Journal of Clinical Immunology, Vol. 2, No. 4, 1982

Histological and Immunological Study in Patients with Rheumatoid Arthritis Showing Isolated Abnormalities of Salivary Scintigraphy

A. JANIN-MERCIER,[1,6] B. SAUVEZIE,[2] J. M. RISTORI,[2] G. BETAIL,[3] A. VEYRE,[4] and S. RAMPON[5]

Accepted: April 13, 1982

We report the histological and immunological findings in 20 rheumatoid arthritis (RA) patients with impaired salivary scintigraphy but without sicca complex. This condition was stable over 2–5 years and associated with mild infiltrates on lip biopsies. Increased levels of IgG, immune complexes, and antinuclear antibodies were found in seven patients (35 vs 69% in secondary Sjögren's syndrome). Two of the seven patients later developed xerostomia. The above-mentioned immunological abnormalities may increase the likelihood of developing a clinical impairment of salivary function in patients with an abnormal salivary scintiscan, the latter being a frequent, yet often isolated, finding in RA. Antisalivary duct antibodies were not related to any other parameter of salivary gland involvement.

KEY WORDS: Secondary Sjögren's syndrome; rheumatoid arthritis; sequential salivary scintigraphy; lip biopsy; immunopathology.

INTRODUCTION

Sjögren's syndrome is characterized by a dryness of the eyes and the mouth. Sequential salivary scintigraphy (SSS) has been proposed (1) as a sensitive method for a noninvasive evaluation of salivary gland dysfunction. In Sjögren's syndrome, SSS is markedly impaired (2). However, a number of abnormal results were also reported in rheumatoid arthritis (RA) (3) and systemic lupus erythematosus (SLE) (4) patients without keratoconjunctivitis sicca (KCS) or xerostomia (5). Isolated SSS abnormalities could express an asymptomatic involvement of salivary glands. It is unclear whether it represents an early stage of secondary Sjögren's syndrome or a mild form linked to some immunological peculiarities. A distinctive histological appearance of lip biopsy specimens (6, 7) and various immunological abnormalities were reported in Sjögren's syndrome (8). Therefore it could be interesting to define the histological and immunological features associated with isolated abnormalities of SSS and to follow the evolution of the salivary dysfunction.

In the present work, we studied a group of RA patients with abnormal scintiscans but without xerostomia and compared it with secondary Sjögren's syndrome and RA patients with no salivary gland abnormality. The follow-up period was 2 to 5 years. Most patients with isolated abnormalities of SSS showed mild lymphocytic infiltrates on lip biopsy specimens. No definite pattern of immunological abnormalities could be ascribed to this group of patients. The presence of antinuclear antibodies (ANA) was significantly correlated with high levels of IgG and circulating immune complexes (CIC) and this cluster was often observed in patients with severe salivary gland involvement. It is proposed that RA or a disease-associated factor produces a frequent but limited salivary dysfunction. This condition remained stable in most cases. From our data, the evolution to overt xerostomia seems more

[1]Department of Pathology, Hospital Saint-Jacques, Clermont-Ferrand, France.
[2]Clinical Immunology Unit, Hospital Saint-Jacques, Clermont-Ferrand, France.
[3]Laboratory of Immunology, Hospital Saint-Jacques, Clermont-Ferrand, France.
[4]Department of Nuclear Medicine, Hospital Saint-Jacques, Clermont-Ferrand, France.
[5]Department of Rheumatology, Hospital Saint-Jacques, Clermont-Ferrand, France.
[6]To whom correspondence should be addressed at Department of Pathology, Faculté de Médecine, Place H. Dunant, 63 000 Clermont-Ferrand, France.

0271-9142/82/1000-0282$03.00/0

likely to occur in patients with the above-mentioned cluster of immunological abnormalities.

