Low Incidence of Rheumatoid Factor and Autoantibodies in Nigerian Patients with Rheumatoid Arthritis

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Summary: Sera from 53 Nigerian patients satisfying the American Rheumatism Association criteria for a diagnosis of definite or probable rheumatoid arthritis and sera from sick and healthy Nigerian controls were tested for rheumatoid factor, autoantibodies, and immunoglobulin levels. Rheumatoid factor and autoantibodies were found no more frequently in patients with rheumatoid arthritis than in controls. These findings confirm the clinical impression that Nigerian patients with polyarthritis satisfying the criteria for a diagnosis of rheumatoid arthritis differ from Caucasian patients with the disease in a number of important respects. They suggest that either these patients do not have rheumatoid arthritis but a distinct clinical syndrome or that in Nigeria the course of rheumatoid arthritis is modified by genetic or environmental factors.

Introduction

Nigerian patients with polyarthritis who satisfy the American Rheumatism Association criteria for a diagnosis of definite or probable rheumatoid arthritis (*Committee of the American Rheumatism Association*, 1959) show a number of unusual clinical features (Greenwood, 1969a). Nodules and vascular lesions are rarely seen, *x*-ray films of the hands and feet usually show only mild erosive changes, and the overall prognosis of the disease is good. These findings suggest that either Nigerian patients who appear to have rheumatoid arthritis do not have this disease or that in Nigerians the course of the illness is modified by genetic or environmental factors. In order to study this problem further an investigation has been carried out into the incidence in Nigerian patients with rheumatoid arthritis of some of the immunological changes recorded in Caucasian patients with the disease.

Materials and Methods

Sera from the following groups of Nigerians have been studied: (1) 53 patients with polyarthritis satisfying the American Rheumatism Association criteria for a diagnosis of rheumatoid arthritis (classical 2, definite 22, and probable 29)—the way in which these patients were selected and the clinical features of their illness are described elsewhere (Greenwood, 1969a); (2) 136 patients with other forms of arthritis; (3) 135 patients with general medical diseases; and (4) 671 apparently healthy controls.

The age and sex distributions of the members of each group are given in Table I. Patients in groups 1, 2, and 3 were seen at University College Hospital, Ibadan, which is situated in the forest region of Western Nigeria. The control group is composed predominantly of villagers investigated during the course of a population survey into the prevalence of joint disease in Western Nigeria carried out in conjunction with Dr. A. S. Muller and Dr. H. A. Valkenburg, of the University of Leiden. Many of these apparently healthy controls were suffering from parasitic infections.

All sera were tested for rheumatoid factors reacting with human gammaglobulin and rabbit gammaglobulin, a latex fixation test being used, and the human erythrocyte agglutination test (Valkenburg, 1963). Sera giving a titre of 1:640 or greater in the former test and 1:32 or greater in the latter test have been considered positive. Sera from most of the control subjects were tested for rheumatoid factor at Leiden, the remainder at Ibadan. Cross exchange of sera established that comparable results were obtained in the two laboratories. Selected sera were also tested for rheumatoid factor with the F.2 tanned sheep cell agglutination test (Heller *et al.*, 1954) and the sensitized sheep cell agglutination test (Ball, 1963). Euglobulin fractions, prepared with 1% boric acid (Badin and Levesque, 1961), and macroglobulin fractions obtained on gel filtration on Sephadex G. 200 were also tested for rheumatoid factor activity. Sera from patients with rheumatoid arthritis were tested for rheumatoid factor inhibitors by the latex inhibition test (Hall *et al.*, 1958).

Sera from patients with rheumatoid arthritis and from randomly selected control subjects were tested for the presence of autoantibodies, immunofluorescent techniques being used. Rat liver, calf thyroid, and human polymorphonuclear leucocytes treated by repeated freezing and thawing (Elling, 1967) were used as substrates for detection of antinuclear factors and human thyroid and rat stomach as substrates for detection of organ-specific autoantibodies. Sera were diluted 1:2 in testing for antinuclear factor to polymorphonuclear leucocytes and an anti-whole-immunoglobulin conjugate (Burroughs Wellcome) was used in this system. In the remaining test systems sera were diluted 1:10 and an anti-IgG conjugate (kindly supplied by the Blood Group Reference Laboratory) was used. Thyroglobulin antibodies were assayed with coated tanned sheep red cells (Burroughs Wellcome). Repeated absorption of sera was often necessary owing to the presence of heterophil agglutinins.

