Cell-mediated immunity in Sjögren's syndrome

H. BERRY, P. A. BACON, AND J. D. DAVIS

Departments of Experimental Pathology and Rheumatology, St. Bartholomew's Hospital, London

The triad of keratoconjunctivitis sicca (KCS), salivary gland involvement (increase in size or xerostomia), and rheumatoid arthritis was described by Sjögren (1933) as a rare syndrome.

Any two of the triad were sufficient to make the diagnosis. Subsequently the syndrome has been extended to include disseminated lupus erythematosus (Morgan, 1954; Ramage and Kinnear, 1956; Bain, 1960), systemic sclerosis (Schaposnik, Bergna, and Conti, 1955), polyarteritis nodosa (Shearn, 1960), and polymyositis (Shearn, 1960), instead of rheumatoid arthritis. More recently it has been described in liver diseases not necessarily associated with an arthropathy (Golding, Bown, Mason, and Taylor, 1970). An immune basis has been suggested for this syndrome, and there is good evidence of altered circulating autoantibodies (Bloch, Bunim, Wohl, and Zvaifler, 1960). Sjögren showed histological evidence of small round cell infiltration in the parotid and sublingual glands, but little work has so far been carried out on specific cell-mediated immunity.

In this study the leucocyte migration test has been used to investigate cell-mediated immunity to parotid and liver antigens in patients with Sjögren's syndrome, with rheumatoid arthritis, and with autoimmune liver disease (chronic active hepatitis and primary biliary cirrhosis). In selected patients buccal salivary gland histology has been studied.

Methods

PATIENT SELECTION

Thirty patients with the full triad of Sjögren's syndrome (26 with rheumatoid arthritis, two with psoriatic arthropathy, one with scleroderma, and one with disseminated lupus erythematosus), 52 patients with classical or definite rheumatoid arthritis with no evidence of keratoconjunctivitis sicca, and 32 patients with autoimmune liver disease, some with keratoconjunctivitis sicca (eighteen with primary biliary cirrhosis and fourteen with chronic active hepatitis), who were attending out-patient clinics, were selected.

A series of 38 patients with other diseases and ten healthy subjects were also studied.

LEUCOCYTE MIGRATION TEST

Washed peripheral leucocytes are allowed to migrate from a capillary tube into a sealed chamber containing antigen in culture medium (Søborg and Bendixen, 1967). The migration is expressed as a percentage of migration of control chambers containing no antigen in the culture medium. Positive biological activity is represented either by stimulation (120 per cent. or over) or by inhibition (80 per cent. or under) of migration.

In this study results are expressed on an activity index by inverting all results over 100 so that an index of 125 appears on our scale as 75.

Antigens

Fresh human cadaver liver and parotid gland taken from a subject who died of unrelated disease were homogenized in saline, ultrasonicated, and freeze-dried. Protein extract, 200 µg., was selected as the antigen concentration, as this was shown to cause no reactivity in initial control experiments.

LIP BIOPSIES

Five patients with Sjögren's syndrome, five with rheumatoid arthritis who showed reactivity in the leucocyte migration test, and five who showed no reactivity had a lip biopsy performed. These were graded in two ways by an independent observer (JDD). The number of lymphocytes per 4 sq. mm. salivary tissue was assessed by the method of Chisholm and Mason (1968): 0 = absent, 1 = slight infiltrate, 2 = moderate infiltrate but less than 1 focus, 3 = 1 focus, 4 = more than 1 focus*.

The histology was also assessed by a simple method of counting the number of lymphocytic foci per 4 sq. mm.

ESTIMATION OF SECRETION

Tear secretion was assessed in all patients by Schirmer's technique, using Whatman No. 41 filter paper strips. The moistened length was measured after 5 min, 10 min. or less being taken as abnormal. Stimulated salivary secretion was estimated by the response to chewing gum over 5 mins., less than 10 ml. being considered abnormal.

Accepted for publication January 6, 1972.

Presented to the Heberden Society on November 27, 1971.

* A focus is aggregate of 50 or more lymphocytes and histiocytes but does not involve plasma cells.

Where indicated the eyes were examined with a slit lamp after installation of 1 per cent. Rose Bengal. Staining of conjunctiva or cornea was considered to be abnormal.

Results

Of 30 patients 28 (93 per cent.) with Sjögren's syndrome showed reactivity to parotid antigen. One of the patients who did not show reactivity has subsequently developed normal tear secretion again, despite completely fulfilling the criteria for Sjögren's syndrome, including staining with Rose Bengal, at a previous assessment. One of the patients showed reactivity to liver antigen (see Table I and Fig. 1).

FIG. 1 Leucocyte migration test to parotid extract, and salivary function

Thirteen of 52 patients (25 per cent.) with definite or classical rheumatoid arthritis showed reactivity to parotid antigen. All these patients showed normal tear and salivary secretion; only one (2 per cent.) showed reactivity to liver.