MATERIALS AND METHODS

Patients

Fifty-five patients were studied. Thirty-seven had classical RA and eighteen had definite RA according to the diagnostic criteria of the American Rheumatism Association (9). Nineteen presented extrasalivary disorders such as vasculitis, myositis, thyroiditis, Felty syndrome, chronic hepatitis, pancreatitis, and renal tubular acidosis.

Diagnosis of Sjögren's Syndrome

KCS was documented by Schirmer's test and confirmed by the slit lamp examination. Diagnosis of xerostomia was based on clinical evidence and measurement of the unstimulated salivary flow rate (3).

Sequential Salivary Scintigraphy

SSS was performed with 5 mCi of sodium pertechnate (Tc 99 m) injected intravenously. Pictures were obtained every 10 min for 90 min. Classification was established according to the following grading criteria.

Class 1: normal scan; definite concentration in salivary glands within 10 min and elimination into oral saliva within 30 min.

Class 2: delay in appearance of parotid concentration but detectable oral activity within 90 min.

Class 3: concentration asymmetry (above 50% in the parotid gland) and/or absence of oral activity after 90 min.

Class 4: absence of salivary gland and oral activities.

After SSS the patients were distributed into three groups.

Group I: 19 patients without KCS or salivary abnormalities.

Group II: 20 patients with abnormal SSS but without xerostomia or KCS (subclinical salivary involvement).

Group III: 16 patients with secondary Sjögren's syndrome.

Histological Grading

Lip biopsy was performed after local anesthesia. Three to five labial salivary glands from each patient were fixed in 10% buffered formalin. Hematoxylin–eosin- and Giemsa-stained sections were examined and graded according to Tarpley *et al.* (7). Normal patients showed no sclerosis destroying the lobular architecture, no mast cells, and no eosinophils in the inflammatory infiltrate. When lymphocytes were observed, they were few and scattered.

Immunological Study

Samples were randomly coded and tested (at a 1/10 dilution) for antisalivary duct antibody (ASD) by indirect immunofluoresence on guinea pig salivary gland using a fluoresceinated goat anti-human globulin (Behring) as a second antibody.

Serum immunoglobulins were studied by quantitative immunodiffusion assay and immunoelectrophoresis.

Serum rheumatoid factor (RF) was tested by the method of Waaler–Rose as modified by Eyquem (10) and by the latex test.

ANA were detected by indirect immunofluorescence (rat liver). The double immunodiffusion method (11) was used for the detection of antibodies to soluble nuclear antigens from calf thymus.

For CIC studies each serum sample was tested in duplicate by the C1q (12) and Raji-cell (13) assays on two different occasions. For each measurement, the results were established with reference to aggregated IgG standards.

Follow-Up

A clinical examination was done every 6 months for 2 years. At least two SSS and immunological studies were performed in each case, including all group I and group III patients at the beginning and at the end of the follow-up period. Twenty-four patients had been included in a previous SSS study, 3 years before, extending the observation period to 5 years.

Statistical Analysis

The distribution of the parameters within the three groups was studied by a chi-square test.

For each quantitative parameter, the differences among the three groups were studied by an analysis

of variance. Without distinction of groups, the correlation coefficients were calculated for each biological parameter plotted against each of the others.

RESULTS

Sequential Salivary Scintigraphy (Table I)

In the patients with subclinical salivary involvement (group II), the SSS was less impaired than in the patients with secondary Sjögren's syndrome (group III). In the group II patients, the repeat SSS was identical in 10 cases followed up over a 5-year period and in 7 cases followed up over a 2-year period. It worsened in 3 patients, and xerostomia appeared in 2 of them. SSS remained unchanged in the other groups.

Histological Findings (Table II)

In group II, the histological grading ranged from class 1 to class 3. Lip biopsies from most group I patients had a normal histological appearance. A few of them had small lymphocytic infiltrates without any lobular destruction.

