ANTINUCLEAR AND PRECIPITATING AUTO-ANTIBODIES IN SJÖGREN’S SYNDROME*

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The diagnosis of Sjögren’s syndrome is usually based on the triad of kerato-conjunctivitis sicca, salivary gland involvement (enlargement and/or xerostomia), and rheumatoid arthritis, but may also be made when any two of these features are present (Sjögren, 1943). In addition, the syndrome has been described in patients with systemic lupus erythematosus (Ramage and Kinnear, 1956; Bain 1960), progressive systemic sclerosis (Oblatt, Fehér, and Csiky, 1958; Bloch, Bunim, Wohl, and Zvaifler, 1960; Bloch, Wohl, Ship, Oglesby, and Bunim, 1960; Shearn, 1960; Stollte, Hanlon, Pease, and Henderson, 1960; Bloch and Bunim, 1963), polyarteritis nodosa (Ramage and Kinnear, 1956; Shearn, 1961), and myopathy or polymyositis (Bunim, 1961; Silberberg and Drachman, 1962), any of which may replace rheumatoid arthritis in the diagnostic triad. The sera of patients with Sjögren’s syndrome have been found very frequently to contain rheumatoid and antinuclear factors, and precipitating and complement-fixing antibodies reacting with a wide variety of the organs and tissues (Jones, 1958; Heaton, 1959; Bloch and others, 1960a and b; Deicher, Holman, and Kunkel, 1960; Anderson, Gray, Beck, and Kinnear, 1961; Thompson, 1962; Beck, 1963; Bloch and Bunim, 1963; Crews and Whitfield, 1963; Vanselow, Dodson, Angell, and Duff, 1963); in addition, several workers have noted the frequent occurrence of thyroglobulin auto-antibodies in patients with the condition (Bloch, Bunim, and others, 1960; Bloch, Wohl, and others, 1960; Anderson, Goudie, Gray, and Buchanan, 1961; Bunim, 1961). The present paper describes the prevalence of several systems of antinuclear and precipitating auto-antibodies in relation to the clinical and laboratory findings in a series of well-documented cases of Sjögren’s syndrome.

Materials and Methods

Patients.—42 patients (40 females, 2 males), from various parts of the United States, suffering from Sjögren’s syndrome, were studied at the National Institutes of Health. Patients were included in the study only when at least two of the three major components of Sjögren’s syndrome could be demonstrated: connective tissue disease (rheumatoid arthritis or other), keratoconjunctivitis sicca, and salivary gland involvement. Patients in whom the sicca syndrome could be attributed to sarcoidosis, macroglobulinaemia of Waldenström, leukaemia, and lymphoma were excluded. The diagnosis of rheumatoid arthritis was based on the criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959).

The 42 patients with Sjögren’s syndrome were separated into five clinical groups:

Group A Eighteen patients with Sjögren’s syndrome with “definite” or “classical” rheumatoid arthritis.

Group B Two patients with Sjögren’s syndrome with “probable” rheumatoid arthritis.

Group C Three patients with Sjögren’s syndrome with progressive systemic sclerosis.

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Group D Three patients with Sjögren's syndrome and myopathy.

Group E Sixteen patients with Sjögren's syndrome with characteristic ocular and oral findings but without evidence of rheumatoid arthritis or other connective tissue disease.

Serological Methods.—Antinuclear antibodies were detected by the indirect fluorescent antibody technique, using cryostat sections of unfixed rat liver by the method described previously (Beck, 1961). The sections were examined under dark-field ultra-violet/blue violet light with a horizontal optical system of the type described by Young (1961). With the technique used in this laboratory, the fluorescent antibody test for antinuclear antibodies was considered positive only when nuclear staining was obtained with sera diluted 1/16 with saline. This level was arbitrarily chosen as the test was positive at 1/16 dilution in only 4 per cent. of a series of 500 hospital patients (none of whom was known to suffer from a connective tissue disease) but was positive in 27 per cent. if sera were used at 1/4 dilution and in 46 per cent. if undiluted serum was used (Beck, 1963). All positive sera were titrated at four-fold dilutions. The pattern of nuclear staining was recorded in each case using the terminology described previously (Beck, 1961, 1962, 1963) as follows:

Homogeneous.—Each nucleus was stained throughout, often with central large irregular areas of somewhat more intense staining, but the nucleoli were unstained.

