansoni (CSM $n = 28$). Anti-ds DNA antibody was detected by an ammonium sulphate precipitation radioimmunoassay, antibodies against extractable nuclear antigen (ENA) were detected and differentiated in RNase-resistant and RNase-sensitive components (Sm and RNP antigens) with an ELISA technique. IgG organ-non-specific and granulocyte-specific antinuclear antibodies (ANA) were detected by immunofluorescence technique with quantitative titration of positive reactions and determination of complement-fixing properties. The results in groups of patients with SLE, RA and OA were of confirmative nature and supported that the different methods detect different systems of autoantibodies and nuclear autoantigens. In CSM it was demonstrated that 23 of 28 cases had positive reactions to the RNase-resistant part of ENA (the Sm-antigen), a significant difference from the three other groups of patients ($P < 0.001$). The antibody was in all cases of IgM class, in seven cases also of IgA class. Antibodies against nuclear material in CSM are probably a consequence of heavy disturbance of the immune system in this chronic infection with great permanent antigen load. It is a matter of discussion, whether production of these antibodies is induced by nuclear material from the host or from the parasite.

Key words: ANA; anti-DNA-antibody; antinuclear antibody; anti-RNP-antibody; anti-Sm-antibody; bilharziosis; ENA, extractable nuclear antigen; immunofluorescence technique; schistosomiasis.

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The immune system is intensely involved in schistosomiasis infection, and the immune status during the various stages of disease may vary and is insufficiently defined (28, 29, 33). Serological techniques have so far been developed mainly for diagnostic purposes to detect antibodies against cercarial, adult or miracidial antigens of the parasite, and thereby define stages of infection and associated immunity (4, 10, 12, 14, 19, 23, 29, 32, 33). The

major aim of experimental studies in infected animal vertebrates has been to analyse the mechanism of protective immunity in order to develop effective vaccination techniques. Some studies, however, have focused on the specific and non-specific suppression of immune responses in chronic schistosomiasis infection, apparently associated with cellular as well as serological factors (6, 18, 20, 24, 31, 33), and attention has been drawn towards more general

changes in the immune system, such as fluctuations in concentrations of immunoglobulins of the various classes in early and late phases of the infection (1, 2, 4, 7, 13), the importance of antigen-burden for immunological non-reactivity, and the immuno-modulating role of immune complexes (3, 11, 15, 22, 27, 33, 37), an obligate and dominant feature of schistosomiasis infections.

Since, evidently, several factors predispose to quantitative and qualitative immunological dysfunction in these chronic infections, one would expect autoimmune phenomena to occur as part of the pathology, and indeed, observations of this kind have been made (8, 11, 26, 28, 33, 37). Jones (11) reported occurrence of antibody against single-stranded and double-stranded DNA in rabbits with experimental *S. japonicum* infection. Similar observations were made in the New Zealand white mouse in experimental *S. mansoni* infection (8), but type, frequency and pathological significance of anti-nuclear antibodies in experimental animals or in man is so far unknown. This study reports occurrence and characterization of some anti-nuclear antibodies in patients with chronic *S. mansoni* infection (CSM) compared with findings in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and osteoarthritis (OA).

MATERIAL AND METHODS

Patients

Serum samples from 22 patients with seropositive RA (age 23–50 years, mean 36 years, 18 females, 4 males), 24 patients with SLE (age 14–60 years, mean 26 years, 23 females, 1 male), 25 patients with OA (age 17–68 years, mean 44 years, all female) and 28 patients with CSM (age 18–40 years, mean 26 years, all male) were studied. All sera were collected from patients in wards and out-patient clinics of two major hospitals in Northern Egypt, and all patients were residents of the rural area surrounding Cairo. The patients were examined and diagnosed by specialists in rheumatology, internal medicine and tropical medicine. The

American Rheumatism Association criteria were used for the diagnosis of RA (25) and SLE (5). The diagnosis of OA was made on the basis of case history, clinical examinations and X-ray findings. Schistosoma infection was diagnosed on the basis of clinical criteria and by microscopic demonstration of the parasite ova.

Anti-DNA-antibody detection by Farr assay

The Farr assay for anti-DNA antibody determination as used in the present study has previously been described in detail (17). The principle of this radioimmunoassay is that a buffer solution containing 200 ng partially ^{14}C -labelled double-stranded DNA (ds-DNA) is added to 10 μl serum sample. After adequate incubation putative immune complexes between DNA and anti-DNA antibody are precipitated with $(\text{NH}_4)_2\text{SO}_4$, and the amount of co-precipitated ^{14}C -labelled DNA is measured on the sediment in a liquid scintillation counter. The analysis was carried out in duplicate, and the daily routine included normal and pathological standards as well as double retesting of all pathological sera. The day-to-day variation was less than 10%. In blood donors the 95% range of this assay was <10% precipitated DNA of added DNA, independent of age and sex. The range in a department of internal medicine for random patients not suffering from SLE was somewhat higher: <15% (16).