Immunoglobulin levels were determined by an immunodiffusion technique (Fahey and McKelvey, 1965) using monospecific sheep anti-human antisera (Burroughs Wellcome). Malaria antibodies were assayed by a fluorescent technique (Voller, 1964) using *Plasmodium falciparum* in human blood films as antigen. Sera were tested for the presence of heterophil agglutinins with 1% fresh sheep red cells.

Results

Rheumatoid Factor

The percentage incidence of positive tests for rheumatoid factor in members of the four groups is shown in Table II. A

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TABLE I.—*Age and Sex Distribution of Subjects Tested for Rheumatoid Factor and Autoantibodies. (Figures for Autoantibodies Given in Parentheses.)*

	5-14		15-24		25-34		35-44		45-54		>55		Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Rheumatoid arthritis	0 (0)	0 (0)	2 (2)	4 (4)	10 (10)	6 (4)	9 (7)	7 (7)	4 (4)	3 (3)	6 (6)	2 (2)	31 (29)	22 (20)
Other arthritides	7 (0)	16 (3)	15 (7)	8 (4)	19 (8)	15 (7)	16 (9)	12 (4)	13 (4)	6 (1)	5 (3)	4 (2)	75 (31)	61 (21)
General medical diseases	3 (2)	0 (0)	13 (7)	11 (5)	13 (6)	17 (16)	20 (13)	7 (5)	14 (11)	11 (9)	18 (11)	8 (4)	81 (50)	54 (39)
Healthy controls	64 (2)	62 (17)	93 (43)	46 (12)	90 (40)	57 (15)	76 (36)	57 (18)	37 (18)	35 (12)	31 (11)	23 (14)	391 (150)	280 (88)
Total	74 (4)	78 (20)	123 (59)	69 (25)	132 (64)	95 (42)	121 (65)	83 (34)	68 (37)	55 (25)	60 (31)	37 (22)	578 (260)	417 (168)

positive latex fixation test was often found in members of the control groups, the incidence increasing markedly with age. The number of positive tests for rheumatoid factor expected in a population group of the age distribution of the 53 patients with rheumatoid arthritis has been calculated on the basis of the figures obtained in the healthy control group. Nine patients would have been expected to have a positive latex fixation test and one a positive human erythrocyte agglutination test. The observed incidence was 7 (13%) and 5 (9%)

TABLE II.—*Percentage Incidence of Positive Tests for Rheumatoid Factor in Nigerian Patients with Rheumatoid Arthritis and in Control Groups.*

	No. Tested	Latex Fixation Test	Human Erythrocyte Agglutination Test
Rheumatoid arthritis	53	13	9
Other arthritides	136	7	4
General medical patients	135	10	11
Healthy controls	671	11	2

respectively. A positive human erythrocyte agglutination test was thus found significantly more often in patients with rheumatoid arthritis than in healthy controls, but the incidence of a positive latex fixation test in patients with rheumatoid arthritis was found to be no higher than in healthy controls of the same age distribution. The incidence of a positive human erythrocyte agglutination test was no higher among the patients with rheumatoid arthritis than among patients admitted to hospital in Ibadan with general medical diseases.

Sera from the 53 patients with rheumatoid arthritis and 200 randomly selected sera from members of the other groups were also tested for rheumatoid factor by the F.2 test and the sheep cell test. The human erythrocyte agglutination test was found to be slightly more sensitive than the sheep cell test in all groups; closely comparable results were obtained with the F.2 test and the latex fixation test.

A number of experiments were carried out to determine whether or not the low incidence of positive tests for rheumatoid factor among the patients with rheumatoid arthritis could be explained by the presence of inhibitors. After preliminary heat inactivation two more sera from patients with rheumatoid arthritis gave a positive latex fixation test, but a similar slight increase in the number of positive tests occurred in the control groups (15 increasing to 18). Euglobulin preparations of sera from 43 patients with rheumatoid arthritis gave no increase in the incidence of positive tests over the values obtained on testing whole sera. No rheumatoid factor activity was found in the macroglobulin peaks obtained on gel filtration at *p*H 7·5 and *p*H 4·0 of individual seronegative sera and of four pools of 10 seronegative sera. Only six of the 46 sera tested failed to inhibit the rheumatoid factor activity of a serum from an English patient with rheumatoid arthritis. Each of these six sera gave a positive latex fixation test. Failure to inhibit rheumatoid factor activity was found in over 90% of sera from American patients with rheumatoid arthritis when this test was used (Hall *et al.*, 1958).