Speckled.—Numerous minute points of fluorescence were seen in each nucleus so that the margin was indistinct.

Nucleolar.—Each nucleolus was uniformly stained, its smooth surface sharply demarcated from the adjacent nucleus, and the heterochromatin was unstained.

Investigations on the corresponding antigens have shown that the *homogeneous* antigen is nucleohistone (DNA-histone) and that the *speckled* antigen is a saline soluble protein, with a sedimentation coefficient between 3:5S and 2:0S. The *nucleolar* antigen has not been identified but is probably a protein associated with nucleolar RNA. The *membranous* antigen is DNA (Beck, 1961, 1962, 1963).

It must be noted that many sera give a mixed pattern of staining at high concentration, but show a characteristic staining pattern near the end-point in the titration. This indicates that a mixture of antibodies of different specificities is present in the serum; only the antibody present in highest titre can be identified conclusively, with the reservation that it is usually possible to identify the pattern of nuclear staining accompanying nucleolar staining.

Precipitatin auto-antibodies reacting with saline extracts of various human tissues were detected by an agar diffusion Ouchterlony plate method, as described by Anderson, Gray, and others (1961) and Anderson, Gray, Beck, Buchanan, and McElhinney (1962). Using this technique, four separate antigen—antibody systems were identified in a large series of patients with Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, and other connective tissue diseases (Anderson, Gray, and others, 1961, 1962). The antibodies (designated anti-Sjd, anti-SjT, anti-DNA, and anti-Lup respectively) ranged in titre up to 1 in 512 and had the characteristic mobility of gamma globulin on immuno-electrophoresis. Three of the antibodies (anti-Sjd, anti-SjT, and anti-DNA) were shown by ultracentrifugation to have sedimentation coefficients of 7S (Anderson and others, 1962). The antigens involved were unidentified specific cellular constituents present in a variety of human tissue extracts.

<table>
<thead>
<tr>
<th>Clinical Group</th>
<th>No. of Patients Studied</th>
<th>No. of Patients with Positive Sera</th>
<th>Nuclear Staining</th>
<th>H : S Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sjögren's Syndrome and Rheumatoid Arthritis</td>
<td>18</td>
<td>10 (56 per cent.)</td>
<td>8 (44 per cent.)</td>
<td>2</td>
</tr>
<tr>
<td>B. Sjögren's Syndrome and &quot;Probable&quot; Rheumatoid Arthritis</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>C. Sjögren's Syndrome and Progressive Systemic Sclerosis</td>
<td>3</td>
<td>2</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>D. Sjögren's Syndrome and Polymyositis</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>E. Sicca Syndrome Alone</td>
<td>16</td>
<td>12 (75 per cent.)</td>
<td>7 (44 per cent.)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>28 (67 per cent.)</td>
<td>17 (45 per cent.)</td>
<td>11 (26 per cent.)</td>
</tr>
</tbody>
</table>

* H : S—Homogeneous; Speckled.

** This patient's serum also contained "speckled" antibody.

*** Two patients' sera also contained "homogeneous" antibody and three patients' sera also contained "speckled" antibody.
but absent from extracts of red cells or platelets, or serum, plasma, or gamma globulin (Anderson and others, 1962). One of the antigens was identified as DNA, but the other antigens are trace constituents of cell extracts which have not been identified. Examples of anti-SjT, anti-SjD, and anti-DNA were shown to react with the patient's own tissues.

The L.E.-cell test used was that developed by Zinkham and Conley (1956). The sera were tested for the presence of rheumatoid factor by the Bentonite flocculation test and the sensitized sheep cell agglutination test, using the methods described by Bozicevich, Bunim, Freund, and Ward (1958) and Ziff, Brown, Lospalluto, Badin, and McEwen (1956) respectively. The tanned red cell agglutination test was used to detect anti-thyroglobulin (Roitt and Doniach, 1958), and the auto-immune complement fixation reaction using human liver as antigen was detected by the method used by Bloch, Wohl, and others (1960).