In group III, the lip biopsy specimens showed class 3 and class 4 changes. In the eight class 4 cases, numerous eosinophils and mast cells were associated with extensive sclerosis destroying the lobular architecture. In all groups, the decrease in the number of acini cells was related to the extension of the lymphocytic infiltrate. A correlation ($R = 0.767$, $P \leq 0.01$) was established between scintigraphic and histological grading (Fig. 1). A discrepancy between marked scintigraphic changes and limited histological abnormalities was observed in two cases with an epimyoepithelial-cell proliferation and lymphocytic infiltrate located within the ductal wall. In repeated examinations, the evolution of the histological changes paralleled the worsening of the SSS.

Table I. Sequential Salivary Scintigraphy (SSS) Results

	SSS class (%)			
	1	2	3	4
Group I ($N = 19$)	100			
Group II ($N = 20$)		65	35	
Group III ($N = 16$)			69	31

Table II. Histological Grading in the Three Groups

	Lip biopsy grade (%)				
	0	1	2	3	4
Group I	58	21	21		
($N = 19$)	(11)	(4)	(4)	(0)	(0)
Group II		25	20	55	
($N = 20$)	(0)	(5)	(4)	(11)	(0)
Group III				50	50
($N = 16$)	(0)	(0)	(0)	(8)	(8)

[a]Digits in parentheses refer to the number of patients who presented both abnormalities. Lip biopsy grades 0 and 1, as well as 3 and 4, were grouped and Yates correlation was applied. $\chi^2 = 37.20$, $P = 0.01$, 4 df.

Immunological Findings

The immunological findings are summarized in Figs. 2 and 3.

ANA were found in 7 group II patients and in 12 group III patients, reaching a high titer in the latter group. Further study did not show any anti-DNA antibody. Two sera contained anti-RNP antibodies.

C1q and Raji-cell assays (Fig. 3) have highly correlated results ($R = 0.515$, $P \leq 0.01$) and showed CIC in 7 group II patients and in 10 group III patients. CIC were also detected in 2 group I patients who later acquired ANA. A polyclonal increase in IgG, IgA, and IgM assessed by immunoelectrophoresis was observed in 5 group III patients and in 2 group II patients who later progressed to Sjögren's syndrome.

ANA (Table III) were correlated with CIC, IgG, lip biopsy, and SSS. For this reason, 5 group I and 7 group II patients with ANA titers $\geq$ 1/200 were separated for further statistical analysis. None of them fulfilled the diagnostic criteria of SLE. They were compared to group III patients. No significant difference was found except in the glandular involvement. It was solely in relation to this ANA subgroup that some evolutionary changes occurred

Table III. Significant Correlations Between Antinuclear Antibody (ANA) Titers and Other Biological Data in All Groups (55 Patients)[a]

	ANA titers
SSS grade	$R = 0.29$, $P \leq 0.05$
Lip biopsy grade	$R = 0.30$, $P \leq 0.05$
RF titers	$R = 0.37$, $P \leq 0.01$
CIC titers (C1q)	$R = 0.29$, $P \leq 0.05$
IgG titers	$R = 0.47$, $P \leq 0.01$

[a]SSS, sequential salivary scintigraphy; RF, rheumatoid factor; CIC, circulating immune complexes.