Autoantibodies

The results obtained on testing the sera of members of the four groups for autoantibodies are given in Table III. Positive tests for autoantibodies were found no more frequently in the patients with rheumatoid arthritis than in subjects in the other groups. Homogeneous staining antinuclear factor was found in only three subjects, two of whom were known to have systemic connective tissue diseases. Granulocyte-specific antinuclear factor was found in only one patient with rheumatoid arthritis. Many sera in each group gave an unusual pattern of speckled staining of the nuclei of rat liver and calf thyroid and sometimes of other tissues. The fluorescent staining was usually only faint, but some sera gave bright speckled staining at a dilution of 1:10.

Antibodies to thyroglobulin were found in only 10 of the 428 sera tested at a titre of 1:25 or greater and in only two at a titre of 1:125 or greater.

TABLE III.—*Number of Positive Tests for Autoantibodies found in Nigerian Patients with Rheumatoid Arthritis and in Controls.*

	No. Tested	Antinuclear Factor (Homogeneous Staining)			Gastric Parietal	Thyroid Cytoplasmic	T.R.C. 1:25 or Greater
		Rat Liver	Calf Thyroid	Polymorphonuclear Leucocytes			
Rheumatoid arthritis	49	0	0	1	0	1	2
Other arthritides	52	2	2	3	1	1	2
General medical diseases	89	0	0	N.D.	1	3	2
Healthy controls	238	1	1	N.D.	3	2	4
Total	428	3	3	4	5	7	10

T.R.C. = Tanned red cell agglutination test for thyroglobulin

Immunoglobulins

The mean immunoglobulin levels obtained in 48 patients with rheumatoid arthritis and 96 age and sex matched controls are given in Table IV. A wide range of distribution was found in each group. Though the mean IgG level was high in both patients and controls, the mean IgG level was significantly lower in the patients with rheumatoid arthritis than

TABLE IV.—*Mean Immunoglobulin Levels in Nigerian Patients with Rheumatoid Arthritis and in Age and Sex Matched Controls. Standard Deviations given in Parentheses. Results expressed as a Percentage of the M.R.C. Normal Human Serum Standard.*

	No. Tested	IgA	IgG	IgM
Rheumatoid arthritis	48	120 (90)	225 (100)	365 (250)
Controls	96	110 (45)	280 (100)	345 (175)

in the controls (difference between means = 55%, standard error = 17·3%). The mean IgM level was high in both groups but did not differ significantly between patients and controls. IgM levels were higher in female than male controls.

Malaria antibodies

The mean malaria antibody titre of 46 patients with rheumatoid arthritis was lower than the mean titre of 46 age and sex matched controls, but the difference between means was not statistically significant. Low malaria antibody titres (1:320 or less), however, were found significantly more often in patients with rheumatoid arthritis than in matched controls (21 compared with 8; $\chi^2=8{\cdot}5$, $P<0{\cdot}01$).

Heterophil antibodies

Heterophil antibodies are found in many Nigerian sera (Greenwood, 1970). The mean titre of 40 patients with rheumatoid arthritis (1:12) was significantly lower than the mean titre of 40 age and sex matched controls (1:24), and significantly more patients than controls had titres at 1:16 or less (23 compared with 11; $\chi^2=7{\cdot}5$, $P<0{\cdot}01$).

Discussion

Rheumatoid factors can be found in the sera of 70-80% of Caucasian patients with rheumatoid arthritis by using standard methods, and even higher values have been obtained with special techniques. Rheumatoid factor reacting with human gammaglobulin, however, was found no more frequently in Nigerian patients satisfying the American Rheumatism Association criteria for a diagnosis of definite or probable rheumatoid arthritis than among healthy controls

and rheumatoid factor reacting with rabbit gammaglobulin no more frequently than among patients with general medical diseases. A number of rheumatoid factor inhibitors have been described (Heller *et al.*, 1954; Ziff *et al.*, 1956; Rantz *et al.*, 1959), and the possibility that the low incidence of seropositivity in Nigerian patients with rheumatoid arthritis was due to the presence of a potent inhibitor was considered. Heat inactivation and the use of euglobulin and purified macroglobulin preparations, however, did not significantly increase the number of sera in which rheumatoid factor activity could be demonstrated.

Many Nigerian sera contain large amounts of IgG, and the possibility that any rheumatoid factor present in these sera was held in a complexed form was also considered. No rheumatoid factor activity was found in the macroglobulin peak of sera fractionated on Sephadex G.200 at *p*H 4·0, conditions under which dissociation of complexes would be expected to occur. These experiments do not completely exclude the existence of a potent inhibitor, as this could also be a macroglobulin, but they suggest that most Nigerian cases of rheumatoid arthritis are truly seronegative.