Results

Antinuclear Antibodies

These were detected by the indirect fluorescent technique in 28 (67 per cent.) of our 42 patients with Sjögren's syndrome and in five (12 per cent.) of 42 control subjects matched for sex and age, including normal laboratory personnel and hospital patients with iron deficiency anaemia. This difference is highly significant ($\chi^2 = 24.16; P < 0.001$). The prevalence of different staining patterns in the different groups of patients with Sjögren's syndrome is shown in Table I. Because of the small number of patients in Groups B, C, and D, comparison is only possible between Groups A and E. The overall incidence of antinuclear antibodies was higher in Group E (75 per cent.) than in Group A (56 per cent.) and this was due mainly to the higher incidence of “speckled” antinuclear antibody and the occurrence of antinucleolar antibody in Group E; the incidence of “homogeneous” antinuclear antibody was the same in both groups. Antinucleolar antibody was not encountered in Group A, although present in one patient with “probable” rheumatoid arthritis in Group B. The “membranous” staining pattern was not given by any of the sera tested in this study.

The titres of antinuclear antibodies found in patients of Groups A and E are compared in Fig. 1. The titres of the different systems of antinuclear antibodies in Group A and E are compared in Fig. 2. These histograms show that higher titres were encountered in Group E as well as the higher incidence and greater variety of antinuclear antibodies described above.

No relationship was found between the age of the patient, the duration of the disease, or the presence of kerato-conjunctivitis sicca or xerostomia, and the presence or titre of antinuclear antibodies in the whole series or in any sub-group. The number of male patients in the series was too small (only two cases) to allow deductions on the relative incidence.
in males and females. There was, however, a direct relationship to the presence of parotid gland enlargement by history or on examination. Thus, of 22 patients with parotid gland enlargement, nineteen had antinuclear antibodies, whereas only ten of twenty patients with no parotid enlargement had positive antinuclear factor tests (χ² = 4.893; P <0.05).

All patients with positive L.E.-cell tests showed high titre "homogeneous" antinuclear antibody, but the converse did not hold. There was no direct relationship between the results of the fluorescent antibody test for antinuclear antibodies and the other immunological reactions such as the Bentonite flocculation test and sensitized sheep cell agglutination test for rheumatoid factor, anti-thyroglobulin, and the auto-immune complement-fixation reaction. The mean γ-globulin level in patients with antinuclear antibodies (2·357 g./100 ml.) was significantly greater (P <0·01) than in patients without antinuclear antibodies (1·514 g./100 ml.).

Precipitating Auto-antibodies

Table II lists the frequency and type of precipitins encountered in this study. As in previous investigations (Anderson, Gray, and others, 1961, 1962), the most commonly detected precipitins in Sjögren's syndrome were anti-SjD and anti-SjT, although anti-Lup was detected in three sera. Failure to detect anti-DNA corresponds with the absence of the nuclear membrane type of staining in the immuno-fluorescent tests. The other three precipitins do not appear to correspond to any of the other patterns of nuclear staining. The incidence of positive precipitin tests was significantly higher in Group E than in Group A patients (χ² = 10·9; P <0·001) and two distinct precipitins were commonly found in Group E sera.

Discussion

Antinuclear antibodies were detected in 28 of our 42 cases of Sjögren's syndrome (67 per cent.). This incidence is somewhat lower than that encountered using the same methods in systemic lupus erythematosus (82 per cent.) and progressive systemic sclerosis (81 per cent.), but is higher than that seen in chronic discoid lupus erythematosus (35 per cent.) and uncomplicated rheumatoid arthritis (24 per cent.) (Beck, 1963). There have been few previous observations on the occurrence of antinuclear antibodies in Sjögren's syndrome and none of them have distinguished the different patterns of nuclear fluorescence. Thompson (1962) and Vanselow and others (1963) tested small groups of patients and found antinuclear antibodies in two of six and two of five cases respectively. Bloch, Bunim, and others (1960) investigated thirty patients (some of them are included in the present series) and detected antinuclear antibodies in 21; the prevalence in patients with rheumatoid arthritis (Group A) and without rheumatoid arthritis (Group E) was eight of thirteen and eleven of twelve respectively. Thus the increased prevalence of antinuclear antibodies in Group E had been recognized, but the increased variety of antinuclear antibodies in these patients had not been known previously.