Anti-RNP and anti-Sm antigen detection by ELISA technique

The technique was performed as described in detail by Struckmann et al. (30). Extractable nuclear antigen (ENA) was derived from calf thymus and prepared according to the principles described by Holman (9), kindly provided by M. Højier-Madsen, State Serum Institute, Copenhagen. Peroxidase-conjugated rabbit anti-human immunoglobulins against γ , μ and α chains were bought from DAKO, Copenhagen. Ribonuclease-A from bovine pancreas (Sigma A4875) (RNase) was used to differentiate between RNase-sensitive ribonucleoprotein (RNP) and RNase-resistant (Sm) anti-

gen. The solid phase material was polystyrene, microtitre flat bottom, Dynatech® (M 129A) and the colorimetric reaction was registered directly in each microtitre well with a Titertek® multiscan spectrophotometer. RNase treatment of antigen for detection of anti-Sm antibody was performed in a special buffer solution before coating the microtitre plates (30). The normal ranges of serum anti-RNP and anti-Sm and the distribution between IgG, IgA, and IgM classes were determined on the basis of findings in 261 normal blood donors. Day-to-day variations were adjusted by always using the same normal and pathological standard sera. Positive reactions exceeded the upper limit of the 95% range of normal blood donors. Readings of Ig class determinations were similarly quantified, the cut-off level being adjusted on the basis of day-to-day standard corrections as indicated. All samples were tested in quadruplicate, coefficient of variation 4% and repeated day-to-day variation 8%.

Antinuclear antibody (ANA) detection by immunofluorescence assay (IMF)

The serum samples were examined for the occurrence of organ non-specific antinuclear antibodies (ON-ANA) and granulocyte-specific antinuclear antibodies (GS-ANA) by indirect IMF using fluorescein isothiocyanate labelled Ig fractions of rabbit antisera against human γ and μ chains (DAKO, Copenhagen). The technique is described in detail elsewhere (36). Complement binding properties of the ANA were detected by IMF in a double layer technique with a labelled Ig specific for human C3 (DAKO, Copenhagen). Positive reactions were titrated on a logarithmic scale and all readings were carried out with blind technique, independently by two of the authors (A.W., H.P.) using a LEITZ orthoplan microscope with incident light illumination equipment as earlier described (35).

Statistics

Results in the four groups of patients were compared by Fisher's exact test.

RESULTS

Anti-DNA-antibodies

Anti-DNA-antibodies as detected by the Farr assay occurred as shown in Fig. 1. There is one slightly positive reaction in RA, a high proportion of strongly positive reactions in SLE, and normal reactions in OA and CSM. The difference between SLE and the other three groups is highly significant ($P < 0.001$). There is no significant difference between any of the other groups. These findings are reproducing an expected pattern of reactivity in the four groups.

Anti-ENA antibodies

(anti-RNP and anti-ENA antibody)

Anti-ENA antibodies as detected by ELISA technique occurred as shown in Fig. 2. In RA two of 22 patients had a positive reaction, in both cases to the Sm-antigen and of IgM class. The great majority of RA patients were negative. In SLE 11 of 24 cases had positive ENA reactions, nine to the RNase sensitive RNP antigen, three to the RNase resistant Sm antigen, one reacted with both antigens. The antibodies were predominantly of IgM class, but

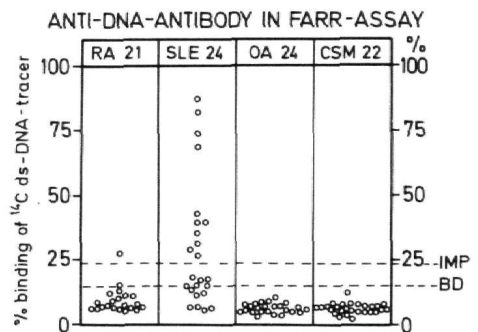


Fig. 1. Anti-ds-DNA antibody in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), osteoarthritis (OA), and chronic schistosomiasis mansoni (CSM). Level indicates per cent binding of ^{14}C -labelled ds-DNA tracer. Upper 95% binding in blood donors (BD) and in patients with random medical disorders except inflammatory connective tissue diseases (IMP) are shown.