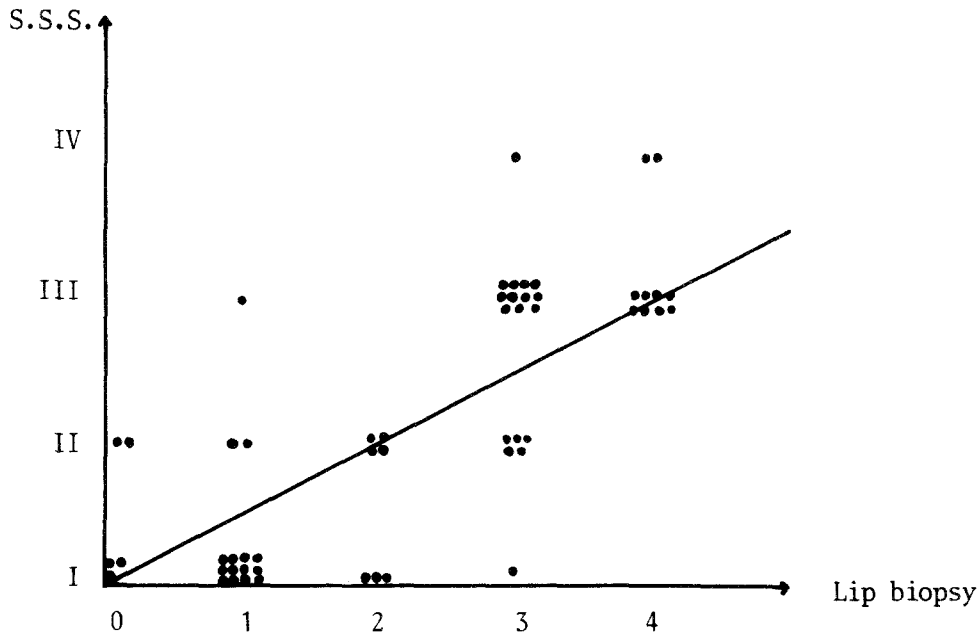

Fig. 1. Correlation between scintigraphic (SSS) and histological (lip biopsy) grading in all groups (55 patients, 53 df). $R = 0.767$, $P \leq 0.01$.

during the follow-up period: 2 group I patients with high titers of CIC later produced ANA as mentioned above, and 2 group II patients with ANA and polyclonal increases in IgG, IgA, and IgM evolved to the completion of Sjögren's syndrome. ASD were observed in each group of patients (Table IV). No correlation was found with xerostomia or histological or scintigraphic grading. They were significantly associated with extrasalivary disorders ($\chi^2 = 9.26$, $P \leq 0.01$, 2 df).

DISCUSSION

Discrepancies between SSS and other examination procedures are mentioned in several works (1, 4, 14). In a systematic study (3) of 100 RA patients without Sjögren's syndrome, we found 53 abnormal SSS including 19 major changes. There was no difference in drug regimen between the patients with abnormal SSS and the other RA patients. Twenty-four age-matched controls showed only five with mild impairment of SSS. Similar results were reported by Katz and co-workers (4): 59% significant but often mild changes in RA. They also found more frequent and marked abnormalities in SLE, suggesting that SSS may have some diagnostic value in this condition. There is little doubt that isolated SSS abnormalities do express a pathological process related to the accompanying disease. However, their significance is still unclear since the current criteria for Sjögren's syndrome do not refer to SSS.

In the present work, we investigated 20 RA patients with marked SSS abnormalities without sicca signs. This condition was stable over a 2- to 5-year period except in 3 cases where SSS worsened. Xerostomia developed in 2 of them. The lip biopsy specimens revealed low-grade abnormalities, but mild lymphocytic infiltrates were often observed, an uncommon finding in RA with normal SSS. Sclerosis which destroyed the lobular architecture in 50% of the Sjögren's syndrome was never observed in isolated SSS abnormalities. Antisalivary

Table IV. Antisalivary Duct Antibodies (ASD) in the Three Groups of Patients[a]

	ASD (%)	
	0	+
Group I ($N = 19$)	37 (7)	63 (12)
Group II ($N = 20$)	65 (13)	35 (7)
Group III ($N = 16$)	37.5 (6)	62.5 (10)

[a]Digits in parentheses refer to the number of patients who presented both abnormalities. $\chi^2 = 3.96$, 2 df, nonsignificant.