In the whole series of cases of Sjögren's syndrome, the ratio of the prevalence of "homogeneous" to "speckled" antinuclear antibodies (H:S ratio) was 1·5. This is somewhat lower than that found in

<table>
<thead>
<tr>
<th>Clinical Group</th>
<th>No. of Patients Studied</th>
<th>No. of Positive Sera</th>
<th>Precipitating Antibodies</th>
<th>Not Identified*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sjögren's Syndrome and Rheumatoid Arthritis</td>
<td>18</td>
<td>1 (6 per cent.)</td>
<td>Anti-SjD Anti-SjT Anti-Lup</td>
<td>1</td>
</tr>
<tr>
<td>B. Sjögren's Syndrome and &quot;Probable&quot; Rheumatoid Arthritis</td>
<td>2</td>
<td>1</td>
<td>1**</td>
<td></td>
</tr>
<tr>
<td>C. Sjögren's Syndrome and Progressive Systemic Sclerosis</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Sjögren's Syndrome and Polymyositis</td>
<td>3</td>
<td>1</td>
<td>1**</td>
<td></td>
</tr>
<tr>
<td>E. Sicca Syndrome Alone</td>
<td>16</td>
<td>13 (81 per cent.)</td>
<td>11 (69 per cent.)</td>
<td>6 (38 per cent.)**</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>18 (43 per cent.)</td>
<td>13 (31 per cent.)</td>
<td>8 (19 per cent.)</td>
</tr>
</tbody>
</table>

* Precipitin factors too weak for identification.
** These patients also had anti-SjD precipitating antibodies.
systemic lupus erythematosus (2.2) and markedly lower than that found in rheumatoid arthritis (4.2) (Beck, 1963) (Table I). It is noteworthy that the findings in Group A (Sjögren's syndrome with rheumatoid arthritis) are very similar to those found previously in uncomplicated rheumatoid arthritis (Beck, 1963). Antinucleolar antibodies were not detected in Group A, nor were they seen in a previous study of rheumatoid arthritis (Beck, Anderson, McElhinny, and Rowell, 1962). Antinucleolar antibody was seen in 33 per cent. of the patients in Group E with the sicca syndrome unaccompanied by a major connective tissue disease, and this is higher than the incidence observed in progressive systemic sclerosis (19 per cent.), systemic lupus erythematosus (1.5 per cent.), and chronic discoid lupus erythematosus (0.67 per cent.) (Beck and others, 1962). Consideration of the titres of antinuclear antibodies found in patients in Group A and E (Fig. I) has shown that high titres are much commoner in Group E.

The precipitating antibodies were more common and of higher titre in Group E than in Group A patients (P<0.001) (Table II). The corresponding antigens could not be identified because they were present in trace quantities in tissue extracts and were unstable on chemical manipulation. The occurrence of three distinct precipitins regarded as auto-antibodies adds to the complexity of the autoimmune phenomena of Sjögren's syndrome, for, in addition to the antinuclear antibodies already discussed, Deicher and others (1960) have described the complexity of complement-fixation reactions involving cytoplasmic constituents in this condition and in the connective tissue diseases.

The striking difference in serological findings in Group E compared to Group A requires comment. There did not appear to be any qualitative differences in the spectrum of pathological changes in salivary glands from patients in these groups. In most patients in Group E, however, the various components of the sicca syndrome appeared to be more fully expressed than in patients in other groups of Sjögren's syndrome. Patients in Group E had more severe xerostomia and greater salivary gland enlargement. Certain other abnormalities, marked hypergammaglobulinaemia, non-thrombocytopenic purpura, neuropathy, pulmonary lesions, and hypersplenism, occurred more frequently or solely in Group E compared to Group A (Bloch, Buchanan, Wohl, and Bunin, in preparation). These manifestations as well as the serological abnormalities may be expressions of the abnormal lymphoid tissue present in greater amounts in organs involved by the sicca complex in Group E patients.

Summary

Tests for antinuclear antibodies were performed by the indirect fluorescent technique in 42 patients with Sjögren's syndrome. Eighteen had rheumatoid arthritis (Group A), two had probable rheumatoid arthritis (Group B), three had progressive systemic sclerosis (Group C), three had polymyositis (Group D), and sixteen had the sicca syndrome alone (Group E).

