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Eosinophil-specific and other granulocyte-specific antinuclear antibodies in juvenile chronic polyarthritis and adult rheumatoid arthritis

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Rosenberg, J. N., Johnson, G. D., Holborow, E. J., and Bywaters, E. G. L. (1975). *Annals of the Rheumatic Diseases*, **34**, 350–353. **Eosinophil-specific and other granulocyte-specific antinuclear antibodies in juvenile chronic polyarthritis and adult rheumatoid arthritis.** Sera from 151 children of whom 112 had juvenile chronic polyarthritis (JCP), and from adults with rheumatoid arthritis (RA), and from healthy pregnant females were tested for the presence of granulocyte-specific antinuclear antibodies (GS-ANA). These were detected in 20% of sera from cases of JCP, in 68% of adult RA, but in none of the controls. Eosinophil-specific ANA were the only ANA present in 18% of positive children and 54% of the positive adults. GS-ANA in children were predominantly IgG and of low titre. Heat-stable GS-ANA were detected in sera from eight children but none bound complement.

The presence of GS-ANA was not significantly associated with sex, age of onset, duration of disease, mean active joint count, mean ESR, nor with the presence of fever, rash, splenomegaly, amyloidosis, pericarditis, or rheumatoid factor.

Antinuclear antibodies (ANA) are, in general, not organ-specific (Beck, 1969), but there are some which react selectively with particular cells. An example of these is granulocyte-specific (GS) ANA, as originally described by Faber, Elling, Norup, Mansa, and Nissen (1964), which react with nuclear antigens of mature polymorphonuclear granulocytes, eosinophils, and monocytes, but not of lymphocytes (Wiik and Munthe, 1972). They have been reported most frequently in sera and synovial fluids of adults with rheumatoid arthritis (RA) (Elling, Graudal and Faber, 1968), in some cases of systemic lupus erythematosus, and occasionally in other conditions (Faber and Elling, 1966). Munthe (1972), using smears of buffy coat leucocytes as the source of nuclear antigen, noted the occurrence of GS-ANA in sera and synovial fluids from a small group of patients with juvenile chronic polyarthritis (JCP).

The aim of the present study was to determine the incidence of these antibodies in a large well-characterized group of children with JCP, the majority of whom had Still's disease, and whether their presence was correlated with particular clinical or laboratory features, following the observation that organ-nonspecific (ONS) ANA is associated with chronic iridocyclitis in Still's disease (Schaller, Johnson, Holborow, Ansell, and Smiley, 1974).

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Materials and methods

PATIENTS

One hundred and twelve patients with JCP (Ansell and Bywaters, 1959) who had been admitted to the MRC Rheumatism Unit since 1972 were studied. They included 98 cases of definite, seronegative, Still's disease, 7 probable Still's disease, 6 juvenile seropositive, rheumatoid arthritis (JRA), and 1 psoriatic arthritis. 32 were male and 80 female The sera had previously been tested for ONS-ANA activity, using cryostat sections of rat liver as the source of nuclear antigen, in a standard indirect immunofluorescence test (Johnson and Holborow, 1973); sera with ANA detectable in dilutions 1 :10 were excluded to avoid masking of GS-ANA by ONS-ANA.

The control sera were from 39 healthy children referred

to an ENT department with minor complaints, from 13 males and 25 females with adult RA, of whom 19 were seropositive, and from 10 healthy pregnant females. All sera were stored at −20°C.

LEUCOCYTE SUBSTRATE PREPARATION

10 ml of normal human venous blood was defibrinated, using sterile paper clips, and mixed with an equal volume of Plasmagel (Laboratoire Roger Bellon, Neuilly, France). After 15 min at 37°C the granulocyte-rich supernatant was mixed with an equal volume of freshly prepared 0·83% ammonium chloride for 15 min at 21°C in order to lyse the erythrocytes in the suspension. The granulocyte concentrate so obtained was washed three times with phosphate buffered saline (PBS) pH 7·4 at 1200 r.p.m. The cells were resuspended in 10 ml of PBS and a few drops cytocentrifuged in order to check the morphology with Leishman's stain. A satisfactory preparation consisted mainly of recognizable granulocytes. The suspension was cytocentrifuged onto Multispot slides (O'Neill and Johnson, 1970), air dried for 10 min at 21°C, and fixed in absolute ethanol for 5 min at 4°C. Slides were stored in plastic bags at −20°C until required.