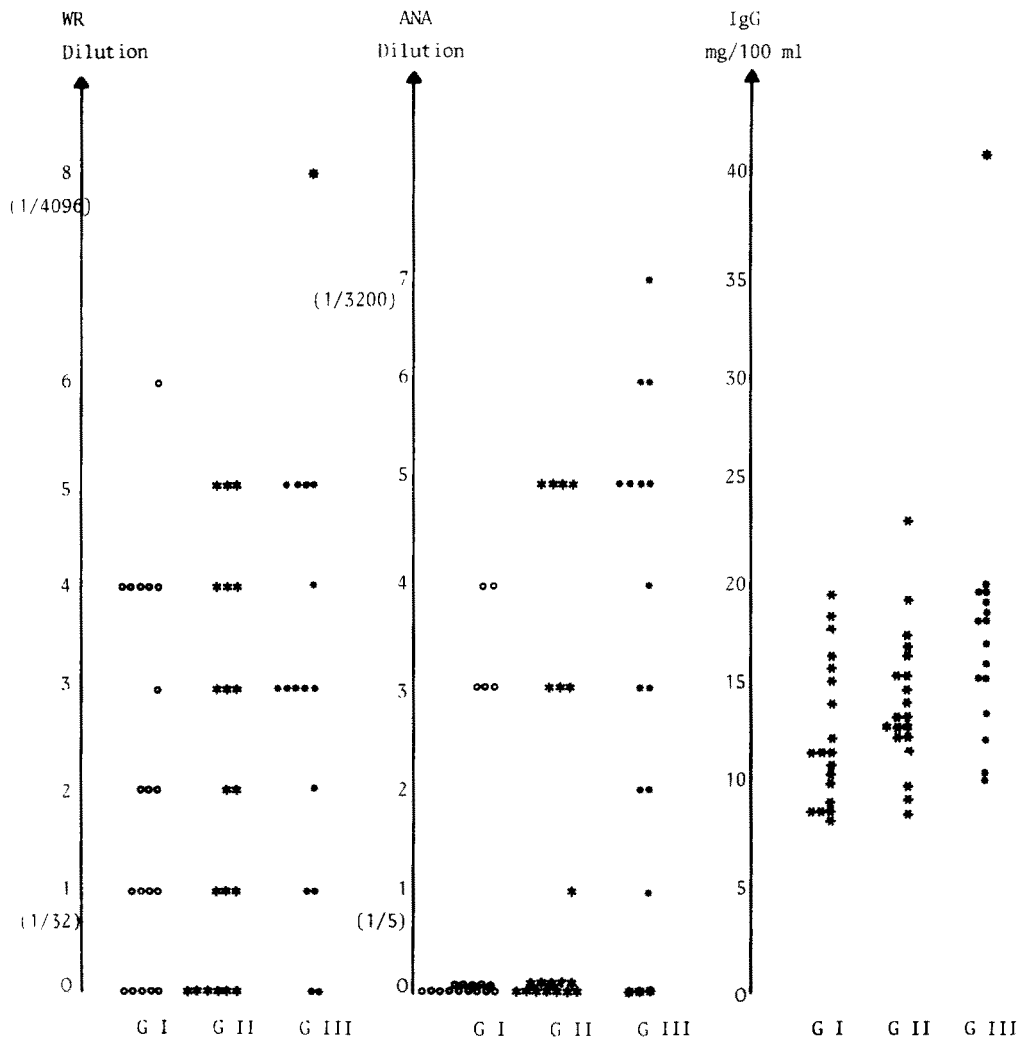

Fig. 2. Distribution of the Waaler–Rose (WR) and antinuclear antibody (ANA) results, as well as IgG levels in the three groups of patients. WR and ANA results are expressed as the logarithm of the reciprocal of the dilution. Each point represents one patient.