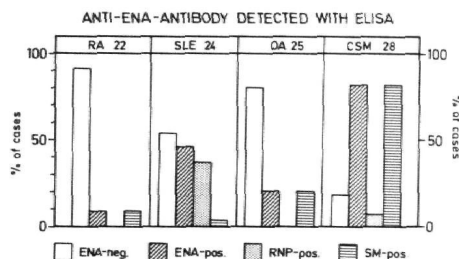


Fig. 2. Anti-ENA antibody in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), osteoarthritis (OA), and chronic schistosomiasis mansoni (CSM). Differentiation into anti-RNP and anti-Sm antibody is indicated.

other classes were represented as well. In OA five cases showed positive anti-ENA reaction, all to the Sm antigen, and either of IgM or IgA class. In schistosomiasis patients anti-ENA antibodies were demonstrated with high frequency: positive reactions were found in 23 of 28 cases, only five being negative. The antibody was in all cases directed against the Sm antigen, in two cases also against the RNP antigen. The antibody was in all cases of IgM class, in seven cases also of IgA class. IgG class antibodies against ENA were not detected.

There is a highly significant difference between the schistosomiasis group and the other three patient groups as to occurrence of anti-Sm antibody. (CSM v SLE: $P < 0.001$, CSM v OA: $P < 0.001$, CSM v RA: $P < 0.001$). There is less difference between occurrence of anti-ENA-antibody in SLE and RA ($P < 0.02$) or OA (P : n.s.). There is a distinct difference between the types of anti-ENA antibodies in CSM and SLE, the former being almost exclusively directed against the RNase-resistant Sm-antigen, the latter almost exclusively against the RNase-sensitive RNP-antigen. IgM is the dominating Ig class in both groups, but even IgA is rather common in the schistosomiasis group. IgG class anti-ENA antibody is seldom demonstrated in any of the groups. In summary, findings in RA, SLE and OA are generally of confirmative nature, but it is unexpected to find this very high frequency of IgM and IgA class antibodies against the RNase-resistant Sm antigen in CSM.

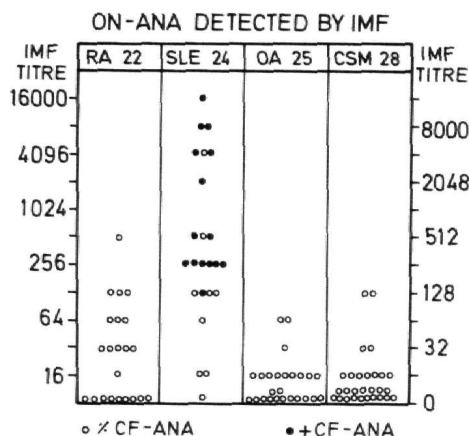


Fig. 3. IgG organ-non-specific antinuclear antibodies (ON-ANA) as detected by immunofluorescence technique (IMF) in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), osteoarthritis (OA), and chronic schistosomiasis mansoni (CSM). Presence of complement fixing (CF) ON-ANA is indicated.

Antinuclear antibodies detected with immunofluorescence technique (IMF-ANA)

IMF-ANA was demonstrated as shown in Fig. 3. In RA 13 of 22 patients had IgG class organ-nonspecific ANA (ON-ANA) in low or medium titres, range between 16 and 512 with a mean of 97, and not complement-fixing. IgG granulocyte-specific ANA (GS-ANA) were demonstrated in four cases at titres of 128, and one of these also had ON-ANA. Complement-fixing GS-ANA were found in only two cases. In the SLE group the typical finding was IgG ON-ANA in 23 of 24 cases, complement-fixing in 14 cases and in rather high titres. ON-ANA was found with a titre range between 16 and 16,000, mean of positive reactions 2175. Complement-fixing ON-ANA was found with a titre range of 32–4096, mean of positive reactions 460. None of the SLE patients had GS-ANA. As expected OA showed low, border-line values of ON-ANA, titres varying between 16 and 64 with a mean value among positive sera of 25. GS-ANA were not found, and the low titred ON-ANA were not complement-fixing.

In CSM IgG ON-ANA were found at low titres in 11 of 32 patients, range 16–128, mean

among positives 39. ANA were not complement-fixing, and GS-ANA were not demonstrated. In summary, SLE patients showed an expected high prevalence of high titred ON-ANA, in many cases complement-fixing. No difference was seen between patients with CSM and OA, both groups in some cases having border-line values of ON-ANA. The prevalence of GS-ANA in RA was somewhat lower than one might expect from comparison with RA patients from Denmark (21). The high prevalence of IgM and ENA antibodies in the CSM group prompted us to look at IgM class ANA as well in these patients. IgM ON-ANA were found in 16 of the 28 sera, showing concordance with the ELISA test in all cases.