IMMUNOFLUORESCENT PROCEDURE

Sera were randomized and tested at a dilution of 1 :10 by standard immunofluorescent technique. The majority of sera had not been previously thawed. Each serum was also screened on a fresh cryostat section of rat liver in order to confirm the absence of ONS-ANA activity. GS-ANA positive and negative sera were included in each run. Fluorescein conjugates reactive with whole immunoglobulin (Ig) and specific for α, γ, and μ chains (Wellcome Reagents Ltd) were used at their optimum working dilutions, as determined by block titrations. Stained preparations were mounted in PBS-buffered glycerol, pH 8, and read without knowledge of their identity within a few hours by one observer (J.N.R.). They were examined with a Reichert microscope equipped with a quartz-halogen lamp; the primary filter was Balzer FITC 3, usually supplemented by BG 38 (Schott and Gen.), and the secondary filter was Kodak-Wratten 12.

Each serum was tested on at least two occasions, and considered positive for GS-ANA only if a positive result was obtained in two consecutive tests. Positive sera were immediately divided and stored at −20°C for further study. Sera from GS-ANA positive cases of JCP were titrated in a doubling dilution series with anti-Ig. They were also examined for complement binding activity, using fresh human serum, stored at −70°C, as the source of complement and fluorescein conjugated rabbit antihuman $\beta_1A/_1C$.

When follow-up samples of sera became available from children with GS-ANA, they were tested in order to determine the persistence of GS-ANA. Fresh sera from GS-ANA negative cases were also examined.

Results

GS-ANA were consistently detected in sera from 22 children with JCP (20%) and from 26 adults with RA (68%), but in none of the control children or pregnant female controls (Table I). Of the remaining cases of JCP, 42 sera were consistently negative for GS-ANA and 48 gave doubtful staining. Sera that gave an equivocal result on first testing invariably gave a doubtful or negative result on retesting. Eighteen of the GS-ANA positive children had definite Still's disease, two had probable Still's disease, and two had JRA. Five were male and 17 female. GS-ANA were detected in 26 cases of adult RA, comprising 9 males and 17 females; 13 of these cases were seropositive.

Table I *Incidence of GS-ANA in JCP and adult RA*

	No. tested	*Consistently positive*	
		No.	*%*
JCP	112	22	20
Adult RA	38	26	68

GS-ANA in children were predominantly of IgG class (95%); 3 sera also gave nuclear staining with anti-IgM and one serum was positive only with anti-Ig. No GS-ANA positive sera reacted only with anti-IgA or anti-IgM. Twenty positive sera were titrated. Eight gave a titre of 1 :10, 7 of 1 :20, 4 of 1:40, and 1 of 1 :80. Twenty sera containing GS-ANA were tested for complement binding activity. However, 12 of these had no demonstrable GS-ANA activity after heating for 30 minutes at 56°C in order to deactivate intrinsic complement activity; none of the remaining 8 heat-stable GS-ANA appeared to bind complement.

The types of leucocytes which gave nuclear staining with GS-ANA positive sera are shown in the Figure; eosinophil-specific nuclear staining was obtained with sera from four of the positive children (18%) and fourteen of the positive adults (54%). No nuclear staining of lymphocytes was observed with any of the sera regarded as GS-ANA positive. Most positive sera, even on dilution, gave a homogeneous nuclear staining pattern, and a few gave peripheral staining. No other nuclear staining variants were seen.

Nineteen further serum samples obtained at follow-up (1–19 months) from 13 children who were initially GS-ANA positive were tested for persistence of

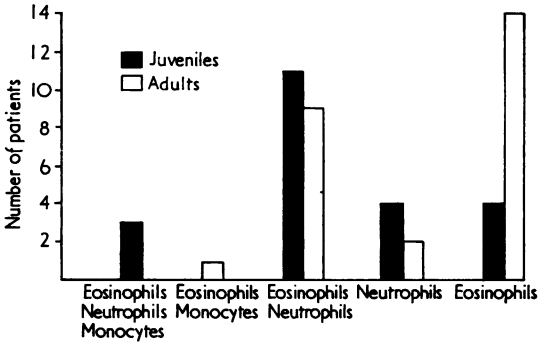

FIGURE *Types of leucocyte showing nuclear staining*

Table II *Analysis of clinical and laboratory findings in 64 cases of JCP*

Mean					*Incidence (%)*						
GS-ANA (no.)	*Sex (M:F)*	*Age of onset (years)*	*Duration of disease (years)*	*Active joint count*	*ESR*	*Fever*	*Rash*	*Splenomegaly*	*Amyloidosis*	*Pericarditis*	*Rheumatoid factor*
+22	5:17	7·6	6·3	21·7	44	40	36	9	14	14	14
−42	12:30	6·9	7·0	24·0	48	52	43	20	17	10	10

GS-ANA; 7 of these were positive (54%). Fresh (unfrozen) sera from 13 GS-ANA negative cases, originally tested on stored sera, were found to be negative.