duct antibodies are common in Sjögren's syndrome and in RA without sicca complex (15), a distribution which could be relevant to our SSS findings. However, no parallel between ASD and SSS results could be found. It is unlikely that their alleged protective effect (15) may account for the subclinical involvement of salivary glands. A cluster of immunological abnormalities suggesting a polyclonal activation of B cells (high levels of Ig, CIC, and ANA) was observed in 35% of the subclinical salivary involvement and in 69% of the secondary Sjögren's syndrome but not in RA with normal SSS. The association of these immunological disturbances may increase the risk of developing a sicca complex in RA with abnormal SSS, as in two cases this cluster appeared shortly before the constitution of xerostomia. The ANA were not anti-DNA or anti-Sm antibodies. None of our patients met the diagnostic criteria for SLE. Thus our data do not support Katz's assumption that isolated SSS abnormalities are restricted to this disease. It is more likely that salivary lesions are related to the imbalance of the immune response as suggested by Miyasaka and co-workers (16). It could be shared by some RA patients, most primary and secondary Sjögren's syndromes, and SLE cases.

There is no evidence that xerostomia is CIC mediated. High levels of CIC and IgG in the blood (17) and Ig and RF synthesis by salivary infiltrate (18–19) were demonstrated in Sjögren's syndrome,