Ten of 32 patients (31 per cent.) with autoimmune liver disease showed reactivity to parotid antigen; 27 of these patients were tested for Sjögren's syndrome and ten had evidence of keratoconjunctivitis sicca. Six of the ten (60 per cent.) showed reactivity to parotid antigen, as did five of the seventeen (29 per cent.) who had no evidence of keratoconjunctivitis

sicca. It was of interest that one patient who had shown reactivity in the leucocyte migration test to parotid antigen without keratoconjunctivitis sicca has subsequently developed this triad. Of 32 patients in this group, 25 (78 per cent.) showed reactivity to liver antigen.

In the control group, one patient (2 per cent.) showed reactivity to liver antigen but more showed reactivity to parotid antigen (this patient also had a positive antinuclear factor).

In the rheumatoid patients there was no correlation between reactivity to parotid antigen and duration of disease, age, and clinical parameters of severity such as the erythrocyte sedimentation rate, articular index, or radiological evidence of hand erosions. Nor was there any correlation with circulating autoantibodies.

LIP BIOPSIES

In the five patients with Sjögren's syndrome, the mean score was 3.4 by Chisholm and Mason (1968), 3.6 by counting foci. In the five patients who showed reactivity to parotid antigen with rheumatoid arthritis and normal secretion, the respective values were 2.2 and 2.5, and in the five patients with non-reactive rheumatoid arthritis 1.8 and 0.5 respectively (Table II). Control biopsies of normal subjects showed no infiltration.

Table II Scores according to Chisholm and Mason (1968) by counting the number of foci in 4 sq. mm.

Diagnosis	Score	Foci score
Sjögren's syndrome	3.4	3.6
Rheumatoid reactors	3.2	2.5
non-reactors	1.8	0.5

In this small series there is a continuous gradation of lymphocytic infiltration between rheumatoid patients not reacting to parotid antigen through rheumatoids with positive reactivity to those with true Sjögren's syndrome although there is some individual overlap (Figs 2 to 4, overleaf). The simpler method of counting foci appears to give a better separation between the three groups.

Table I Reactivity to antigen in leucocyte migration test in disease groups and controls

Diagnosis	Sjögren's syndrome	Rheumatoid arthritis	Autoimmune liver disease	Controls
Total cases	30	52	32	48
Reactivity to Parotid	28	13	10	0
Liver	1	1	25	1

FIG. 2 Lip biopsy from a patient with rheumatoid arthritis and no leucocyte migration test reactivity. $\times 500$

FIG. 3 Lip biopsy from a patient with rheumatoid arthritis and leucocyte migration test reactivity. $\times 500$

FIG. 4 Lip biopsy from a patient with Sjögren's syndrome. $\times 500$

Discussion

Søborg and Bendixen (1967) first reported cellular hypersensitivity to salivary gland extracts which they observed in 50 per cent. of their small series of patients with Sjögren's syndrome. Our results confirm and extend this finding. The higher proportion of positives in the present study may be due to minor differences in the strength and preparation of the parotid extract. Both the antigen concentration and the strength of the individual hypersensitivity can alter the result of the leucocyte migration test (Søborg and Bendixen, 1967) and only testing at serial dilutions which was not done by those authors can show whether a result in the normal range is a true negative or a hidden positive. The response to parotid antigen is not a nonspecific reaction to tissue, as liver in the same concentration produced no reaction except in one patient with Sjögren's syndrome. The finding of keratoconjunctivitis sicca in patients with autoimmune liver disease confirms the report of Golding and others (1970) and is discussed in more detail elsewhere (Bacon and Berry, 1972). The lower percentage of positive reactions to parotid antigen in those patients with liver disease and keratoconjunctivitis sicca was very close to the figure reported by Søborg and Bertram (1968). This difference between the two groups of patients with Sjögren's syndrome could be attributed in part to patient selection. All the first group

had sufficiently severe keratoconjunctivitis sicca to complain about it spontaneously and were already attending the Ophthalmic Department. The keratoconjunctivitis sicca in the liver patients were found only on specific survey and was not a source of complaint. The association of a high proportion of positive responses to parotid antigen in the leucocyte migration test in both groups suggest that the keratoconjunctivitis sicca found in autoimmune liver disease is indeed a true part of the spectrum of Sjögren's syndrome, as suggested by Shearn (1971), rather than a separate disorder. The finding of reactivity to parotid antigen in a quarter of the rheumatoid patients in the absence of any clinical evidence of keratoconjunctivitis sicca is of considerable interest, especially as the same proportion of patients with liver disease but no keratoconjunctivitis sicca reacted similarly. In the small highly selected series of patients who had lip biopsies, there was a close association between lymphocytic infiltration and a positive response to parotid antigen *in vitro*. The *in vitro* method thus appears to be a good test for the pathological changes underlying Sjögren's syndrome. It detects lymphocytic hypersensitivity before infiltration of the glands has progressed sufficiently to produce atrophy of secreting tissue and consequent clinically apparent disease.