An increased incidence of antinuclear factor in the sera of Caucasian patients with rheumatoid arthritis has been found on many occasions, figures varying in relation to the substrate used and the immunofluorescent technique employed. Ward *et al.* (1964), using calf thyroid as antigen and employing a similar technique to that used in the present study, found antinuclear factor in 19% of a series of 220 English patients with rheumatoid arthritis. Alexander *et al.* (1960) and Elling (1967) have found a much higher incidence of antinuclear factor when polymorphonuclear leucocytes have been used as antigen. In this study none of the 49 sera from Nigerian patients with rheumatoid arthritis was found to give a positive test for homogeneous antinuclear factor with calf thyroid and only one with polymorphonuclear leucocytes. Some workers, but not others, have found an increased incidence of organ-specific autoantibodies in Caucasian patients with rheumatoid arthritis. No increased incidence was found in the present study.

Variable results have been obtained in studies of immunoglobulin levels in Caucasian patients with rheumatoid arthritis possibly owing to the failure to use age and sex matched controls in some series. Most studies, however, have shown a rise in one or more of the immunoglobulin classes in patients with the disease. In a recent study in which age and sex matched controls were used (Rhodes *et al.*, 1969) a rise in mean levels of IgA, IgG, and IgM was found. Mean levels of IgG and IgM, but not IgA, were found to be high in Nigerian patients with rheumatoid arthritis and in controls, as previously noted in Nigeria (Turner and Voller, 1966) and in other parts of tropical Africa. The mean IgG level, however, was significantly lower in the patients with rheumatoid arthritis than in the controls. It is of interest that a similar depression of IgG levels was found in a group of Nigerian patients with an acute form of polyarthritis, for which no cause could be found, who may have been suffering from an unusual form of rheumatoid arthritis (Greenwood 1969b).

Nigerian patients with rheumatoid arthritis were found to have lower malaria antibody levels and lower titres of heterophil agglutinins than age and sex matched controls. These findings, taken in conjunction with the low IgG levels, suggest that this group of patients shows a diminished humoral response to the repeated infections to which residents of a tropical African country are exposed. It is possible that this diminished humoral response is consequence of their disease. Several studies of the immune response of Caucasian patients with rheumatoid arthritis, however, have shown a normal or enhanced humoral response (Rhodes *et al.*, 1969). These findings may therefore indicate that individuals showing a poor immune response to the antigenic stimulus of repeated infections are more liable to develop rheumatoid arthritis—indirect evidence for the hypothesis that the effects of repeated parasitic infections may have some protective action against the development of autoimmune disease (Greenwood, 1968).

During the course of this study a number of interesting incidental findings emerged. The frequent occurrence in Nigerian sera of an IgM rheumatoid-factor-like antibody, of heterophil agglutinins, and of an unusual form of speckled antinuclear factor were unexpected findings that are being further investigated. Organ-specific autoantibodies were found infrequently in the 428 sera tested, and antibodies to thyroglobulin were also uncommon. The overall incidence of thyroglobulin antibodies at a titre of 1:25 or greater in women over 35 years old (2·5%) is very much lower than the value of 19% for women over 40 years old obtained by Dingle *et al.* (1966) in a series of healthy English subjects. This finding is of interest in relation to the low incidence of lymphocyte aggregates in Nigerian thyroids and the low incidence of adult myxoedema and Hashimoto's thyroiditis in Western Nigeria (Taylor, 1968) and probably in some other parts of tropical Africa (Greenwood, 1968).

The results of this immunological study support the clinical findings that Nigerian patients satisfying the American Rheumatism Association criteria for a diagnosis of rheumatoid arthritis differ from Caucasian patients with the disease in a number of important respects, though the initial features of their joint disease are typical of the condition. These observations can be explained in one of two ways. It is possible that this group of patients do not have rheumatoid arthritis but a distinct clinical syndrome which must be added to the recognized forms of seronegative polyarthritis such as Reiter's syndrome, psoriasis, and ulcerative colitis, all of which diagnoses were excluded so far as was possible among patients in the present study. In this case true rheumatoid arthritis must be an exceedingly rare disease in Western Nigeria. Alternatively, these patients may be thought to have rheumatoid arthritis modified in some way by genetic or environmental factors, possibly by the effects of parasitic infections. This latter possibility is being further studied.

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