DISCUSSION

The four patient groups investigated in the present study were not entirely comparable as to sex and age. In Egypt CSM is prevalently a disease of the male, and an expected characteristic higher prevalence in females is apparent in the RA, SLE and OA groups. The OA group naturally differs from the others as to age. The findings in these studies must therefore be interpreted with a formal reservation for possible differences in sex- and age-associated occurrence of antibodies to nuclear components in these patients (34). Since an association of this type as sole explanation of the differences between the groups is improbable, the reservation is disregarded in the following discussion.

Profound disturbance of the immune function is a characteristic feature of schistosomiasis and is probably a consequence of life-long parasitic infection with a heavy antigen burden, which has an impact on pathogenesis and pathology through the various stages of the disease. Occurrence of antibodies of organ-specific type, antiimmunoglobulin type and/or antibodies to nuclear material have been reported sporadically in animal experimental models and man (8, 11, 26, 28, 37) but the frequency and specificities of such autoantibodies in human infection are unknown.

This study shows that antibodies against ENA, in particular against the RNase-resistant

part, the Sm antigen, is a very common feature of CSM. For comparison, other patient groups with chronic inflammatory connective tissue diseases were examined, and a significant difference in patterns was observed. Anti-DNA antibody, selectively directed against double-stranded DNA, as found in SLE, was not a feature of CSM. Also antibody against the RNase-sensitive part of ENA was infrequent in CSM. The anti-ENA antibodies in CSM were typically of IgM and not of IgG class. However, seven cases had anti-ENA antibodies of IgA class, which is seldom found in the other disorders compared. The finding suggests that there may be an association in some cases between the chronic parasitic infestation of mucous membranes and a response of anti-ENA antibody formation of IgA class.

Certain comparisons of findings in the individual patients in the three different test systems are of interest. SLE showed a high degree of concordance between positive or negative reactions in the anti-DNA antibody test and the ANA-IMF. But quantitatively, the titres in ANA-IMF were uncorrelated to the levels of anti-DNA antibody detected in the Farr assay, confirming that the two test systems detect different sets of antigen-antibody systems. In CSM the 11 ANA IMF-positive cases were all found among the 23 cases with positive anti-ENA antibody (RNase resistant) reaction, but additional 12 sera with ELISA detectable anti-ENA antibodies were negative with the ANA IMF, leaving five cases negative in both test systems. This is probably explained by the differences in Ig classes looked for. In the OA group, which in no case showed detectable anti-DNA antibody with the Farr assay, slightly positive IgG ANA IMF reactions were found in 12 cases. These were all different from the five cases showing positive ENA ELISA reaction due to IgM or IgA antibodies. In RA the ANA IMF test was positive in 16 cases, which did not include the two cases with positive IgM anti-ENA antibody reaction in the ELISA technique.

Several reasons for development of autoantibodies in CSM can be imagined. The occurrence of antibodies against nuclear material may signify either a true autoimmune phenomenon

indicating that ribonucleoprotein and associated molecules belonging to the host have participated as antigens in stimulation of an auto-immune response in a qualitatively dysregulated immune system. Specific and non-specific immune suppression exerted by serum factors or by mononuclear blood cells is an established feature of human and experimental schistosoma infections (6, 18, 20, 24, 31, 33), and a coexisting failure in the regulation of autoreactive clones is a probable additional pathogenetic possibility in the antigen-overloaded immune system.

Another explanation of the occurrence of antibodies against nuclear components in CSM could be that antibody production against nuclear antigen is induced by antigenic nuclear components from the parasites, either cercaria, adult worms or miracidia. Such antibodies might well be detected with the techniques used and might then represent merely a perfectly adequate B-lymphocyte response to parasitic nuclear antigens without further pathogenetic significance. This explanation seems rather probable, since antibodies against nucleic acids and their associated proteins generally cross-react in a species non-specific manner. There is indeed suggestive information in favour of this possibility (29). So far we may conclude that the antibodies against nuclear components in CSM may be an epiphenomenon, and no causal relation to the pathogenesis has been established. Probably antigen-antibody complexes with this particular specificity would constitute part of the total pool of circulating and potentially damaging immune complexes in CSM, although this aspect still remains to be proven.

The titres of antibodies to ENA were moderate, comparable to values found in SLE. Since the specificity to RNase-resistant and RNase-sensitive antigen differs so clearly in CSM and SLE, there is only limited risk of wrong diagnostic interpretation, if appropriate methods are used. The high prevalence of chronic schistosomiasis in certain countries, however, implies that concurrence of the two disorders must be a comparatively frequent event which one must be prepared to meet. Here the clinical assays for detection of antibodies against

nuclear components should be carefully differentiated and interpreted, since patterns as described in this study are frequent in chronic schistosomiasis without any other inflammatory connective tissue disease.

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