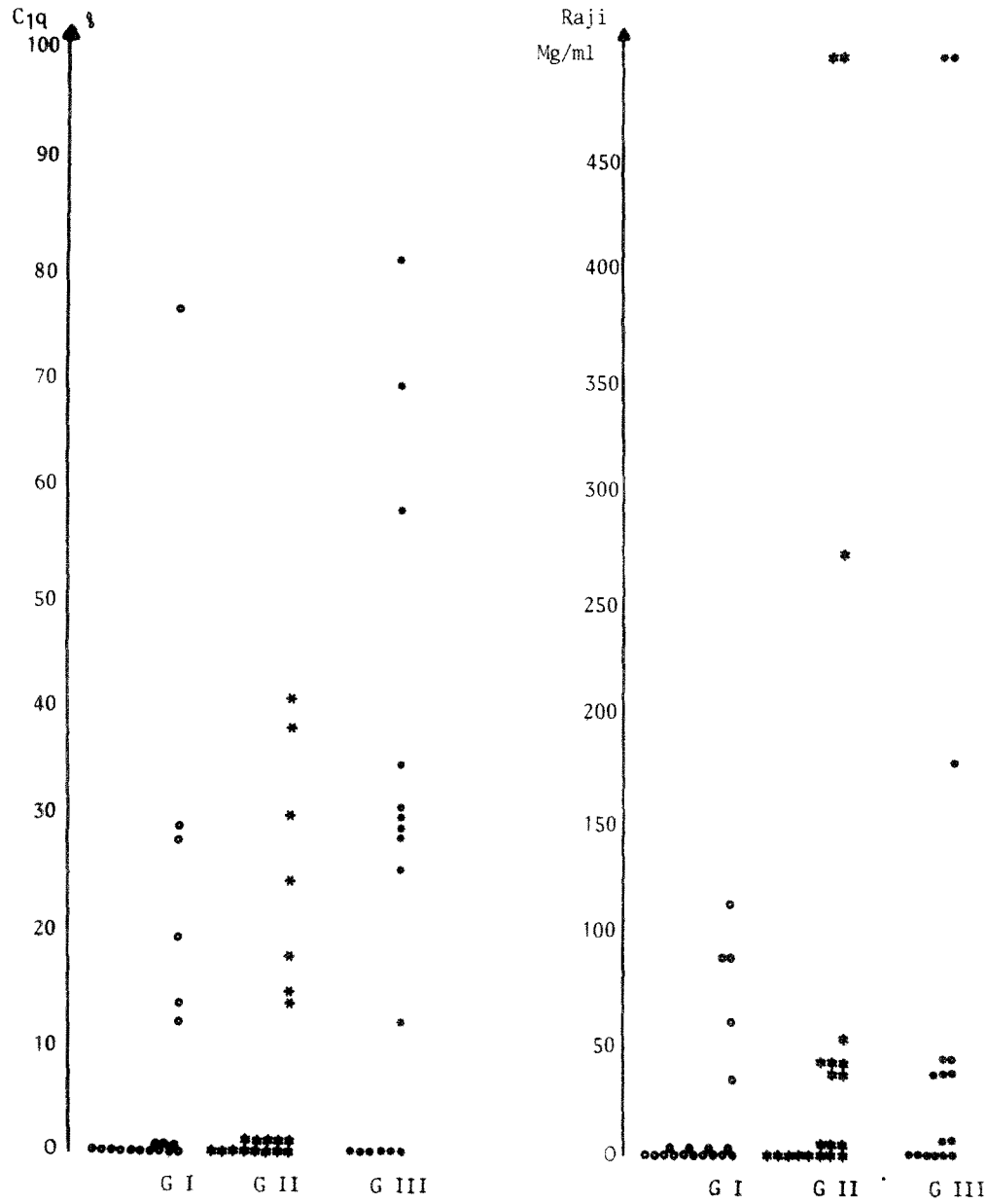

Fig. 3. Distribution of the circulating immune complexes in the three groups by two assays: Clq (left) and Raji cell (right). Each point represents the mean value of two measurements in each patient. Patients were considered as having CIC when at least one method gave a positive result (Clq binding > 12%; Raji-cell binding > 33 mg) in duplicate.

but it is difficult to credit the lymphocytic infiltrate with the whole production of CIC and circulating autoantibodies. In a recent study (20), the levels of CIC were not related to the degree of lymphocytic infiltration on lip biopsies.

These immunological abnormalities appear neither the primary cause nor the mere consequence of salivary infiltrate, and therefore an alternative hypothesis should be sought. We propose that RA produces a frequent subclinical salivary involvement detectable only by SSS (3). In most cases, this condition is stable. In some patients an impairment of the immunoregulatory mechanism could promote the extension of the lesions. The immunoregulatory imbalance may be more pronounced in SLE accounting for Katz's results. The nature of the subclinical salivary involvement detected by SSS should be further investigated in order to explain

the discrepancy among SSS, clinical examination, and lip biopsies. SSS may evaluate a function of the ductal cells (21), whereas the reduction of salivary flow and the ensuing symptoms may result primarily from acinar lesions of the major salivary glands. Minimal vascular changes, which are assumed to initiate lupus vasculitis and rheumatoid synovitis, could be demonstrated as an early expression in the sensitive SSS.

ACKNOWLEDGMENTS

The authors wish to thank Dr. P. Verroust, INSERM U 64 Hospital Tenon, for the measurement of circulating immune complexes in one series of sera, and Dr. A. P. Peltier for the anti-RNP tests. Mrs. M. C. Brunel provided excellent secretarial assistance.