Of the 42 patients, 28 (67 per cent.) had positive antinuclear factor tests, the incidence and titre of the antibodies being greater in patients of Group E than in Group A. There was a direct relationship between the presence of antinuclear factor and parotid enlargement indicated by history or examination, and a direct relation between the titres of antinuclear factor and the gamma globulin levels.

Study of the morphological pattern of nuclear fluorescence showed that the prevalence of "homogeneous" antibody, was the same in Groups A and E, but that "speckled" antinuclear antibody was more common in Group E.

With the exception of one patient with "probable" rheumatoid arthritis (Group B), antinucleolar antibody was found exclusively in patients of Group E. "Membranous" antinuclear antibody (anti-DNA) was not detected in any of the sera. All patients with positive L.E.-cell antibody had high titre "homogeneous" antinuclear antibody, but the converse did not hold.

Precipitating auto-antibodies reacting with saline extracts of various human tissues were detected by an agar diffusion Ouchterlony plate method in eighteen of the 42 patients (43 per cent.). Three distinct precipitating auto-antibodies were found (anti-SjD, anti-SjT, and anti-Lup). As with the antinuclear antibodies, the incidence and titre of the precipitating auto-antibodies were higher in Group E than Group A.

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REFERENCES

SJÖGREN'S SYNDROME

Trans. Ass. Amer. Physicians, 73, 166.

DISCUSSION

Dr. R. E. H. Partridge (Edinburgh): How many patients with the sicca syndrome alone later developed rheumatoid arthritis?

Dr. Buchanan: None.

Prof. E. G. L. Bywaters (Taplow): How long was the follow-up period?

Dr. Buchanan: Between one and five years. This illustrates the innocuous nature of rheumatoid factor, assuming it is the same factor as in patients with rheumatoid arthritis.

Dr. W. A. Bourne (Brighton): What were the ages?

Dr. Buchanan: The mean age was about 50 years, with no difference between sicca syndrome and rheumatoid patients. There were only two males.

Dr. C. Croft (Plymouth): What sort of respiratory complications were there?

Dr. Buchanan: Infections, pulmonary infiltrates, pleurisy, and pneumonia of low-grade severity but taking a long time to clear—comparable perhaps to infections of salivary glands.

Dr. K. W. H. Walton (Birmingham): You mentioned high levels of gamma globulin. Did you estimate levels of γ1A—this is found in bronchial secretion.

Dr. Buchanan: Immunoelctrophoresis of serum showed diffuse hyperglobulinaemia with elevation of all three types. Salivary secretion was difficult to obtain but the three immune globulins were also present there.

Dr. T. M. Chalmers (Edinburgh): Did any of these patients have myositis? Was there any difference here between those with sicca syndrome alone and those with rheumatoid arthritis?

Dr. Buchanan: Muscle biopsies showed focal aggregations of lymphocytes in patients with the sicca syndrome, with about the same incidence as in rheumatoid arthritis. Nor was there any difference in the incidence of arteritis.

Prof. J. H. Kellgren (Manchester): Was there any difference in the severity of the sicca syndrome in those with and without rheumatoid arthritis? Patients with rheumatoid arthritis tend to be screened for the sicca syndrome, whereas those presenting with sicca syndrome only would have a more advanced degree of it.

Dr. Buchanan: Yes, eye and salivary involvement was more severe in the sicca syndrome alone. This was reflected in antibody titres.

Dr. W. R. M. Alexander (Edinburgh): The respiratory complications sound like primary atypical pneumonia. Were cold agglutinins looked for?

Dr. Buchanan: Yes and they were absent. Primary atypical pneumonia is pretty rare and unlikely to be present in all these patients.

Prof. E. G. L. Bywaters (Taplow): Is there any difference between the Sjögren's syndrome seen in the United States and that in Scotland?

Dr. Buchanan: No. We are now testing 58 sera collected in Glasgow. They seem to be identical to those in the present series except that gastric antibodies are much more common in the Glasgow patients.

Prof. E. G. L. Bywaters: The effect of haggis, perhaps.