The finding of cell-mediated immune reactivity *in vitro*, which correlates with the histological evidence of infiltration with small lymphocytes, suggests that this may have a pathogenetic significance in Sjögren's syndrome. Some support for this concept can be adduced from the two patients who were noted to develop dry eyes a few months after the test *in vitro* had been found positive. No systematic study of the progress of these patients has yet been made.

Keratoconjunctivitis sicca occurs in a variety of disorders which are associated with immune complex deposition: in the synovial tissue in rheumatoid arthritis, in the renal and vascular lesions of disseminated lupus erythematosus (Koffler, Schur, and Kunkel,

1967), and in the liver in certain hepatic diseases (Almeida, Zuckerman, Taylor, and Waterson, 1969). However, there is no direct evidence of immune complex deposition in the salivary tissue in Sjögren's syndrome and the evidence points to a cell-mediated pathology. This suggests that keratoconjunctivitis sicca may have a separate aetiology although it is a disorder which occurs more frequently in patients with other autoimmune diseases than as an isolated symptom.

Summary

Of thirty patients with Sjögren's syndrome, 28 (93 per cent.) showed reactivity to parotid extract antigen in the leucocyte migration test. Thirteen (25 per cent.) of 52 patients with rheumatoid arthritis without evidence of Sjögren's syndrome similarly showed reactivity. Ten (31 per cent.) of 32 patients with autoimmune liver disease were found to have keratoconjunctivitis sicca and six (60 per cent.) of these ten reacted to parotid antigen. Five (29 per cent.) of seventeen patients without keratoconjunctivitis sicca also reacted to parotid antigen. Liver reactivity was seen in 25 (78 per cent.) of 32 patients with autoimmune liver disease but not in other groups. Lip biopsies on fifteen of these patients (five with Sjögren's syndrome, five with rheumatoid arthritis who exhibited reactivity to parotid antigen, and five with rheumatoid arthritis who did not react) showed a correlation between the degree of lymphocytic infiltration and reactivity in the leucocyte migration test.

We are grateful to Prof. B. Amor and Mme A. Kahan, Hôpital Cochin, Paris; Dr. D. Holdsworth, Sheffield Royal Infirmary; and Dr. A. M. Dawson and Dr. H. Wykeham Balme for permission to use their patients. HB is in receipt of an Aylwen Bursary from the Board of Governors, St. Bartholomew's Hospital. We should also like to thank Prof. D. A. Willoughby for his consistently stimulating advice and help.

References

- ALMEIDA, J. D., ZUCKERMAN, A. J., TAYLOR, P. E., AND WATERTON, A. P. (1969) *Microbios*, **2**, 117 (Immune electron microscopy of the Australia-SH antigen)
- BACON, P. A., AND BERRY, H. (1972) *Gut*, **13**, 427 (Cell-mediated immune reactivity in liver disease)
- BAIN, G. O. (1960) *Canad. med. Ass. J.*, **82**, 143 (Pathology of Mikulicz-Sjögren disease in relationship to disseminated lupus erythematosus)
- BLOCH, K. J., BUNIM, J. J., WOHL, M. J., AND ZVAIFLER, N. J. (1960) *Trans. Ass. Amer. Phycns*, **73**, 166 (Unusual occurrence of multiple tissue component antibodies in Sjögren's syndrome)
- CHISHOLM, D. M., AND MASON, D. K. (1968) *J. clin. path.*, **21**, 656 (Labial salivary gland biopsy in Sjögren's disease)
- GOLDING, P. L., BOWN, R., MASON, A. M. S., AND TAYLOR, E. (1970) *Brit. med. J.*, **4**, 340 (The sicca complex in liver disease)
- KOFFLER, D., SCHUR, P. H., AND KUNKEL, H. G. (1967) *J. exp. Med.*, **126**, 607 (Immunological studies concerning the nephritis of systemic lupus erythematosus)
- MORGAN, W. S. (1954) *New Engl. J. Med.*, **231**, 5 (The probable systemic nature of Mikulicz's disease and its relation to Sjögren's syndrome)

- RAMAGE, J. H., AND KINNEAR, W. F. (1956) *Brit. J. Ophthalm.*, **40**, 416 (Keratoconjunctivitis sicca and the collagen diseases)
- SCHAPOSNIK, F., BERGNA, L. J., AND CONTI, A. (1955) *Rev. clin. esp.*, **59**, 102. (Sindrome de Sjögren y lupus eritematoso disseminado)
- SJÖGREN, H. (1933) *Acta ophthalm. (Kbh.)*, Suppl. 2 (Zur kenntnis der keratoconjunctivitis sicca)
- SHEARN, M. A. (1960) *Ann. intern. Med.*, **52**, 1352 (Sjögren's syndrome in association with scleroderma)
- (1971). "Sjögren's syndrome", in "Major Problems in Internal Medicine", vol. 2. Saunders, Philadelphia
- SØBORG, M., AND BENDIXEN, G. (1967) *Acta med. scand.*, **181**, 247 (Human leucocyte migration as a parameter of hypersensitivity)
- AND BERTRAM, U. (1968) *Ibid.*, **184**, 319 (Cellular hypersensitivity in Sjögren's syndrome)