Analysis of clinical and laboratory features is shown in Table II. There was no significant difference between the persistently positive and negative GS-ANA groups.

Discussion

Leucocytes employed as the source of nuclear antigen in this study were obtained from healthy adults, and we confirmed that background fluorescence due to reactions attributable to blood group isoantibodies was minimal provided that red cells were lysed in the preparation, as shown by Gibson and Quarles (1969). Of the various fixatives that we tested the best contrast between positive and negative results was obtained with cells treated with ethyl alcohol. In order to show GS-ANA activity, Elling (1967a) recommended repeated freezing and thawing of the cell preparations. However, it is evident that the combination of trauma during defibrination and treatment with alcohol provides preparations in which the nuclear antigens involved are in a reactive state, as reported by Wiik and Munthe (1972). Furthermore, the preservation of morphology in such preparations enables cells to be confidently identified under the fluorescence microscope.

Sera were screened at a dilution of 1:10. This differs from some earlier studies in which undiluted sera were employed. We found that the use of undiluted serum resulted in unacceptable levels of non-specific staining. A further possible hazard associated with testing undiluted serum has been pointed out by Ritchie, Bayles, and Harter (1965) who noted false negative reactions in 8% of rheumatoid sera tested undiluted with class-specific conjugates. In our laboratory prozones in immunofluorescent tests due to competition between antibodies of differing immunoglobulin classes have been observed (Johnson and Holborow, 1973).

GS-ANA were detected in the sera of 20% of cases of JCP, and 68% of patients with adult RA. Comparison with results obtained in other series is difficult because of variations in the technical procedure employed, and consequent differences in sensitivity of the immunofluorescence method, as discussed by Nairn (1968). In making such comparisons it is also important to distinguish granulocyte-*reactive* ANA from granulocyte-*specific* ANA and to take into account the incidence of Felty's syndrome and other cases with neutropenia. The incidence of GS-ANA in JCP and adult RA in the present study is similar to that previously found in this laboratory in an earlier study, in which leucocytes were prepared by freezing and thawing (G. D. Scharf and Y. Johnson, unpublished). Munthe (1972) studied undiluted sera from 25 cases of JCP and obtained an incidence of GS-ANA of 44% which was increased to 80% after absorption of ONS-ANA. The incidence of GS-ANA in adult rheumatoid arthritis in the literature ranges from 18–75% (Elling and others, 1968; Faber, Wiik, Friis, and Jensen, 1973). Granulocyte-*reactive* ANA have been detected in 4–60% of sera from cases of JCP (Bluestone, Goldberg, Katz, Marchesano, and Calabro, 1970; Munthe, 1972) and in 65–88% of cases of adult RA (Alexander, Bremner, and Duthie, 1960; Elling, 1967b). Variable persistence of the antibody and noncorrelation with disease activity accords with previous findings for ONS-ANA in this laboratory in adult RA (Ward, Johnson, and Holborow, 1964).

GS-ANA in children were predominantly IgG, as has been previously noted (Munthe, 1972), and in 3 of the sera activity was also found in the IgM fraction. The detection of the latter reactions, however, may have been attributable to the uptake of antiglobulin (rheumatoid) factors at the site of reaction of IgG antibody as discussed by Barnett, Condemi, Leddy, and Vaughan (1964). The GS-ANA activity was lost in sera from 12 cases of JCP after heating to 56°C for 30 minutes and retained in 8 cases. Heat lability of ANA in RA but not SLE sera was reported by Johnson and Holborow (1961), but in that study the sera were heated at 65°C. Complement binding by GS-ANA has been shown to be correlated with high titres of IgG antibody in Felty's syndrome and other cases of RA with neutropenia (Wiik and Munthe, 1974). None of the GS-ANA in the present series had a titre greater than 1:250 or was associated with neutropenia, and all those tested failed to bind complement.

The high incidence of *eosinophil-specific* ANA in the JCP and adult RA patients was striking. Faber and

Elling (1967) reported a single instance of such antibody in a study of patients with hepatic cirrhosis. None of our patients showed any evidence of liver disease, and their eosinophil counts were all within normal limits. The significance of this unexpected finding, indicating a remarkable degree of nuclear antigenic specificity, requires further elucidation.

There is some evidence that ONS-ANA may be involved in the perpetuation of chronic synovial inflammation (Zvaifler and Martinez, 1971). GS-ANA might also have some pathogenic significance in both adult and juvenile joint disease. Such antibodies may be locally produced and indeed predominate over ONS-ANA in neutropenic synovial fluid (Elling and others, 1968), and may form complexes with nuclear antigens released as a result of neutrophil activation and degranulation after exposure to other complexes present in synovial tissue. The possible involvement of GS-ANA in maintaining synovial inflammation through such a mechanism merits further study.

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