Auto-anticorps antinucléaires et précipitants dans le syndrome de Sjögren

Résumé

On rechercha les anticorps antinucléaires par le procédé de fluorescence indirecte chez 42 malades atteints de syndrome de Sjögren. Parmi ces malades il y eut 18 cas d'arthrite rhumatismale (Groupe A), 2 cas d'arthrite rhumatismale probable (Groupe B), 3 cas de sclérose disséminée progressive (Groupe C), 3 cas de polymyosite (Groupe D), et 16 cas de syndrome sicca seul (Groupe E). Chez 28 (67 pour cent) sur 42 malades les tests pour déceler le facteur antinucléaire furent positifs et la fréquence ainsi que le titre des anticorps furent plus grands chez les malades du Groupe E que chez ceux du Groupe A. Il y eut un rapport direct entre la présence du facteur antinucléaire et l'hypertrophie pariétodienne, existante ou passée, et aussi un rapport direct entre les titres du facteur antinucléaire et les niveaux de la globuline gamma.
L'examen de l'image morphologique de la fluorescence nucléaire montra que la fréquence de l'anticorps "homo-gène" fut la même dans les Groupes A et E, mais que l'anticorps antinucléaire "tacheté" fut plus commun dans le Groupe E. A l'exception d'un malade atteint d'arthrite rhumatismale "probable" (Groupe B), l'anticorps antinucléaire fut trouvé exclusivement chez des malades du Groupe E. L'anticorps antinucléaire "membraneux" (anti-ADN) ne fut décelé dans aucun sérum. Tous les malades accusant le phénomène L.E. eurent un titre élevé de l'anticorps antinucléaire mais le réciproque ne fut pas vrai.

Les auto-anticorps précipitants, réagissant avec des extraits en l'eau physiologique de différents tissus humains, furent découverts par la méthode d'Ouchterlony (plaques de diffusion sur agar) chez 18 sur 42 malades (43 pour cent). On trouva trois différents auto-anticorps (anti-SjD, anti-SjT, et anti-Lup). Comme pour les anticorps antinucléaires, la fréquence et les titres des auto-anticorps furent supérieurs dans le Groupe E que dans le Groupe A.

Auto-anticuerpos antinucleares y precipitantes en el síndrome de Sjögren

SUMARIO

Se determinaron los anticuerpos antinucleares por el procedimiento de fluorescencia indirecta en 42 enfermos con síndrome de Sjögren. Entre estos enfermos hubo 18 casos de artritis reumatoide (Grupo A), 2 casos de artritis reumatoide probable (Grupo B), 3 casos de esclerosis diseminada progresiva (Grupo C), 3 casos de polimiositis (Grupo D), y 16 casos de síndrome sicca solo (Grupo E). En 28 (67 por ciento) de los 42 enfermos el test para el factor antinuclear fué positivo; su frecuencia y el título de los anticuerpos fueron mayores en los enfermos del Grupo E que en los del Grupo A. Se notó una razón directa entre la presencia del factor antinuclear y de la hipertrofia parotidea, presente o pasada así como una razón directa entre la concentración del factor antinuclear y el nivel de la globulina gamma.

Un examen del aspecto morfológico de la fluorescencia nuclear reveló que la frecuencia del anticuerpo "homo-généo" fué igual en los Groups A y E, pero el anticuerpo antinuclear "moteado" fué más común en el Grupo E. Con excepción de un enfermo con artritis reumatoide "probable" (Grupo B), el anticuerpo antinuclear fué encontrado exclusivamente en enfermos del Grupo E. El anticuerpo antinuclear "membranoso" (anti-ADN) no fué observado en ningún suero. Todos los enfermos con células L.E. acusaron cifras altas del anticuerpo antinuclear, pero no hubo relación reciproca.

Los auto-anticuerpos precipitantes, que reaccionaban con extractos de diferentes tejidos humanos en solución salina, fueron evidenciados por el método de Ouchterlony (difusión sobre placas con agar) en 18 de los 42 enfermos (43 por ciento). Encontrarono tres diferentes auto-anticuerpos (anti-SjD, anti-SjT, y anti-Lup). Como en el caso de los anticuerpos antinucleares, la frecuencia y la concentración de los auto-anticuerpos fueron superiores en el Grupo E que en el Grupo A.