REFERENCES

1. Schall GL, Anderson LG, Wolf RO, Herdt JR, Tarpley TM, Cummings NA, Zeiger LS, Talal N: Xerostomia in Sjögren's syndrome. Evaluation by sequential salivary scintigraphy. JAMA 216:2109–2116, 1971
2. Daniels TE, Powell MR, Sylvester RA, Talal N: Evaluation of salivary scintigraphy in Sjögren's syndrome. Arth Rheum 22:809–814, 1979
3. Rampon S, Bussiere JL, Sauvezie B, Hullin JP, Veyre A, Lafaye C, Rivier A: Etude systématique de la scintigraphie salivaire séquentielle au cours des polyarthrites rhumatoïdes et des collagénoses. A propos de cent cinquante examens. Rev Rheum 42:185–194, 1975
4. Katz WA, Ehrlich GE, Gupta VE, Shapiro B: Salivary gland dysfunction in systemic lupus erythematosus and rheumatoid arthritis. Diagnostic importance. Arch Intern Med 140:949–951, 1980
5. Bloch KJ, Buchanan WW, Wohl MJ, Bunim JJ: Sjögren's syndrome. A clinical, pathological and serological study of fifty five cases. Medicine 44:187–231, 1965
6. Greenspan JS, Daniels TE, Talal N, Sylvester RA: The histopathology of Sjögren's syndrome in labial salivary gland biopsies. Oral Surg 37:217–229, 1974
7. Tarpley TM, Anderson LG, White CL: Minor salivary gland involvement in Sjögren's syndrome. Oral Surg 37:64–74, 1974
8. Moutsopoulos HM, Chused TM, Mann LD, Klippel JH, Fauci AS, Franck MM, Lauwley TJ, Hamburger MD: Sjögren's syndrome (sicca syndrome): Current issues. Ann Intern Med 92:212–226, 1980
9. Masi AT, Medsger TA: Epidemiology of the rheumatic diseases. *In* Arthritis and Allied Conditions, DJ MacCarthy (ed). Philadelphia, Lea and Febiger, 1979, p 13
10. Eyquem A: Immunopathologie de la polyarthrite rhumatoïde, des colagénoses, des affections thyroïdiennes, hépatiques et gastriques. Cours Immunol Gen Serol Inst Pasteur 6:199–216, 1971
11. Peltier AP, Gaudreau A, Haim T: Les anticorps spécifiques d'antigènes nucléaires solubles en pathologie rhumatismale et inflammatoire. L'actualité rhumatologique 1978 présentée au Praticien, Paris, Expansion Scientifique, 1978, pp 222–236
12. Zubler RH, Lange G, Lambert PB, Miescher PE: Detection of immune complexes in unheated sera by a modified 125 I C1q binding test. Effect of heating on the binding of C1q by immune complexes and application of the test to systemic lupus erythematosus. J Immunol 116:232–235, 1976
13. Theofilopoulos AN, Wilson CB, Dixon FJ: The Raji cell radioimmune assay for detecting immune complexes in human sera. J Clin Invest 57:169–182, 1976
14. Alarcon-Segovia D, Gonzalez-Jimenez, Garza LR, Maisterrena I: Radioisotopic evaluation of salivary gland dysfunction in Sjögren's syndrome. Am J Roentgenol Radium Ther Nucl Med 112:373–379, 1971
15. McSween RNM, Goudie RB, Anderson JR, Armstrong E, Murray MA, Mason DK, Jasani MK, Bayle JA, Buchanan WW, Williamson J: Occurence of antibody to salivary duct epithelium in Sjögren's syndrome disease, rheumatoid arthritis and other arthritides. A clinical and laboratory study. Ann Rheum Dis 26:402–411, 1967
16. Miyasaka N, Sauvezie B, Pierce D, Daniel TE, Talal N: Decreased autologous mixed lymphocyte reaction in Sjögren's syndrome. J Clin Invest 66:928–934, 1980
17. Lawley TJ, Moutsopoulos HM, Katz SI, Theofilopoulos AN, Chused TM, Frank MM: Demonstration of circulating immune complexes in Sjögren's syndrome. J Immunol 123:1382–1387, 1979
18. Talal N, Asofsky R, Lightbody P: Immunoglobulin synthesis by salivary gland lymphoid cells in Sjögren's syndrome. J Clin Invest 49:49–54, 1970
19. Anderson LG, Cummings NA, Asofsky R, Hylton MB, Tarpley TM, Tomasi TB, Wolf RO, Schall GL, Talal N: Salivary gland immunglobulin and rheumatoid factor synthesis in Sjögren's syndrome. Natural history and response to treatment. Am J Med 63:456–463, 1972
20. Fischbach M, Char D, Christensen M, Daniels T, Whaley K, Alspaugh M, Talal N: Immune complexes in Sjögren's syndrome. Arth Rheum 23:791–995, 1980
21. Logothetopoulos JH, Myant NB: Concentration of radioiodure and thiocyanate by the salivary glands. J Physiol 134:180–194, 1956